WORKING
PAPER

Project Retrosight

Understanding the returns from cardiovascular and stroke research

Case Studies

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Preface

This report presents a set of 29 case studies of cardiovascular and stroke research grants funded in three countries between 1989 and 1993. The case studies focused on the individual grants but considered the development of the investigators and ideas involved in the research projects from initiation to the present day.

Basic biomedical and clinical cardiovascular and stroke research grants awarded in Australia, Canada and the UK were selected through a stratified random selection approach that aimed to include both high- and low-impact grants. Case studies were constructed around the Payback Framework[1], a research evaluation framework that is both a tool for evaluating a range of potential *outputs* from research and a logic model that provides a mechanism for conceptualising the *process* through which outputs are created.

The structured case studies were used to assess the impact of the research grants in detail and to draw conclusions about the relationship between impact and a range of factors. The key messages from the project are as follows.

- The cases reveal that a large and diverse range of impacts arose from the 29 grants studied.
- There are variations between the impacts derived from basic biomedical and clinical research.
- There is no correlation between knowledge production and wider impacts.
- The majority of economic impacts identified come from a minority of projects.
- We identified factors that appear to be associated with high and low impact.

This report presents the case studies. As far as possible, they are presented in the form used in the analysis. Case studies were initially approved by the PI for the research examined and each case study was then peer reviewed by independent researchers in the cardiovascular and stroke field. These reviews were used by the rating panel in assessing the impacts of the research described. Twenty-four of the case studies were reviewed by two independent researchers; for the remaining five we were only able to secure one reviewer in each case.

[1] Buxton, M., and S. Hanney. "How can payback from health services research be assessed?" Journal of Health Services Research and Policy 1 (1996): 35–43.

The Policy Report[2] presents the project's findings and their implications for policy, and the Methodology Report[3] provides a detailed account of the methodology used, including the review process.

This work was led by RAND Europe in collaboration with the Health Economics Research Group (HERG) at Brunel University. RAND Europe is an independent not-for-profit policy research organisation that serves the public interest by improving policy-making and informing public debate. The Health Economics Research Group is a Specialist Research Institute of Brunel University dedicated to conducting accessible, policy-relevant research of a high academic quality focused on improving the efficiency and cost-effectiveness of resources devoted to health care and to research.

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[2] Wooding, S., S. Hanney, A. Pollitt, M. Buxton, and J. Grant. *Project Retrosight. Understanding the returns from cardiovascular and stroke research: Policy Report*. Cambridge, UK: RAND Europe, MG-1079-RS, 2011.

[3] Pollitt, A., S. Wooding, S. Hanney, M. Buxton, and J. Grant. *Project Retrosight. Understanding the returns from cardiovascular and stroke research: Methodology Report*. Cambridge, UK: RAND Europe, TR-925-RS, 2011.

Contents

Acknowledgements and detailed authorship

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Project Retrosight team

Jonathan Grant (RAND Europe), Martin Buxton (HERG), Stephen Hanney (HERG) and Steven Wooding (RAND Europe) devised the methodological approach and analysis with input from the Retrosight team. Steven Wooding managed and coordinated the project, supported by Eddy Nason (RAND Europe), Sharif Ismail (RAND Europe), Sue Kirk (RAND Europe) and Alex Pollitt (RAND Europe). Statistical analysis was provided by Laura Staetsksy (RAND Europe).

The Australian case studies were carried out by Rob Mercer (Instinct and Reason), Angela Mitchell (Instinct and Reason) and Christine Latif (National Heart Foundation of Australia); the Canadian studies by Laura McAuley (Canadian Institutes of Health Research), Heather Mustoe (Canadian Institutes of Health Research) and Kimberly-Anne Ford (Canadian Institutes of Health Research); the UK studies by Stephen Hanney, Sharif Ismail, Sue Kirk, Sonja Marjanovic (RAND Europe) and Eddy Nason. The case study bibliometric analysis was coordinated by Linda Butler (Australian National University) and Sharif Ismail and carried out by Kumara Henadeera (Australian National University) and Thed van Leeuwen (CWTS).

CHAPTER 1 To conduct studies of a high density lipoprotein conversion factor

1.1 Overview of case study grant

Today it is widely known that high blood cholesterol is a risk factor for cardiovascular disease (CVD). The idea of 'good' and 'bad' cholesterol is also broadly accepted; 'good cholesterol', bound to high-density lipoprotein (HDL), helps to unblock blood vessels, while 'bad cholesterol', bound to low-density lipoproteins (LDL), clogs blood vessels. This is not just known in the scientific community; it is well known by the public and the idea has been adopted by the food industry, where it has spawned brands, sub-brands and product ranges.

This case study examines the evolution and impacts of a National Heart Foundation of Australia (NHFA)-funded basic research grant, awarded from 1989 to 1991, titled 'To Conduct Studies of a High Density Lipoprotein Conversion Factor' (grant reference: G88M2544). The principal investigator (PI) was Dr Philip Barter (now Professor) and the research was conducted at the Baker Institute and later at the University of Wollongong. It was a basic research project with a 'large' grant (the award was for just under Aus$112,500 over three years; roughly equivalent to Aus$40,000 each year) running from 1989 to 1991.

The research conducted through this grant focused on HDL, looking in particular at the different forms it could take and the factors which influence the partitioning of cholesterol between the different forms and influence the ability of HDL to drain cholesterol from tissues. When this work was conducted, little was known about HDL. The HDL particles were known to transport cholesterol in the body and high levels of HDL had been observed to reduce the risk of heart disease, but little was known about their biology. The team had found an 'HDL conversion factor', which changed the properties of HDL by converting it between different forms. Their work investigating this eventually revealed information about how the balance between LDL- and HDL-bound cholesterol is maintained and the role of fatty acids in this balance.

The project was successful at many levels. It led to a greater understanding of HDL and identification of its implications. It completed a story in relation to the dynamic state of HDL. It led to new techniques, a significant step forward in scientific understanding and the development of new research areas. Professor Barter and at least two members of the team, Rye and Lagrost, continue to practice today, working in similar fields of research,

and have international reputations. For example, Rye is one of the few people in the world able to construct in-vitro HDL.

1.2 Introduction to case study

1.2.1 Scientific background

At the time of the grant application it was known that levels of blood cholesterol were connected to risk of atherosclerosis and resulting cardiovascular diseases such as heart attack and stroke. It was also established that these risks differed between the two major forms of cholesterol. High levels of low-density lipoprotein (LDL) were known to increase the risk of cardiovascular disorders, while increased levels of high-density lipoprotein (HDL) reduced the risk of such conditions. However, there was a lack of consensus at that time as to whether this knowledge could lead to treatment or prevention activities, as it was not well established how this mechanism was controlled and how cholesterol levels could be reduced.

HDL protects against CVD through its crucial role in reverse cholesterol transport (RCT). This is the process by which cholesterol is transported from the tissues of the body to the liver for excretion. This reduces the level of cholesterol in all tissues, thereby reducing the build up of cholesterol plaques on the walls of arteries, which obstruct the flow of blood, are responsible for atherosclerosis and can cause acute events such as heart attacks. In contrast, LDL delivers cholesterol from the liver to the tissues of the body, hence its effect on increasing CVD risk.

The RCT process also involves a complex mix of other components in the blood. One of the key steps is the esterification of cholesterol on the surface of HDL, catalysed by lecithin:cholesterol acyltransferase (LCAT). This incorporates the cholesterol esters into the core of the HDL, leaving the surface depleted of free cholesterol, thereby generating a concentration gradient that promotes the continued flux of cholesterol out of the tissue and into the blood. Therefore, the way in which this esterification process is regulated is crucial to the RCT process.

Professor Barter's team, as a part of previous work funded by the National Heart Foundation of Australia, had investigated the role of HDL particle size in this process. HDL in human plasma was known to comprise several discrete subpopulations, which differ in terms of particle size, density, lipid content and apolipoprotein composition. These different size particles are inter-convertible, and the size of HDL particles had been found to affect the rate of esterification. This discovery prompted the team to further investigate HDL heterogeneity, with particular interest in factors regulating HDL particle size and the implications for regulation of plasma cholesterol esterification.

1.2.2 The case study approach

The case study based on this research grant involved a combination of face-to-face interviews with Professor Barter and another member of the research team, dialogue with another team member, review of the grant application and supporting documents, and a review of the relevant literature.

1.3 Stage 0 – topic identification

Prior to this grant application, Professor Barter had been conducting research in this field for a number of years. According to Barter, a chance observation combined with his interest in the field led to the grant application. In this section we look at the key influences on topic identification for this project more closely. These are:

1. previous research experience – prior work and personal interest
2. funding environment
3. well-established PI.

1.3.1 Previous research experience

The main impetus for this grant application was the team's discovery of a plasma factor that changes the size of HDL particles. As outlined previously, HDL particles can vary significantly in size, and the size of the particles is significant for function, including in the RCT process, which has strong implications for a range of cardiovascular diseases. Therefore, the discovery of this 'HDL conversion factor' was considered significant. This discovery, and some initial work on the conversion factor, is described in the paper 'Isolation of a High-Density Lipoprotein Conversion Factor from Human Plasma: A Possible Role of Apolipoprotein A-IV as its Activator' (Barter et al., 1988). This discovery came as part of a background in research in this field in general, and on HDL in particular, which had formed part of Professor Barter's research portfolio for a number of years. A number of the research projects he led contributed to a growing body of knowledge in this field and increased Barter's own interest in this field. The team was making a steady stream of discoveries that in turn prompted new avenues of investigation, leading to the discovery of the HDL conversion factor.

The team had conducted some initial investigation of the HDL conversion factor using National Heart Foundation of Australia funding already held at that time. This work is detailed in the grant application (Grant application, 1988) and followed three main strands, investigating the interaction of the conversion factor with other components in the blood.

The first of these looked at apolipoproteins and whether any of these acted as an activator or inhibitor of the HDL conversion factor. While apoliproprotein (apo) A-I, apo A-II, apo C and apo E were all found to have no effect, apo A-IV was found to stimulate the conversion factor. These findings were published around the time of the grant application[1]. Further investigation into this discovered that lower concentrations of apo A-IV stimulated activity of the conversion factor but in contrast higher concentrations inhibited the activity. Professor Barter believed that this observation was potentially of great importance for three key reasons: 'Firstly, it provides a clear physiological role for a relatively abundant apolipoprotein, the function of which has been previously unknown. Secondly, the concentrations of apoA-IV required to produce both the stimulatory and inhibitory effects were within the range reported in vivo. Thirdly apoA-IV exists both as a component of lipoproteins (mainly HDL) and in a 'free' form unassociated with the major lipoprotein

[1] As documented in Barter et al. (1988), in press at time of application.

fractions; the observations that the relative proportions of 'free' and HDL-bound apoA-IV vary markedly raises an obvious question: do the effects of apoA-IV on the conversion process relate to activity of the 'free' or the HDL-bound fraction?' (Grant application, 1988).

The HDL conversion process was also found to be profoundly stimulated by the addition of either very-low-density lipoprotein (VLDL) or LDL to an incubation mixture containing HDL, the conversion factor and the lipid transfer protein. Work by the team had shown the effect of VLDL to be concentration dependent within the range found in normal human subjects. This indicated that the phenomenon may be of importance to the physiological regulation of HDL particle size. However, the mechanism of this stimulation was unknown and hence the grant proposed further investigation.

Finally, preliminary studies had shown evidence of an interaction between the conversion factor and LCAT. In particular, the conversion factor was found to promote the production of small HDL particles, which had previously been observed in patients with familial LCAT deficiency, a genetic disorder that limits or completely prevents the production of LCAT. It was thought that these small, highly reactive HDL particles might act as nucleating points for LCAT, which is why they are not seen normally in the presence of LCAT, and that their presence may enhance the rate of cholesterol esterification.

These three avenues of investigation formed the three aims defined for the research performed and provided evidence of interesting phenomena upon which the research could be built.

1.3.2 Funding environment

Topic identification was also influenced by the funding environment. Clearly, this area of HDL research could have significant implications for CVD, and taking a CVD angle to the research project made it easier to get funding than submitting a purely curiosity driven application. Indeed, at the time of application, Professor Barter currently held another grant from the National Heart Foundation of Australia, 'HDL and Plasma Cholesterol Esterification', funded from 1986 to 1988.

Professor Barter had both a basic research and clinical background. While this particular grant was a biomedical project, as a clinician it was clear that Barter was interested in research that was relevant to the health outcomes, and the potential health implications of this work are mentioned in the application.

1.3.3 A well-established PI

As noted above, Professor Barter had been active, and successful, in the field of HDL for several years. At the time of this application, he was Deputy Director of the Baker Institute, one of the leading institutes at the time.

The grant submission was, in Professor Barter's opinion at least, highly speculative, given the original nature of the work proposed. However, Barter had the advantage of being well established with a successful track record, and indeed both reviewers rated Barter's track record as 'outstanding'. From the time of receiving his first competitive research grant in 1975 (Grant in Aid, National Heart Foundation of Australia, Aus$10,500) to the awarding of this particular grant in aid, Barter had been awarded Aus$492,000 in grant in

aids (just under Aus$1.9 million in research funding overall), notably from the National Heart Foundation of Australia and National Health and Medical Research Council (NHMRC), plus a five-year programme grant from the NHMRC for Aus$250,000 per year and a block grant (five years) from the NHMRC to the Baker Institute for the same amount[2]. He already had 68 papers in peer-reviewed journals, most of moderate impact but the best for this field. He also had a significant research capacity, with eight doctor of philosophy (PhD) students working with him.

Professor Barter believes his background gave him an advantage over younger or newer researchers who had yet to prove themselves and so had to 'play the game' to achieve funding. Furthermore, the funding background meant that Barter had the luxury of applying for funding to answer questions that were of interest. That said, the speculative nature of the application led Barter to be uncertain about its successful award, but he believed that if the National Heart Foundation of Australia had not funded this project it would 'probably be funded by someone else' (Barter interview, 2007).

1.3.4 Curiosity

Clearly, there was significant interest at this time in a greater understanding of the cholesterol transport process and the role of HDL in reducing CVD risk. The proposed study into the newly discovered HDL conversion factor provided the possibility of investigating this. Furthermore, the background research had alluded to a function for apo A-IV, which had not been previously established, so this was clearly an issue of academic curiosity.

Though this work had obvious potential health implications, it would also provide the opportunity to look at broader issues such as the physiology of HDL and its different forms from the position of pure academic curiosity. Indeed, the initial observations had piqued the interest of the two grant reviewers, particularly in regard to the possible identification of a role for apo A-IV (Grant-in-Aid Assessor Report, 1988):

- 'The idea that apo A-IV might be involved in this process is an exciting proposition for a function for this apoprotein. The work could have an important bearing on our understanding of the role of high density lipoprotein and apolipoprotein A-IV in lipoprotein metabolism'
- 'The proposed role of conversion factor is obscure, but it is obvious that more information about its activity is needed. Apolipoprotein A-IV has no known functions; perhaps Barter has found one'.

1.4 Interface A – project specification and selection

The grant application was submitted in the sole name of Professor Barter. This grant application built on significant preliminary data and the track record of Barter and his research group: 17 publications were cited in the application (including one in press) from Barter and relevant to this work and a further 19 cited as other major references of Barter

[2] Data taken from PI's curriculum vitae.

(some of which were co-authored by the requested research assistant (RA)). The HDL conversion factor at the centre of this project had itself been identified by Professor Barter's team previously (specifically by Rye). In addition, a previous National Heart Foundation of Australia-funded project was directly related to this study.

Three specific project aims were listed:

1. to define the effects of apo A-IV on activity of a recently identified HDL conversion factor
2. to define the effects of the lipid transfer protein VLDL and LDL on activity of the HDL conversion factor
3. to define the physiological significance of very small HDL particles formed by the HDL conversion factor: specifically to define their role in regulating plasma cholesterol esterification.

These were based on prior preliminary work which had shown that these were areas of potential research interest. A detailed research plan for each of these avenues was included in the application (Grant application, 1988).

The assessors and interviewers were very positive about the application. Positive comments were made on the concept itself, the proposed approach ('The proposed experiments make sense and should provide answers to the questions raised' (Grant-in-Aid Assessor Report, 1988)), and also on Professor Barter and his team. The assessors clearly recognised the potential of the project, noting the potential health benefits despite the very basic nature of this research. The assessors and interview panel all recommended the project be funded:

- 'This is a most interesting proposal. The work proposed is so original that it is difficult to be critical of it. The applicant has broken new ground with the concept of a high density lipoprotein conversion factor and his work must be clearly allowed to develop…The work could have an important bearing on our understanding of the role of high density lipoprotein and apolipoprotein A-IV in lipoprotein metabolism and hence our understanding of atherogenesis and protection from atherosclerosis' (Grant-in-Aid Assessor Report, 1988).
- 'Funding is recommended in view of the strong record of productivity from this group and the need for further information about conversion factor' (Grant-in-Aid Assessor Report, 1988).
- 'Enthusiastic and lucid explanation of the status and progress in this unique project' (Grant-in-Aid Report of Interview, 1988).

One assessor had concerns about the time that would be required – this appears to have been further explored at interview with the outcome that the interviewer was satisfied that 'Plans are appropriate for the time scale of the project' (Report of Interview, 1988).

The budget request was for Aus$39,000 per annum for an RA (Aus$32,938 in the first year, Aus$36,231 in the second year and Aus$39,854 in the third) and Aus$8,000 per annum for consumables. Both assessors acknowledged the need for an experienced assistant on the project, but one was concerned that the budget requested for consumables seemed high and should be further justified. It appears the budget was approved without changes.

However the grant provided just under Aus$112,500 over three years, roughly equivalent to Aus$40,000 per annum, so just under the request.

The grant application notes that the Aus$8,000 requested for consumables was a contribution to this rather than the total cost which in previous work had exceeded Aus$10,000 (Grant application, 1988).

1.5 Stage 1 – inputs to research

The most important inputs to this research were the skills and experience of the project team and the money required to support them.

This project involved a large number of different researchers from the laboratory, though the majority of the work was conducted by Rye, Chang and Lagrost. However, the financial input from this grant only covered the salary of one RA (Chang) and a proportion of the consumables, so other funding sources made a contribution. All three of these researchers had relevant experience, particularly Rye, who had discovered the conversion factor in earlier research. Barter's input was also significant as he had been working in this area for several years.

Other factors such as the change in location during the project, and collaboration, particularly with Enholm, also made an impact on the research conducted.

1.5.1 Funding

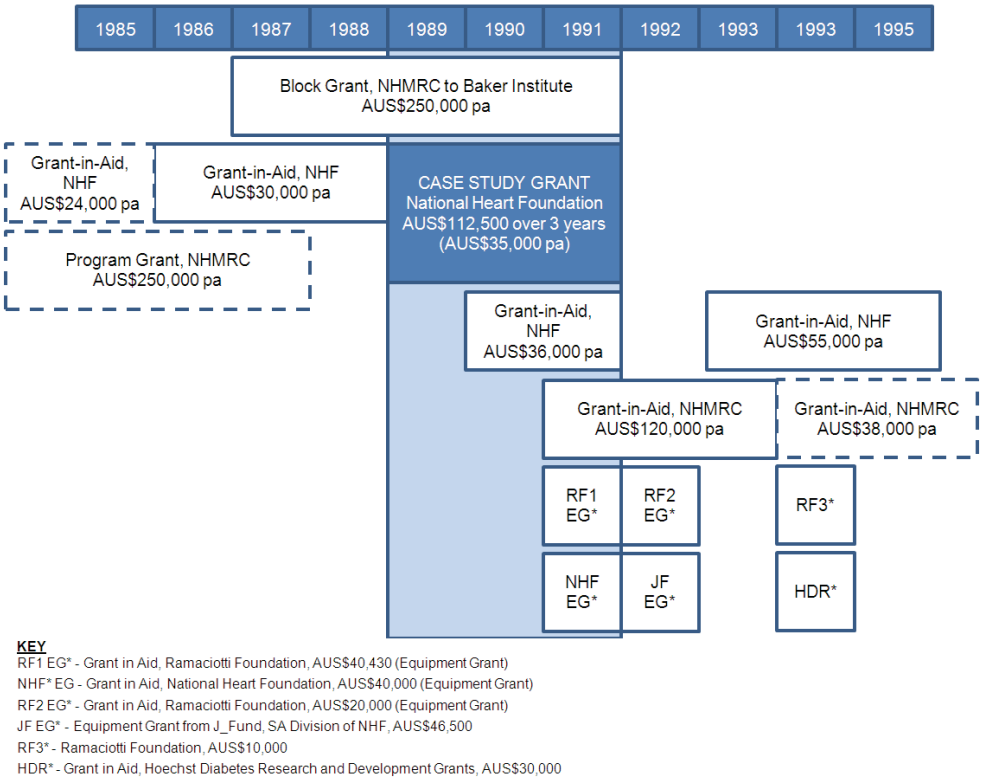
Grant funding

Funding from the grant provided for an RA and a considerable contribution to the consumables required. The RA was deemed to be of considerable importance to the project as particular skills and experience were required to undertake the experiments involved, and this was commented on by reviewers: 'The support requested is essentially [the RA's] salary. [The nominated RA] is highly trained and clearly an important element in continued progress with this work' (Grant-in-Aid Assessor Report, 1988).

Other funding

Other funding available to Professor Barter at the time of this grant included an NHMRC grant, a National Heart Foundation of Australia grant and a block institute grant for the Baker Institute from the NHMRC. Subsequent to the award of this grant, Barter was awarded a further National Heart Foundation of Australia grant (Rye and Ghiggino were also named on this application). According to Barter, the National Heart Foundation of Australia grant was about a quarter of the funding needed to do the research, and it can be difficult with small amounts of funding to see exactly where it is being used. The application notes: 'The [research] plan outlined...includes only those components for which NHF [the National Heart Foundation of Australia] support is requested' (Grant application, 1988), indicating that financial support from other sources was important to the bigger research picture. Figure 1-1 illustrates the range of funding won by the laboratory around that time. The majority of funding comes from the National Heart Foundation of Australia and NHMRC, though there are also a few small grants from the Ramaciotti Foundation and a Hoescht diabetes research and development grant.

Figure 1-1 Diagram showing funding available to Professor Barter's during the period 1985–1995



KEY
RF1 EG* - Grant in Aid, Ramaciotti Foundation, AUS$40,430 (Equipment Grant)
NHF* EG - Grant in Aid, National Heart Foundation, AUS$40,000 (Equipment Grant)
RF2 EG* - Grant in Aid, Ramaciotti Foundation, AUS$20,000 (Equipment Grant)
JF EG* - Equipment Grant from J_Fund, SA Division of NHF, AUS$46,500
RF3* - Ramaciotti Foundation, AUS$10,000
HDR* - Grant in Aid, Hoechst Diabetes Research and Development Grants, AUS$30,000

Overall, Professor Barter's laboratory was set up well and had funding from other sources including clinical funding. In addition, they conducted chemical trials at the laboratory, which provided an extra source of income.

1.5.2 Knowledge and expertise

The grant built directly on previous research conducted by this group; this was something Professor Barter had been working on for many years, gradually establishing the building blocks of understanding around HDL and cholesterol and triggering the key question at the heart of this grant. In the application for the grant, Barter was able to refer to the outcomes of previous studies undertaken by the group, on occasion supported by this funder, and detail how the key trigger for this application arose. All aims of this grant derived directly from previous research and expertise and this was acknowledged by the reviewers. The productivity of the team was noted in the review of the award: 'Funding is recommended in view of the strong record of productivity from this group and the need for further information about conversion factor' (Grant-in-Aid Assessor Report, 1988).

The PI

The project was borne out of Professor Barter's interest in HDL and was interest driven, and he brought to it considerable prior experience. He also provided a track record rated by reviewers as 'outstanding', which helped to secure this, and other, funding. According to Barter, also of importance to this project was his skill in detecting leads: 'One of my

strengths is the ability for being able to discern between meaningless observations and those that really revealed something new.' (Barter interview, 2007)

It would appear too that Professor Barter's ability to manage a team and play to their combined strengths was important to this project. This is explored further in the discussion of the research process. Furthermore, it would appear that his attitude and personality were important in the dissemination of the research findings.

The grant was basic research, yet Professor Barter also has a clinical background. Barter believes his clinical training contributes to his perspective on research and enables him, in contrast to researchers who do not share this background, to 'live with uncertainty', meaning that he would put projects to one side if they were not making sense or working and move on, focusing on other things rather than getting bogged down.

The RA

The RA on this grant was Chang, a masters student who acted as RA and technical assistant. He was named on the grant application, which notes that he had 18 months of experience with studies using the proposed techniques 'and a continuation of his involvement is essential'. Reviewers agreed with this. The grant funding covered his salary.

The rest of the team

Professor Barter and Chang were the only researchers named on the grant application. However, as the project commenced the team grew from its initial inception as Barter was able to move staff onto this project from others. In particular, Kerry Anne Rye and Laurent Lagrost both contributed to this research.

Rye had worked with Professor Barter's laboratory previously as a PhD student. She had been involved in HDL research and during her PhD had identified the conversion factor that was the focus of this research project in question. Following her PhD award, Rye moved to the United States and the University of Illinois, where she continued to study the conversion factor for around two and half years before taking the decision to return to Australia. As a consequence, Rye was not personally involved with the grant application, but she was back at the Baker Institute when the funding came through. Rye was a key player in this team and her contribution was particularly noted by Barter at interview. Two years after this project she received funding towards her postdoctoral research. Rye's involvement with this research focussed on sequencing and exploration of the very small HDL particles as per the third aim of the project.

Lagrost joined the team as a postdoctoral fellow in January 1990; he came from the University of Dijon, where he was involved in identifying and characterising the activity of the HDL conversion factor. His PhD thesis was mainly focused on the role of apo A-IV in lipoprotein metabolism and his expertise was in working out the putative implications of lipid and protein factors in modulating CETP–lipoprotein interactions, in particular the CETP-mediated HDL conversion process. While Rye focussed on the HDL particles, Lagrost focussed on the fatty acids component of the project.

Also involved in the project were a number of other students and postdoctoral researchers, including Harvey Newnham, Moira Clay, Linus Rajaram, Angela Denim and Neil Heim. A number of these names can be seen in the associated publications; however, most of

them made a minor contribution to this particular project, focusing on other work ongoing in the laboratory at the time. Harvey Newnham was an endocrinologist and PhD student, who was involved in research regarding fatty acids; at the time there were several fatty acid related projects being undertaken in Professor Barter's laboratory. Clay was a PhD student at the Baker Institute; however, she left the group when it moved to Wollongong in September 1990. Linus and Rajaram worked with Barter to drive some other fatty acid projects that were downstream, while Lagrost drove this main one. However, the majority of the work on this project was conducted by Chang, Rye and Lagrost, with advice from Barter.

Clinical experience

The research on this grant involved clinicians: Professor Barter himself had a clinical background and Newnham was also a clinician.

Professor Barter is a firm believer in using both basic researchers and clinicians on projects, largely due to their ability to bring a different perspective to research: 'Having clinicians brings a different perspective…they often do it a different way…a better way. Also, the clinicians are not the same after doing basic research. They should be pressured to do some basic research, because once they have done such research they are always better clinicians. However, the good basic researchers are often non-clinicians' (Barter interview, 2007).

1.5.3 Techniques

The team primarily used a range of standard techniques for this study. Some key techniques used include immunoblotting, gradient gel electrophoresis and gel permeation chromatography. No specific techniques that served as an input to research had been developed in advance of this work. However, the development of techniques surrounding HDL in-vitro by Rye was a significant output of this research, as described in Section 1.7.2.

1.5.4 Reputation

Both Professor Barter and the Baker Institute had strong reputations, which was probably a factor in winning this and other funding. However, this was not a key input into research, except for it being easier to attract funding and high-quality researchers, which act as inputs to research in themselves.

1.5.5 Time

This was not identified as a significant input to this research project.

1.5.6 Equipment, infrastructure and space

The application was made when Professor Barter was based at the Baker Institute in Melbourne, Australia; in the course of the award the laboratory moved to the University of Wollongong. The Baker Institute was a significant facilitator of this research. It provided not just block funding as noted above but also an excellent reputation, a laboratory assistant and, according to the grant application, 'all major items of equipment required for this project are available in the Institute' (Grant application, 1988). Barter did not mention the move to Wollongong at interview. However, this change of environment must have had some impact on the research conducted. Firstly, it is likely that there was

some delay in the work due to the need to establish the group in the new facility. Secondly, a number of the researchers (eg Clay) did not move to Wollongong with Barter. This change in staff is likely to have affected the way in which the research was conducted. The facilities provided at Wollongong were of similar quality to those available to the group at the Baker Institute.

1.5.7 Consumables

The application estimated that approximately Aus$10,000 per year would be required for consumables. The grant provided for 80% of this requirement, with the rest coming from other funding sources.

1.5.8 The research question

From Professor Barter's perspective, knowing the research question to ask is a very crucial input to successful research. From knowing which question to ask, the group, and Rye in particular, were able to understand the structural function of HDL and discover how to get HDL to act in the laboratory, out of the body, as it would in vivo. Barter said, 'It's not about knowing all the answers…it is about deciding on what the key question is to ask, which can then lead to identifying a whole series of questions to ask' (Barter interview, 2007).

1.5.9 Collaborations

Collaborations were important to this project and again are a fundamental in Professor Barter's approach: 'To get the answers you need facilities and different capabilities. You can't get all you need in one facility and so you need to look around at other facilities around the world that can provide what you need and what you can provide to them that they don't have' (Barter interview, 2007).

In the course of the project, Professor Barter suggests that four or five laboratories around the world were involved and have been involved in subsequent work. However, Rye can only recall one collaboration at that time, with Christian Enholm, Head of Clinical Biochemistry at the National Institute of Health in Helsinki, Finland. Barter had already established a close link with Enholm through a previous sabbatical. He spent around a year with the project team in Australia before returning to Helsinki in 1988, during which time he worked with Rye on apo A-IV. Rye believes Enholm would have played a valuable role at the conception stage of the project and made a lot of intellectual contributions. She suggests he probably also helped to draft the grant application.

A further related collaboration existed between Rye and Professor Dan Rader from the University of Philadelphia, although this was more concerned with spin-offs from the original observation than this particular grant itself.

1.6 Stage 2 – research process

Details of the planned research approach are laid out in the grant proposal and fall under three main categories, as outlined previously (Grant application, 1988):

1. 'To define the effects of apolipoprotein A-IV on activity of a recently identified HDL conversion factor.' This was to include concentration-dependence studies to

find the changeover point where apo A-IV turned from an activator to an inhibitor. It also would study whether apo A-IV is incorporated into HDL during conversion, and if so, into which subpopulation. Standard techniques such as immunoblotting, gradient gel electrophoresis and gel permeation chromatography were to be used.

2. 'To define the effects of the lipid transfer protein, VLDL, and LDL on activity of the HDL conversion factor.' Here, dose-dependent studies of each lipoprotein fraction were planned, at varying concentrations of HDL, HDL conversion factor and lipid transfer protein. Time course studies were also to be conducted, as well as studies whether artificial lipids could mimic the effects of natural lipoproteins on HDL conversion. The interaction with LCAT, including concentration dependence and time course studies, as well as reversibility of the conversion process, was also to be explored
3. 'To define the physiological significance of very small HDL particles formed by the HDL conversion factor: specifically, to define their role in regulating plasma cholesterol esterification.' Research was to include both simultaneous and sequential studies of conversion factor and LCAT in order to establish the overall effect of factors that activate or inhibit the conversion factor.

This plan followed directly from the preliminary work that had already been conducted. According to Professor Barter, the project was relatively simple and focused on the HDL conversion factor. He believes that the design led to a clear answer: 'the project was single-minded and would either work, or not, and it did' (Barter interview, 2007).

However, over the course of the project, it became apparent that some of these areas of research were less significant than others. In fact, by the first progress report, the work had taken a different direction with the identification of the nature of the conversion factor. As it was understood to consist of two components, the interplay of these became an area of more significant research focus and forms the basis of much of the work that was published during the grant (Rye, Hime and Barter , 1995; Barter, 1991; and Lagrost and Barter, two articles in *Biochimica Et Biophysica Acta*, 1991). The conversion factor was actually found to represent an interaction between the cholesteryl ester transfer protein (CETP) and non-esterified fatty acids (NEFA). CETP is responsible for the transfer of esterified cholesterol between HDL and LDL forms, and NEFA modulates the transfer activity of CETP. Therefore, research focused on this interaction between NEFA and CETP and the dependence on NEFA type, such as saturation and length of the carbon chain. However, there was some work on the impact of apolipoproteins and lipoproteins an the action of CETP published soon after the initial studies concerning the nature of the HDL conversion factor (Rye, Garrety and Barter, 1992; Newnham and Barter, 1992; and Lagrost and Barter, 1992).

One of the significant outputs of this work was the development of a new technique for the preparation of reconstituted HDL. This is an approach that allows the creation of in-vitro HDL, which closely mimics in-vivo HDL in terms of appearance and behaviour but is isolated from the other components of human plasma, can be modified as required and is extremely homogenous. This enables a wide range of extra experiments. The impact of this technique is explored in more detail in Section 1.7.2.

1.6.1 The role of the PI

Professor Barter was not directly involved in laboratory research for this project. He suggests this is because laboratory work was not one of his strengths: 'I was banned by the others because I'm a bit clumsy...it's not my strength...My strength is to recognise the unexpected in the results and have a feel for what is important' (Barter interview, 2007).

According to Professor Barter, his role was one largely of directing, guiding, reviewing and writing – in his words: 'my role was to bring the results to fruition' (Barter interview, 2007). His situation at the Baker Institute, and later at Wollongong, meant that he was able to move other researchers in his laboratory onto this project as required.

He also claims to be proactive in ensuring that his staff develop and their careers progress, taking care to give guidance to his team which was appropriate to their level of experience. For example: 'With the post docs I would have a quick look at the results – but they would then take it away and work on it...I let them run with it. Whereas with the research assistants I would tell them more what needed to be done' (Barter interview, 2007).

1.7 Stage 3 – primary outputs from research

The key output of this research was in the area of knowledge production. A large number of highly cited papers can be directly attributed to this project, and the knowledge generated denoted a significant advance in the understanding of HDL and LDL and cholesterol transport. The work also involved a large number of researchers within the group and, for two of these, formed a basis for their future research career.

1.7.1 Knowledge production

As shown in the two progress reports, the key knowledge produced concerns the nature and behaviour of the HDL conversion factor. It was established that the conversion factor actually represents the interaction between CETP and NEFA, where CETP is responsible for transferring cholesterol between HDL and LDL forms and NEFA modulates this transfer activity. Experiments showed that with no NEFA present, CETP promotes an equal exchange of cholesteryl esters between HDL and LDL, so the amount of cholesterol present in each of the two main forms is balanced. But in the presence of NEFA, CETP promotes a significant transfer of cholesteryl esters from HDL to LDL form. So, when these fatty acids are present, cholesterol is switched from the health-promoting HDL form to the damaging LDL form. The NEFA also promotes interactions between HDL and VLDL, which mean that HDL particles that are formed are small and lipid poor, explaining the presence of the smaller HDL species observed in the preliminary experiments. Experiments on different types of NEFA showed that fatty acids with a carbon chain longer than 10 units were most damaging, particularly those with 12, 14 or 16 units, and that unsaturated fatty acids were more damaging than saturated ones. The behaviour was also seen to be concentration dependent.

The over-riding conclusion was that unesterified fatty acids may have an important physiological role in regulating the proportion of cholesteryl ester present as HDL or LDL. Increased concentration of NEFA, which may be associated with obesity, smoking, diabetes and chronic stress, may well underlie the low concentration of HDL found in

these conditions. This clearly has significant health consequences, as well as representing a significant change in understanding the physiological balance between different lipoproteins and the relationship between the different fractions of HDL that had been observed.

It is interesting to note that these outputs, though significant, differ significantly from those imagined at the inception of this project. For example, the potential role for apo A-IV was a possible outcome of great interest for the proposal reviewers. However, this was not a significant output of this work.

The key findings outlined above were published in a series of seven papers over the period 1990 to 1995(Barter, 1991; Lagrost and Barter, two articles in *Biochimica Et Biophysica Acta*, 1991; Rye, Hime and Barter, 1995; Clay et al., 1990; and Barter et al., 1990). Further details about the interaction of CETP with HDL and the influence of other factors on this behaviour were published in a further five papers over a similar period (Clay et al., 1992; Lagrost and Barter, 1992; Newnham and Barter, 1992; Rye, Garrety and Barter, 1992; and Rye, Garrety and Barter, 1993). These include studies on the role of apolipoproteins and VLDL in the interaction.

According to Professor Barter, no single paper published as a result of this grant has been transformatory, but the family of papers altogether amounted to a significant knowledge output. Altogether, sixteen peer-reviewed articles are directly attributable to the grant, generating a total of 570 citations. A further 13 are indirectly attributable to this work, with a further 597 citations, and indeed the results spawned a stream of work concerning the properties and behaviour of HDL and its physiology for the laboratory. More details about the publication output of this grant are presented in Table 1-1 and Figure 1-2.

Table 1-1 Publication output and impact of directly related publications

Number of journal articles:	16
Number of articles included in citation analysis:	16
Total number of citations (all papers):	570
Aggregate relative citation impact:	1.01 (Class III)
Self-citations:	6%

Distribution of publications across relative citation impact classes:

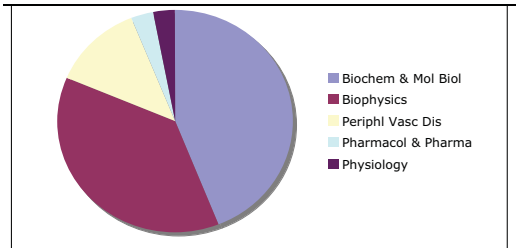
	Class I (uncited)	Class II (>0 and <0.8 citations)	Class III (0.8 to 1.2 citations)	Class IV (1.2 to 2.0 citations)	Class V (>2.0 citations)
Number of publications		9	2	4	1
Proportion of total output		56%	13%	25%	6%

Most highly cited publication[5]:	Clay, M.A., H.H. Newnham, T.M. Forte and P.J. Barter, 'Cholesteryl Ester Transfer Protein and Hepatic Lipase Activity Promote Shedding of Apo A-I from HDL and Subsequent Formation of Discoidal HDL', *Biochimica Et Biophysica Acta*, Vol. 1124, 1992, pp. 52–58
Times cited:	108

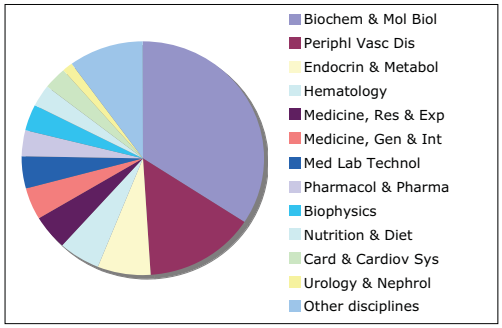
[5] In addition, 13 publications were indirectly linked to this grant. All of these publications were indexed in Web of Science, received 597 citations in total, for a relative citation impact of 10.83. Seven were in relative citation impact Class II, one in each of Class II and IV, and four in Class V, while their self-citation rate was 6%.

Figure 1-2 Knowledge diffusion: fields of research of output (a), fields of citing papers (b) and countries of citing papers (c)

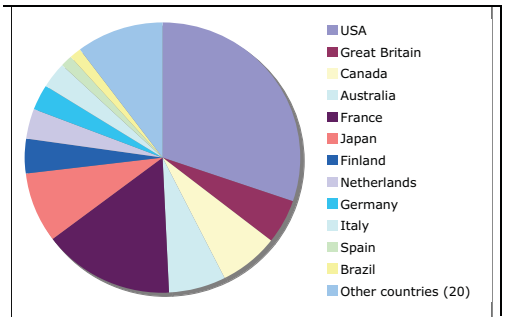
(a)



(b)



(c)



Professor Barter indicated the main publication arising from this grant to be 'Effects of Various Non-Esterified Fatty acids on the Transfer of Cholesteryl Esters from HDL to LDL Induced by the Cholesteryl Ester Transfer Protein', co-authored with LaGrost and published in *Biochimica Et Biophysica Acta* in 1991. This generated 44 citations.

The most highly cited publication directly attributable to this grant was 'Cholesteryl Ester Transfer Protein and Hepatic Lipase Activity Promote Shedding of Apo A-I from HDL and Subsequent Formation of Discoidal HDL', co-authored by Clay, Newnham and Forte, published in *Biochimica Et Biophysica Acta* in 1992, generating 108 citations.

1.7.2 Benefits to future research and research use

This project had a significant impact on the careers of Rye and Lagrost in particular, acting as a springboard for a lot of the work they do today, particularly for Rye. There was also some more minor impact on the careers of a number of the other researchers involved,

including Professor Barter. In terms of targeting future research, the development by Rye of a new method to produce in-vitro HDL is significant and continues to be used today, and CETP has been investigated by the laboratory for a number of years subsequent to the grant. Barter also suggests that a significant amount of subsequent funding stemmed from this work.

Capacity building and career development

The PI on this grant was Dr Philip Barter (now Professor). Professor Barter is a basic research scientist who is also trained as a clinician. At the time, Barter was an established researcher and Deputy Director at the Baker Medical Research Institute in Victoria. Today, he is currently Director of the Heart Research Institute, Australia. He continues to be actively involved in heart research, is involved in a number of clinical trials and continues to publish and speak on the area of HDL. His reputation takes him all over the world to international meetings, and he reviews around 100 papers a year in his role on the editorial board of a number of scientific journals. It is not clear to what extent this grant contributed to his career progression. However, it is likely to have contributed to some extent as part of a broader stream of research conducted over a longer period.

Professor Barter believed that part of his role was to develop capacity and he took care to nurture his team as described in the previous section on inputs to research. This is confirmed by a member of the team (Rye). Rye has now adopted this approach herself and suggested two further benefits of this management approach. Firstly, this style helps to determine the people who are going to continue in a career of research – 'the people who are never going to be able to do it only find out when given the opportunity' (Rye interview, 2009). Secondly, it offers greater scope for learning across the whole team: 'It moves projects in directions I wouldn't have thought of in a million years' (Rye interview, 2009).

A significant output of this grant was the contribution to the development of one of the research team in particular; indeed Professor Barter believes that she perhaps gained the most from this research project. Rye had previously been a PhD student with Barter and her work had identified the conversion factor at the centre of this research. After two and a half years in the United States she returned to Australia and the Baker Institute and was quickly brought in to work on this project as a postdoctoral researcher.

Rye's area of interest and expertise lay in the small HDL particles produced by the conversion factor, which turned out to be one of the drivers of HDL metabolism. Rye published a number of papers in the *Journal of Biological Chemistry* (Rye et al., 1995, 1997, 2003) documenting how these particles are formed and their properties, but notes that these papers were not produced from the grant in question but rather from NHMRC funded work. Rye believes it was involvement in this grant played a significant role in establishing her as an independent scientist. Following involvement with this grant she received funding from the National Heart Foundation of Australia in her own right, receiving her first sole investigator grant in 1999. She was also the first recipient of a National Heart Foundation of Australia Senior Career Research Fellow (1999–2001) and was a Principal Career Research Fellow (National Heart Foundation of Australia) 2002–

2006[3]. She has spent a further 10 years working on small HDL particles, receiving a numerous grants (including six from NHMRC in quick succession) and publishing a number of papers in peer-reviewed journals. In particular, experience from this project developed Rye's expertise in protein interactions (how proteins talk to each other) and the technique of making HDL in test tubes, which is today an industry in its own right, and today is approached in her own right by people from around the world for assistance. Rye said, 'I do this [creation of in-vitro HDL] a lot not only in Australia but a third of what I do, I do with people in the US and Europe' (Rye interview, 2009).

Having spent 10 years post-doctoral bench-side and doing things herself, Rye finally stepped out of the laboratory but continued to be active as a career researcher. Her own research group in 2009 comprises seven postdoctoral fellows, four research assistants, seven PhD students and one undergraduate student. Through her own admission, her management style reflects that of Professor Barter's at the time of the grant as described above.

Today, Rye's positions include Professor at the Faculty of Medicine, University of Sydney, and Head of the Lipid Research Group at the Heart Research Institute, Australia, and she is a member of a number of scientific and professional bodies and committees. Still active in research, the main focus of her research today is the metabolism, remodelling and anti-inflammatory properties of HDL in vitro and in vivo. She continues to work with the approaches she has developed over the last 15 years for synthesising different types of reconstituted HDL and uses these very small particles to answer important questions in the areas on inflammation and HDL metabolism. Both her publication record and invitations to speak and deliver plenary lectures internationally and nationally in Australia are extensive. She is involved in national and international collaborations, including with Christian Enholm, who was involved with this specific grant.

Laurent Lagrost was a postdoctoral student and played an important role in the project. It is believed (by Rye) that this helped his career development. Rye said, 'He was a success, the grant definitely helped him as the manuscripts that evolved out of this grant set the scene for what he did as a postdoc fellow' (Rye interview, 2009).

Lagrost was involved with the group for 18 months and was responsible for moving the fatty acids component ahead while Rye focused on the very small HDL particles. Over that period, Lagrost published five articles, all of which dealt with CETP and its implication in neutral lipid exchange and HDL conversion; these included three of the papers published directly on this research, including the paper indicated by Professor Barter as the single key publication for this project: 'Effects of Various Non-esterified Fatty Acids on the transfer of cholesteryl esters from HDL to LDL induced by the cholesteryl ester transfer protein' (*Biochimica Et Biophysica Acta*, 1991).

Rye believes that what came out of this grant was a springboard for what Lagrost is doing today, but if he had not been involved in this grant he would have been involved in another and ultimately would have gone down a similar path. Lagrost agrees with this, suggesting that the postdoctoral research had a significant impact on his career, though

[3] These fellowships were later abolished.

more through the association with the group than this particular grant: 'It has probably been of significant help at several steps of my career to mention that I had been part (even if it was for only an 18-month period) of the renowned group headed by Philip Barter' (Lagrost interview, 2009).

Lagrost returned to France and kept working in the lipoprotein and atherosclerosis field. He is currently leading a research group at the Medicine Faculty of Dijon and is the Deputy Director of an INSERM Research Centre, where 220 senior researchers, technicians, and postdoctoral and doctoral fellows are involved in cancer, cardiovascular and lipid research. Rye said, 'In terms of long term output, the results [of this grant] have been quite substantial for the two of us' (Rye interview, 2009).

The project also played a part in the career development of Moira Clay, although to a lesser degree than for Rye. A PhD student at the time of the grant, Clay was able to write some 'some really nice papers' (Barter interview, 2007) from this grant. However, Clay was not involved in the full duration of the grant, leaving the group for the United States shortly before the group moved to Wollongong. Clay has stayed in research but has moved from bench research to the policy arena, holding the position of research manager at National Heart Foundation of Australia along the way. Today she does a lot of political networking and lobbying. She is currently Associate Director at the Children's Cancer Research Institute, Sydney, and organises career development programmes for postdoctoral researchers.

While a lot was made of Chang's expertise in the grant application, it is not known what impact the project had on his career development. He is first author on three papers published in collaboration with Professor Barter and this may have impacted upon his career. However, we cannot be sure of this as he is no longer in contact with Rye or Barter, and it is not clear whether he remained active in academic research.

According to Professor Barter, as part of this grant, he also trained researchers who stayed in research in Australia and overseas, and this is likely because, as described above, a large number of researchers were involved in this project. However, it is not clear to what extent this work had on their careers, particularly as many of them were also working on other projects in the laboratory. Harvey Newnham co-authored with Barter two of the eight publications arising from this grant. Like Lagrost, he was involved in the fatty acids component of the research. After completing his PhD he returned to endocrinology. Garrety and Forte are other named authors on directly attributable publications. It is unknown if involvement with this grant affected the career development of these researchers and others involved in the work.

Professor Barter favoured the inclusion of clinicians in basic research, largely due to their ability to bring perspective. By using clinicians in this project, there were also possible benefits in terms of capacity building. Barter believes that clinicians who are serious about academic positions should do at least two to three years in basic research because 'if they have done research they will always be thinking'.

Targeting of future research

New techniques

A key new technique arising from this grant was for the creation of in-vitro HDL that acted like in-vivo HDL. According to Rye, this technique does not have a particular name but is referred to as preparation of reconstituted HDL and has been a springboard for her career.

Rye has developed a unique expertise in assembling these HDL and knows more than anyone about how they work and their properties and structure, which means that she gets asked to join in a lot of projects. Others have tried to copy this approach, in some cases with Rye's assistance. However, as far as Rye knows, none of them have been successful. This is most likely because the process is very difficult and time consuming. Therefore, it is a very important technique for the group. Rye said, 'This particular technique is still the driving force of the whole group and given the amount of time that we have been doing this it is probably will stay that way' (Rye interview, 2009).

This technique is important as it enables HDL to be rebuilt in such a way that it is identical to HDL produced in vivo but because it is rebuilt from individual components isolated from human plasma it is purer and homogeneous and can be freely modified. In contrast, HDL from human plasma is heterogeneous and tends to contain other components as purification is not perfect, meaning that it is often difficult to draw firm conclusions as to whether effects are due solely due to the HDL. Rye said, 'I start off with purified protein and assemble the particles just as it happens in people but I do it in a test tube. The benefit is that you don't have all these other things, the only thing you end up with is particles that contain what you put in. I know exactly what's there and what they look like...you can systematically change one thing at a time and if you are looking for a certain effect you can pinpoint it exactly' (Rye interview, 2009).

Future research areas

The knowledge produced through this study was a significant change in the understanding of HDL and its behaviour, as evidenced by the high citation rate of the publication related to the grant both directly and indirectly. Therefore, in the sense that it represented significant new thinking, it can certainly be regarded as significant in targeting future research both for this research groups and others.

In a more direct way, 13 papers are identified as an indirect output of this grant, and indeed the properties and interactions of HDL became a significant research focus of the group. It is difficult to establish how much this is as a result of the grant, as HDL had already formed a significant part of their research focus prior to this grant. However, the novel findings published here strengthened their reputation and Professor Barter suggested that it elevated the group to become a 'world leader' in this field; though this is difficult to validate.

Further research following from this grant is not constrained to the field of cardiovascular research. For example, the observation that the lipid-free form of HDL had anti-inflammatory properties has consequences for other conditions, notably rheumatoid arthritis. These anti-inflammatory properties are still an area of ongoing research for Rye today.

However, much of the further research that has come out of the project is related to CVD and the fundamental role of HDL in atherosclerosis and related conditions.

Funding for future research

As can be seen in Figure 1-1 Professor Barter received many grants in the time following this project. Barter believes that this grant grew 'heaps' of money, in the range of Aus$1.5–Aus$2.5 million. Furthermore, Rye also went on to receive funding for her own research as a result of her involvement in this grant; these included joint grants with Barter, progressing to chief investigator grants and then to sole investigator grants from the National Heart Foundation of Australia, NHMRC and pharmaceutical industry. More of Rye's lead investigator grants were funded by NHMRC than National Heart Foundation of Australia as these projects were submitted to both but picked up by NHMRC, which Rye concedes was good as NHMRC funding tended to be of a greater value. Such was Rye's success in obtaining funding that she withdrew her involvement from a five-year NHMRC grant because at the time it was only possible to hold six chief investigator grants. She estimates that her total funding to date as sole investigation exceeds Aus$3 million.

Figure 1-1 in 1.5.1 shows Professor Barter's grant history in the five years following the grant period. The 1991–1993 grant in aid from NHMRC of Aus$120,000 per annum was concerned with the concept of factors causing the size of HDL particles to change – an idea that arose directly from the grant in question. The group also won a grant in aid from NHMRC from 1996 to 1998. This was Rye's first grant as chief investigator and on this she continued her research on the very small HDL particles; as chief investigator, she had a further two grants from NHMRC concerned with this subject area. A further grant in aid from the National Heart Foundation of Australia concerned with inflammation was awarded to Cockerill, Barter and Rye for the period 1996–1998, and a 1998–1999 grant in aid, also from the National Heart Foundation of Australia, brought together Barter, Rye and Clay working on inflammation.

1.8 Interface B – dissemination

Barter said, 'At the start it wasn't realised how big an issue HDL was. The complexity frightened people. The project and the dissemination of the results aimed at demystifying it' (Barter interview, 2007).

The dissemination approach adopted by Professor Barter was broad although financial support for dissemination was limited to that available from the supporting institute. A lot of the dissemination described below describes Barter's broader research portfolio than the specific findings of this project. However, this work made a significant contribution to the understanding of HDL and, as such, will have had an impact on the picture of HDL that Professor Barter is trying to describe.

Dissemination of information around this project was probably aided by Professor Barter, who had strong communication skills and was willing to address academic, clinical and lay audiences.

In addition to the publications noted above, Professor Barter was invited to present his work at a number of conferences and other scientific meetings. This has amounted to more than 50 presentations over the years, and some of the work discussed in these presentations will have included reference to the work conducted as part of the grant, although it is not clear to what extent. There have also been a number of book chapters on HDL, to which the research conducted here will have contributed (Rye, Clay and Barter, 1999; Barter and Rye, 1999; and Barter, 2005).

HDL is now part of graduate and postgraduate clinician training and Professor Barter has contributed to the teaching of clinicians through involvement with Lipids Online (Baylor College of Medicine, 2010) producing 62 slides (for presentations in PowerPoint format) for this initiative, which he says he often sees reproduced elsewhere. As with most of the measures discussed here, this is a reflection of Barter's broader research portfolio and reputation.

According to Professor Barter, it was not his intention to spread the message beyond academic peers, but it has happened. After giving lectures people would come up to him and ask where they could find out more. In the early 2000s he was approached by a publisher from the UK who had heard about him and asked him to write a book on HDL for people who were interested in the topic but had little background knowledge. Barter says that for three years he declined this invitation but eventually 'gave in and wrote it'. With an original working title of 'HDL for Idiots', Barter says the book was written in very simple language and intended to demystify HDL, which has now assumed a level of importance that was not anticipated and is a complicated story. The book, *High Density Cholesterol: The New Target: A Handbook for Clinicians* (Barter and Rye, 2005), has been very successful and is now in its third edition, having sold around 50,000 copies (according to Barter). Again, this represents Barter's broader research interests rather than being a specific output of this work.

Professor Barter believes strongly that the public can be a powerful force of support and can be of great assistance in reaching the government. Barter also believes that attitudes in the research community about talking to the general public about research have changed a lot, with the majority now recognising this is important to do. Barter takes the time to speak with lay audiences, making no assumptions about levels of knowledge. In our interview he commented on a talk he had given in a town hall in Adelaide after which a couple of people came up to him and commented this was the first time they had ever understood this subject, and it transpired they were cardiologists. The Heart Research Institute, of which Professor Barter is Director, today holds an open house four times a year to talk with the public about what they are doing. Barter is also involved in communicating via television and has made many appearances in the United States and Europe but interestingly has done very little in his home of Australia – he suggests that this is because the Australian media are not currently interested in this area. For example, he recently had a paper in the *New York Journal* and was on all the evening news networks in the US and page three of the *New York Times* and featured in the *Wall Street Journal*, yet the Australian media declined the story.

1.9 Stage 4 – secondary outputs

It is difficult to quantify some of the secondary outputs from this research. In a broad sense, the understanding of the importance of HDL and its health implications has led to significant changes in policy. However, this research is only a contributor in building up the picture of HDL we have today. Looking at current clinical guidelines regarding CVD (National Heart Foundation of Australia, 2009), stroke (NSF, 2005) and diabetes (NHMRC, 2005) in Australia, there is only one reference to work by Professor Barter (Barter and O'Brien, 2000), in the diabetes guidelines, and no reference to work directly resulting from this project. Barter was a contributor to *Lipid Management Guidelines, 2001 (*National Heart Foundation of Australia and Cardiac Society of Australia and New Zealand, 2001). Editorial comment on these guidelines included the note that they 'provide an excellent overview of the current evidence on the cardiovascular disease (CVD) benefits of cholesterol lowering' (Jackson, 2001). One paper by Barter, entitled 'High Density Lipoproteins and Coronary Heart Disease' (Barter and Rye, 1996), is referenced in these guidelines but this is a later paper and does not directly reference any of the work conducted by the group during this grant. Again, therefore, this is likely to be an output of Barter's broader research interests rather than this specific grant. Similarly, Barter was involved in the preparation of the National Heart Foundation's 'Position Statement on Lipid Management' (Tonkin, 2005) and again the same paper is cited (Barter and Rye, 1996). However, research mentioned in the *European Cholesterol Guidelines Report* (Hockley and Gennill, 2005) is a piece of work by Barter discussing the potential of CETP as a therapeutic target (Barter and Kastelein, 2006). Although much later than the work in this project, it can clearly be seen that this work is a result of the workstream initiated by work in this grant.

Changes in the curriculum for medical students have also been made to reflect new understanding of the importance of HDL. However, it is not possible to quantify the importance of this research in these changes.

Professor Barter has had many advisory roles to industry and policy makers, nationally and internationally, though again this is not linked exclusively to this grant, and there is no evidence of specific outputs from this grant contributing to policy though these positions. A list of the positions he has held over the last 20 years is presented below:

- Scientific and professional bodies
  - Australian Atherosclerosis Society
    - 1993–1996 Chairman
    - 1998–2003 Treasurer
  - National Heart Foundation of Australia
    - 1996–1997 Chairman, Diet and Heart Disease Committee
    - 1986–1992 Chairman, National Medical and Scientific Advisory Committee
    - 1997–1999 Chairman, Grants Committee

    - 1992–1997 Chairman, Lipid Study Data and Safety Monitoring Committee
    - 1988–2001 Chairman, Nutrition and Metabolism Advisory Committee
    - 2002–ongoing Chairman, Nutrition and Metabolism Advisory Committee
    - 1999–2003 Chairman, Research Committee
  - National Health and Medical Research Council
    - 1998–1999 Chairman, RGIC
  - International Task Force for Prevention of Coronary Heart Disease
    - 2003–ongoing Member, Board of Directors
  - Future Forum and Future Forum Editorial Board
    - 2001–2004 Chairman
  - AZTECC (An international training program for emerging opinion leaders)
    - 2001–ongoing Chairman
  - Metabolic Syndrome Institute (International)
    - 2003–2004 Chairman
  - International Atherosclerosis Society
    - 2003–ongoing Member, International Executive
    - 2006–ongoing Secretary
    - 2009–1012 President Elect
- Industry consultation (current)
  - 1998–2004 Merck, Sharpe and Dohme, Australia; Member Joint Cardiovascular Advisory Board
  - 2000–ongoing Pfizer, Australia; Executive member, CVL Grants Committee
  - 2000–2006 Laboratoire Fournier; Member, HDL Expert Panel
  - 2001–ongoing Pfizer International; Member, International Atherosclerosis Scientific Advisory Board
  - 2002–ongoing AstraZeneca International; Member, Rosuvastatin Scientific Advisory Committee
  - 2002–2008 Pfizer International; Member Scientific Advisory Board for development of new CETP inhibitor drug

– 2004–2008 Sanofi-Aventis; Member Scientific Advisory Board for development of rimonabant, a novel weight reducing agent

No patents were developed from this specific grant. Professor Barter noted that patenting could have been inhibitory of further research if put in place at that time – and it would probably have expired before they were in a position to do anything about it. Commercial activity was also against the grain of academic research at the time. Barter said, 'Academics didn't do commercial things in those days' (Barter interview, 2007).

There is, however, a broader potential for the findings and subsequent research to be used to develop new treatments. For example, there is the potential for a new field of cholesterol-lowering drugs. This study led to the identification of new targets for pharmaceutical intervention to improve health outcomes, particularly CETP. Other spin-offs from this research include the discovery that HDL can be modified, and the functional implications of the anti-inflammatory properties is an area that could be explored further and could lead to treatments, although there is no evidence of this having taken place at this stage

1.10 Stage 5 – adoption by practice and the public

It is clear that there is now a good understanding of the importance of HDL and cholesterol more broadly in CVD among health practitioners. Indeed, this is also understood by many in the general public, and this is reflected by the number of products marketed as containing 'good' cholesterol and being heart healthy. However, it is by no means clear to what extent this change is a result of this particular piece of work, which is only one part (if a reasonably significant part) of the wide range of research that has been conducted in this area and which has led to a step change in understanding of this field.

However, there are a number of more specific examples where the outputs of this research, and the follow-on research from the group, have made a clear contribution. One such example is a recent clinical trial in which Professor Barter was involved. He was asked in 2005 to design a project on the potential for a new field of cholesterol-lowering drugs. This was a large international trial to test the protective effects of inhibiting CETP and, as such, was closely related to the work conducted in this grant. Tests were undertaken with rabbits and then clinical trials were undertaken. Unfortunately unexpected side-effects resulted and some people died in the trials. The deaths related to off-target effects of the drug causing complications that may have been unrelated to inhibition of CETP. Barter said, 'The exposure of the problems with the trials means it will need a drug company that is brave enough to give it another go' (Barter interview, 2007).

At the time of interview, it appeared that this had stopped the progress of this particular approach towards treatment, as no drug companies were willing to run trials. Professor Barter believed this may be revisited in the future and in the meantime he has been looking at new targets without direct involvement from pharmaceutical companies at least in the early stages. Further to interview, however, new trials have commenced with other CETP inhibitors.

1.11 Stage 6 – final outcomes

It is possible that the findings of this grant have already started to have wider health benefits in terms of their contribution to the general understanding of the role of cholesterol and in establishing the link between cholesterol and CVD risk. However, this is difficult to confirm, as although the findings of this study were significant, they only formed part of a wider research picture. What we can say is that the overall improved understanding of HDL and lipid risk factors for CVD has started to improve health outcomes in Australia and worldwide. It is also possible that these findings could have impacts on health in a more specific way, through the development of new mechanisms to control cholesterol, but this has yet to occur. The findings also had potential implications for other conditions, such as arthritis and diabetes, but there is no evidence so far of health benefits resulting from this grant in these areas.

1.12 Additional observations

There are some additional observations around the background and personality of the PI, Professor Barter, which may be pertinent to the assessment of this case study.

Professor Barter had both a basic and clinical research background. He describes his background as 'very very strange' – from studying mathematics at high school he moved into medicine, specialising in gastroenterology. He became interested in the liver and cholesterol production and his PhD in biochemistry focused on cholesterol. He worked overseas in a basic research laboratory before working overseas for three years. He came back to Australia as a senior lecturer in biochemistry. He based himself initially in Adelaide and then in Melbourne, where he held the position of Deputy Director of the Baker Research Institute. At the time of the application, he already had 68 papers in peer-reviewed journals, most of moderate impact but the best for this field. He had already attracted just under Aus$1.9 million in research funding, including a Aus$250,000 per annum grant from the NHMRC the previous year. He had eight PhD students. It could be said that he was a good prospect for the National Heart Foundation.

1.12.1 Factors influencing success of outputs

As far as we could assess, there were no barriers to the success of outputs from this grant, while in contrast we saw many factors that we believe to be facilitators to successful outputs, perhaps most notably Professor Barter himself. We really felt that Barter's personality would mean that he could overcome barriers. On the facilitator side:

- As a clinician, he is motivated to see his research translated.
- As a clinician researcher, he is networked to apply his findings and influence peers.
- Potential health benefits were clear, which made funding easy, made interest high and also attracted industry interest and later support.
- Personality and skills of the PI were clearly important to his success.
- Willingness to seek and commit to collaboration: Professor Barters' motivation to get the answer means that he is not only willing to collaborate but that he would

seek out collaborations to achieve his desired research. He credits this with his research success.

Professor Barter also made some comments about the funding system, and the National Heart Foundation of Australia's approach to funding in particular, which may be pertinent.

The application was, by Professor Barter's admission, highly speculative, with no certainty that it would work, yet the National Heart Foundation took a punt and gave it a go; something that Professor Barter has great respect for, as he sees this approach holding great potential benefits for research: 'This was the benefit of NHF [National Heart Foundation of Australia] funding in the past, where it picked up the most exciting projects, which are those which may not pay off, but if they do, there can be a huge leap forward and also gave an important opportunity for young researchers…Too much of the funding is now about whether there is certainty about getting a result...which discriminates against young people' (Barter interview, 2007).

1.13 Summary of case study impacts

The payback framework describes the process from research to several categories of impact. The categories allow us to compare across different case studies. Table 1-2 shows, in point form and by impact category, some of the impacts, described more fully above, that have emerged from this grant.

Table 1-2 Payback

Payback category	Impacts from case study
Knowledge production	• Large number of publications, including 16 directly attributable peer-reviewed articles producing a total of 570 citations • Significant progress in the understanding of HDL, the partitioning of cholesteryl ester between different lipoproteins and the identification of fatty acids as a regulator of this process
Research targeting and capacity building	Capacity building • One postdoctoral researcher went on to gain funding in her own right and today is highly successful • Another member of team involved in policymaking today • Large number of research collaborations for Professor Barter • Clinician involvement Benefits to future research and use • New techniques • Further research in this area for the group and better targeting for other groups through increased understanding of HDL • Funding obtained for continued research in this and related areas
Informing policy and product development	• Some input into clinical practice guidelines • Education (public and clinical) • Contributed to body of work that led to PI's advisory role to industry and policymakers both in Australia and internationally • No patents or direct product development from this grant by Professor Barter
Health and health sector benefits	• HDL recognised as an indicator of high CVD risk • Clinical trials of CETP for cholesterol reduction with Professor Barter
Broader social and economic benefits	• Contributing to understanding of the cholesterol story and establishing the link with CVD • Relevance to other diseases • Economic benefits related to research funding and capacity building and improved cardiovascular health • Possible health benefits through treatments developed from in vitro HDL and CETP

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CHAPTER 2

Fibrillin deficiency in Marfan syndrome and overlapping syndromes (familial mitral valve prolapse, familial ascending aortic aneurysm): diagnosis and risk stratification

2.1 Overview of case study grant

Marfan syndrome (MFS) is an inherited connective tissue disorder that affects various parts of the body including the heart, eyes and joints. The cardiac problems are the most severe and often fatal. It has a prevalence of at least one in 5,000. In the early 1990s studies identified it as being caused by mutations in the gene that encodes for fibrillin-1 on chromosome 15. Much of the early genetic research regarding MFS involved international collaborations. The syndrome is manifest in many different forms (phenotypes) and there are many different mutations in the gene that encodes for fibrillin-1. Furthermore, many of the symptoms of MFS are shared by other syndromes and, therefore, it was important to conduct research on them to establish how far they too were caused by mutations in the gene that encodes for fibrillin.

Dr Anne Child is a clinical geneticist and has been a leading researcher in the international MFS field since her interest was sparked by a patient in the 1970s. She built up the largest database of families with MFS and overlapping syndromes in the United Kingdom (UK). Originally she did not have access to her own molecular genetics laboratory, but, with funding from a series of grants (mainly from the British Heart Foundation (BHF)), by 1993 she had successfully collaborated with various research teams in the UK and abroad. This case study focuses on a project funded by the British Heart Foundation (BHF) in 1993 to enable Child to conduct further collaborative research on fibrillin-1 deficiency in MFS and overlapping syndromes. While some key breakthroughs had already been made, and this research project did not directly lead to many more publications, it did contribute to Child gaining access to her own molecular genetics facilities. The continuing mutation analysis conducted in Child's laboratory – to which the case study project made a small but essential contribution – has, in turn, made a major contribution to recent publications of the international database of mutations in the gene that encodes for fibrillin-1.

As a leading clinician in this field, and Medical Advisor and Medical Director of the patient and research organisations in the UK (the Marfan Association (UK) and the Marfan Trust respectively), Dr Child is well placed to provide 'official', research-informed advice for clinicians and patients, including through publications from the Marfan Trust and the BHF. The international research, and the work of her laboratory in providing a screening service, made an important contribution to the National Health Service (NHS) decision to provide genetic screening for MFS. There has been a cumulative effect of the international research stream in terms of contributing to improving diagnostic and prognostic tests, risk stratification, raising awareness and developing and applying effective therapies. Health gains have resulted, in particular, from the diagnostic tests informing the provision of improved preventive management for relevant patients. The work of Child's clinic and many others has helped push the average age of death of MFS patients considerably higher. This provides a substantial health gain for some people with MFS[1], and many of them can remain active in the workforce for longer. Finally, the database of phenotypes and mutations built up by Child and her team is currently being used in a major trial of an existing drug, Irbesartan, that might provide further major benefits for patients.

2.2 Introduction to the case study

2.2.1 Overview

Marfan syndrome (MFS) is an inherited connective tissue disorder with a prevalence of at least one in 5,000. It is named after the French physician, Antoine Marfan, who first described the syndrome in 1896. Because it is dominantly inherited, each child of an affected parent has a 50% chance of inheriting MFS. It is a complex syndrome, with the symptoms varying from person to person, but symptoms characteristically occur in the eye, skeletal and cardiovascular systems (Pyeritz and McKusick, 1979). The classic model of MFS produces a tall thin body build but the most severe problems are usually various cardiac ones, hence it is an important topic in cardiovascular research.

The cardiac problems include mitral valve prolapse (MVP) (heart valve weakness) and ascending aortic root dilation or ballooning, and dissecting, or tearing, aortic aneurysms. The latter cardiovascular manifestations create the most severe, often fatal problems for the patients. Dominantly inherited marfanoid body build is also noted in many patients with overlapping syndromes: familial MVP and dominantly inherited (non-Marfan) ascending aortic aneurysms (AsAA). The project on which this case study is based was a wide-ranging study conducted by Dr Anne Child from St George's Hospital Medical School, London, of the role of the deficiency in fibrillin-1, a connective tissue protein, in MFS and some of the overlapping syndromes.

Dr Child is a clinical geneticist who trained at the Hospital for Sick Children, London, with Professor Cedric Carter, and then at Guy's Hospital, London, with Professor Paul Polani, another of the founding fathers of medical genetics (Harper, 2007). She first started researching into MFS after a couple came to her genetic counselling clinic: the

[1] Dr Anne Child reported a recent estimate of 18,000 people being affected by MFS in the UK alone.

husband explained that he had MFS and they asked if there was a prenatal test available to ensure unaffected offspring. At that time no such test was available because the cause of MFS was unknown. Child wanted to address this issue and was funded by the BHF as early as 1979 to conduct research into diagnostic methods in MFS. In 1983 she moved to St George's, London, and received BHF funding to research MFS and other disorders such MVP. In 1985 she became Medical and Genetic Advisor and Founding Member of the Marfan Association, which became the main patient group in the UK in relation to MFS.

From 1986 Dr Child received further funding from the BHF that enabled her to set up a database of families with MFS. Over the years she also established databases of families with various other genetic disorders, including several that overlapped with MFS. In 1988 she helped to found, and became Medical Director of, the Marfan Trust, a medical research charity that raises funds for research into MFS. At the international level she also played a key role in forming the Marfan Syndrome and Related Disorders Study Group. She was able to contribute data from her families to international collaborations. International collaboration was assisted by the John Fyffe Travelling Fellowship from the BHF, which was regularly awarded to Child, thus facilitating her international visits to conferences and research units to learn about progress in treating MFS.

The international collaboration involved researchers and teams in various countries who sometimes conducted independent studies and sometimes collaborated in their attempts to locate the gene responsible for MFS. In 1990 the Finnish team led by Professor Leena Peltonen reported the first genetic linkage of MFS to three polymorphic markers on chromosome 15 using DNA samples provided by an international collaboration co-organised by Dr Child (Kainulainen et al., 1990). In one of the subsequent studies, various researchers, including Dr Child, came together to study more families and use more markers and were therefore able to provide a more precise location of the MFS locus (Kainulainen et al., 1991). These studies also made it increasingly clear that MFS was caused by errors in a single gene.

Also in 1991, two studies had independently linked the syndrome to a gene (*FBN1*) on chromosome 15 that encodes the protein fibrillin-1 (Lee et al., 1991, and Dietz et al., 1991). Fibrillin-1 is one of the proteins that provide strength to connective tissue.

Having identified the gene it was important to establish the nature of the mutations and the role of fibrillin-1 deficiency, therefore there was still a considerable amount of research to be undertaken in this field, and the case study focuses on Dr Child's role in this.

2.2.2 The case study approach

For this case study the principal investigator (PI), one of her collaborators and one of her research team were interviewed. Documentary and bibliometric analysis was conducted on various papers from the PI describing her stream of research, and various websites were accessed, including that of the Marfan Trust. Archival review was conducted using the original application and the PI's curriculum vitae, both of which were supplied by the PI following the initial interview.

2.3 Stage 0 – topic identification

Several overlapping factors were the main drivers behind the identification of the topic for the specific 1993 BHF-funded project described in this case study. These include Dr Child's continuing clinical interest in developing ways to improve patient care and the opportunities to help her patients by using her ever-expanding database in research projects, including international collaborations that were making important breakthroughs in the advancement of knowledge in this field.

2.3.1 Expanding database and continuing international collaboration

Dr Child continued to build up the database of families with MFS and related disorders through continued clinical referral of patients and their families. In 1991 she had received a two-year grant from the BHF to study genotype–phenotype correlation in 52 families from the UK with MFS. This stream of work was further supported by another two-year grant in 1992, in this case from the Arthritis and Rheumatism Council, to study phenotype–genotype correlation in families with either MFS or joint hypermobility syndrome.

International collaboration continued, and the important role played by Dr Child in a range of collaborations is illustrated by the following three papers, which were three of the major papers on MFS published in 1992:

- Sarfarazi, M., P. Tsipouras, R. Delmastro, M. Kilpatrick, P. Farndon, M. Boxer, A. Bridges, C. Boileau, C. Junien, C. Hayward, D. Brock and A.H. Child, 'A Linkage Map of 10 Loci Flanking the Marfan-Syndrome Locus on 15q: Results of an International Consortium Study', *Journal of Medical Genetics,* Vol. 29, 1992, pp. 75–80.
- Tsipouras, P., R. Delmastro, M. Sarfarazi, B. Lee, E. Vitale, A.H. Child, M. Godfrey, R.B. Devereux, D. Hewett, B. Steinmann, D. Viljoen, B.C. Sykes, M. Kilpatrick, F. Ramirez and the International Marfan Syndrome Collaborative Study, 'Genetic Linkage of the Marfan Syndrome, Ectopia Lentis, and Congenital Contractural Arachnodactyly to the Fibrillin Genes on Chromosome-15 and Chromosome-5', *New England Journal of Medicine*, Vol. 326, 1992, pp. 905–909.
- Kainulainen, K. Y., A. Child, M. Puhakka, L. Ryhanen, A. Palotie, I. Kaitila, L. Peltonen 'Two Mutations in Marfan-Syndrome Resulting in Truncated Fibrillin Polypeptides', *Proceedings of the National Academy of Sciences USA,* Vol. 89, 1992, pp. 5917–5921.

The importance of the role played by Child is indicated by her being the last named author on the first collaborative paper and the only researcher named on both the second and third papers, which have been cited 189 and 101 times, respectively.

In the first paper, members of an international consortium co-organised by Dr Child and Dr Tsipouras pooled their data for a joint analysis in an attempt to determine the precise location of the gene for MFS and the order of 10 DNA markers on chromosome 15 (Sarfarazi et al., 1992).

The second paper describes the International Marfan Syndrome Collaborative Study, which examined the genetic linkage of MFS and related syndromes. In the highly cited paper in the *New England Journal of Medicine* (Tsipouras et al., 1992) they addressed two main questions: is more than one gene that encodes for fibrillin-1 implicated in causing MFS, and are the genes that encode for fibrillin genetically linked to other phenotypically related disorders, such as MVP and ectopia lentis? The findings were that MFS 'is probably caused by mutations within or very close to the chromosome 15 fibrillin gene. Genetic linkage analysis could be used for diagnosis at the molecular level both prenatally and postnatally' (Tsipouras et al., 1992). Ectopia lentis was also linked to the gene that encodes for fibrillin-1 on chromosome 15, whereas congenital contractural arachnodactyly was linked to the gene that encodes for fibrillin-2 on chromosome 5. There was no linkage of MVP to the gene that encodes for fibrillin-2; and the studies could not determine whether there was a link to the gene that encodes for fibrillin-1 (Tsipouras et al., 1992).

In the third paper Dr Child's collaboration with the Finnish team continued (Kainulainen et al., 1992). They identified two mutations in the gene that encodes for fibrillin and suggested that most MFS families carry their own distinct mutation.

Turning to the specific project that forms the basis of this case study, in part Dr Child's broad application to the BHF represented a request for further funding to enable her to continue making her important contribution to the fast-evolving stream of research in MFS, as described above, and to continue collaborating with key researchers in this field. Hence the reference to diagnosis and risk stratification.

In particular, in 1993 the John Fyffe Travelling Fellowship funded her visit to the third International Marfan Symposium held at Portland, Oregon. At this symposium the progress in phenotype–genotype correlation was reported and, in addition, several papers indicated that fibrillin deficiency is a large disease category and important in the aetiology of many overlapping cardiovascular syndromes.

From her previous BHF-funded work, Dr Child had a large database of families with MFS, as well as families with MVP and with AsAA. At the 1993 meeting in Portland, Child and three other members of the Marfan and Related Disorders Consortium decided to join forces to try to answer the question as to whether MVP and AsAA could at least partially be explained by fibrillin deficiency.

Patients with MVP suffer from leaking heart values and those with AsAA have aortic walls that rupture. Primary MVP syndrome is one of the commonest cardiac abnormalities and whilst most of those affected are asymptomatic and have a good prognosis there are rare complications (including severe mitral regurgitation, infective endocarditis and sudden death), that make the condition important to recognise. As part of the background to the application for the project being studied in this case study, Dr Child argued that, 'The association of mitral valve prolapse with certain inherited diseases (secondary mitral valve prolapse) including Marfan syndrome…supports the hypothesis that mitral valve prolapse may be due to abnormal connective tissue biosynthesis which has been demonstrated at the molecular level in collagen, in several of these conditions' (Child, 1993). Child had various families available from a previous study of patients with significant MVP who had been ascertained from the regional echocardiography database of St George's Hospital (Child, 1987). Studies of some of these families, and the work of others, led to the suggestion,

made in the application, that a connective tissue precursor fibre, such as fibrillin-1 on chromosome 15q, 'is the primary deficiency in these families, leading to measurable secondary deficiency in collagen production' (Child, 1993).

2.3.2 Clinical interest

A key driver for Dr Child in the 1993 application, as with her original motivation for conducting research into MFS, was the wish to improve medical therapy for the patients she saw in her clinics. This related to both the patients with MFS and those with overlapping syndromes. In relation to MFS, some of the initial major breakthroughs had already been made – locating the gene on chromosome 15, and then identifying the mutations in the gene that encodes for fibrillin-1 as being the cause of MFS – but it was felt important to conduct further research to develop a good prognostic test that would allow those patients at most risk to be identified. This would be particularly valuable in relation to cardiac risk, where it could guide the geneticist, cardiologist and cardiac surgeon in their choice of frequency and type of follow-up and aid decisions as to the best time for surgical intervention prior to the dissection of the aortic root. Furthermore, not only was it generally considered important, for severe genetic disease, to detect specific causative mutations in order to be able to develop prenatal diagnosis, but also 'specific therapy for any of the three conditions, aimed at halting their relentless progression, could only be developed once the underlying mechanisms were elucidated' (Child, 1993).

2.4 Interface A – project specification and selection

The proposal made to the BHF consisted of various elements, some of which essentially involved a continuation of existing projects and collaborations, especially those funded by the previous BHF grant. As a clinical geneticist, Dr Child did not have her own genetic laboratory and had to rely on various collaborations to carry out the genetic testing required. As set out in the proposal, however, she had already undertaken considerable preparatory work in selecting and taking samples from individuals and families from her databases.

The proposal was developed over a number of months and, as set out in the application, there were four main aims of this specific project (Child, 1993):

1. to further correlate phenotype in MFS with genotype (mapping of the gene for fibrillin-1)
2. to correlate Marfan phenotype with fibrillin production abnormalities evident by immunoprecipitation
3. to establish fibrillin production abnormalities as a diagnostic/prognostic test in MFS
4. to elucidate the pathophysiology of familial MVP and AsAA by determining the proportion of cases due to primary fibrillin-1 deficiency.

To achieve these aims, a variety of studies were to be pursued with the various collaborators, but the studies were to contribute to the different aims in diverse ways:

- family history and physical examination

- fibroblast immunoassay for fibrillin assembly abnormalities with Dr (now Professor) Cay Kielty of the University of Manchester, UK (Kielty is a basic connective tissue biochemist; sometimes working in collaboration with Child, she had already attempted to characterise how the expression of different forms of fibrillin affected the assembly of microfibrils – one of the building blocks of connective tissue)
- screening for fibrillin gene mutations with Professor Leena Peltonen and the National Institute of Health, Helsinki, Finland using techniques adopted in their previous collaborations (Kainulainen et al., 1992) (patients were to be studied for fibrillin gene mutations using a variety of techniques including gene sequencing by a trained postdoctoral molecular geneticist in the molecular genetics laboratory in Helsinki)
- linkage to the gene that encodes for fibrillin on chromosome 15q21 in two and three generation families with Dr Mansoor Sarfarazi of the University of Connecticut, Farmington, Connecticut, United States, using techniques adopted in the previous study of overlapping syndromes (Tsipouras et al., 1992).

The specification of the project was somewhat unusual in various ways. It was a very broad application covering a number of activities, many of which were to be undertaken by collaborators not funded by the project. The main request for funding was for some of Dr Child's time and for the employment of a technician to work with her in providing the samples to facilitate the work of her collaborators. Therefore, the planned activities of Child were set out in some detail and included:

- selecting suitable patients from MFS, MVP and AsAA databases and referring clinicians
- arranging for patient participation and reporting back to patients and clinicians at the end of the study
- taking family history, and examining each patient and family member fully according to a standardised examination sheet, in patients from 25 MFS families, 24 MVP families and 10 AsAA families for whom this had not already been done
- performing 50 full-thickness skin biopsies using a sterile technique and taking 372 blood samples for DNA extraction
- arranging transport of tissue samples and fibroblast cultures to Kielty and Peltonen and blood samples to Sarfarazi
- adding all the pedigree and phenotype data generated by this study to the MFS, MVP and AsAA databases for analysis and phenotype–genotype correlation
- performing phenotype–genotype correlation with the molecular geneticist and protein chemist (Child, 1993).

The application set out that after training, the technician would be required to grow up to 15 flasks of fibroblasts for each of 59 probands (ie the family member whose phenotype leads to the family being investigated) and a similar 15 flasks for a minimum of one other

affected member per family. Three of each 15 were to be frozen down to form a cell bank for future years.

The proposal was funded to the full amount requested. It is too long ago now for Dr Child to recall any details but there did not seem to be any major objections to the proposal.

2.5 Stage 1 – inputs to research

In this section we first set out the inputs that were originally contributed to the project, but, as described in more detail later, in essence the project transformed and continued in a somewhat different way as a result of further resources being put into a continuing stream of work. These resources are described at the end of this section.

2.5.1 Facilitators

The grant from the BHF was for £88,647, which covered three tenths of Dr Child's salary for two years from mid 1994 and the salary of a technician for 15 months. In terms of reputation, Child was a leading member of the international collaborations working on the genetics of MFS and related disorders. As noted, the key contribution she made was as a clinical geneticist who had built up databases of families but at this time she needed to collaborate with other researchers and teams. The reputation of her collaborators in their respective fields was also high. Professor Peltonen, in particular, was already a member of the International Council of the Human Genome Organisation (HUGO) and in 1992 had won the Antoine Marfan Award from the National Marfan Foundation in the USA. In 1995 Peltonen was to become chair of the Medical Research Council of Finland.

2.5.2 Knowledge and expertise

Collectively Dr Child and her collaborators had considerable knowledge and expertise in the various areas in which they were contributing to the project. Her clinical expertise would ensure the necessary diagnostic accuracy to provide precise data for linkage analyses. The Helsinki laboratory was the first to link the MFS gene to chromosome 15 and the Connecticut laboratory had undertaken the important work on overlapping syndromes. However, this was still a very new field and limitations as to what could be achieved soon emerged.

2.5.3 Techniques

Some of the techniques had been used with success in previous studies, but others were really being developed and the gene for fibrillin-1 was particularly complex, with 65 coding segments (exons).

2.5.4 Samples/study recruits

In terms of recruiting families with the various disorders, Dr Child was uniquely well placed. She had the largest database of MFS and MVP families in the UK and, as noted, played a key role in the patient association for MFS in the UK.

2.5.5 Consumables/space

The grant provided about £11,000 for the consumables at St George's and also about £5,000 shipping expenses to transfer the samples to the various collaborators. As noted,

Dr Child did not have her own molecular genetics laboratory, but there was space for her technician to operate.

2.5.6 Continuing project inputs

As is discussed in later sections, more progress was made in some parts of the project than others (and there were some publications). But, when Dr Child moved in 1994 to take up an additional post at the Heart Science Centre, Royal Brompton and Harefield Hospital Trust, as well as maintaining her Honorary appointments at St George's Hospital and Medical School (St George's Hospital Medical School, 2009) further opportunities arose to support this stream of work. The leading heart surgeon at the Harefield Hospital, Sir Magdi Yacoub, 'has performed more transplants than any other surgeon in the world' (Royal Society, 2007). One of his many interests was how to improve the care for cardiac patients with MFS; for example a paper from Sir Magdi Yacoub, Dr Child and others explored ways of dealing with malfunction of the aortic valve and drew on experience from 158 patients, 68 of whom had MFS (Yacoub et al., 1998). This paper is a major contribution to the field and has been cited about 150 times. Yacoub and Child were able to secure institutional funding for a series of research fellows who worked on the continuation of this project.

According to one of the Research Fellows part of the BHF project was continuing at the Harefield, on a less formal basis but just flowing on and, 'it would be rather difficult in terms of the science to find any break'. Clearly this continuation did not amount to an entirely new project for which a separate proposal had been put to a funding body, therefore, for the analysis in this case study, it seems reasonable to consider it as additional funding for the BHF project. In total the funding for the series of Research Fellows was quite considerable because over a period of years there were at least three research fellows: Drs Junaid Shabbeer, Serena Rooker and Philip Johnson. The Heart Science Centre, which was part of the National Heart and Lung Institute of Imperial College, London, also provided the laboratory space where the work could be conducted. Furthermore, Sir Magdi Yacoub brought his enormous surgical expertise to this continuation of the project, a main aim of which had always been, as noted above, to help inform surgeons as to the best time for intervention.

2.6 Stage 2 – research process

It proved more feasible to make good progress on some of the areas of this wide-ranging project than others, although in terms of timing it is not easy to make a distinction between the specific work undertaken in this project funded by the BHF and the earlier one from 1991 to 1993 and the project developed in ways not originally foreseen.

In terms of the attempts to correlate Marfan phenotype with fibrillin production abnormalities evident by immunoprecipitation, one paper described the progress made in relation to a mutation in a specific MFS patient who died following an operation to replace his aortic root and mitral valve (Kielty et al., 1995). An account of this paper is presented here, rather than in the next section – primary outputs – where the papers containing the knowledge produced are usually described, because this account helps to explain some of the limitations and why the continuation of the project developed a rather

different emphasis. The fibrillin mutation in the patient, presumably one from Dr Child's database, was analysed in the Helsinki laboratory by the techniques described above. It was identified as being a single point mutation in the gene that encodes for fibrillin-1.

An extremely detailed analysis was then conducted by Dr Kielty of cell cultures from various tissues of the patient to investigate the effects of this mutation on fibrillin expression and deposition, and the consequences in terms of microfibril assembly and organisation. As set out at the start of the discussion section of the paper by Kielty et al., 'The relationship between defined mutations in *FBN1*, fibrillin defects, microfibrillar abnormalities and clinical phenotype is still not understood, despite the recent accumulation of mutation and biochemical data' (Kielty et al., 1995). The paper concludes by reporting that, 'This is the first such comprehensive assessment of the relationship between genotype and phenotype for an individual patient.'

It soon became apparent that, especially given the complexity of fibrillin, the level of detailed analysis conducted on the fibrillin from this one patient would have been impractical for Dr Kielty to replicate for the number of patients in Dr Child's database. While, in the case above, the nature of the mutation had been identified by the Helsinki team, for the cell cultures coming directly from Child's patients the mutations were unknown. It became clear from the perspective of a basic cell matrix biologist that it would be easier to start from known mutations in the gene that encodes for fibrillin-1– as generated recombinantly in Kielty's laboratory – than from patients in whom the phenotype produced by the abnormality was known but the mutation itself had not been identified. From the point of view of the main aim of Child too, this collaborative approach, while well worth trying and capable of making some progress as described in an earlier paper (Kielty et al., 1994), turned out not to be the best way to make progress and it was clear it would be better to focus more on mapping the mutations. Furthermore, the processes in terms of the overlapping syndromes were limited because it turned out that they were not caused by a fibrillin deficiency.

However, the focus on mapping mutations in the gene that encodes for fibrillin-1 to further correlate phenotype in MFS with genotype meant that increasing emphasis was given to the first aim of the project and, with the development of the capacity to undertake the mapping at the Heart Science Centre, Harefield, it became less of a collaborative project. The methods used for mutation detection by Dr Johnson, one of the research fellows, became increasing sophisticated. A considerable amount of time was spent on the mutation mapping, but Johnson also compared the normal protein with the mutated protein, as intended in aim two of the project, and spent some time working on the overlapping syndromes.

As mentioned above, one of the outcomes of the funding from this project was to keep Dr Child working generally in this field of MFS and overlapping syndromes. Therefore, it also helped enable her to collaborate with Dr Dianna Milewicz and her team from the University of Texas, who were investigating the genes and mutations responsible for overlapping syndromes such as AsAA and congenital contractural arachnodactyly (CCA), a condition phenotypically related to MFS but without the problems with the eyes and heart.

2.7 Stage 3 – primary outputs from research

It is difficult to be precise about the primary outputs produced by the specific project that is the central focus of this case study. The reasons for this include: the existing collaborations that were ongoing at the time this project started and that had, in part, been funded by the BHF; the subsequent continuation of this project at a related location; and the way in which follow-on work not only built on the methods and findings of the earlier studies but also specifically incorporated the findings into the ever-expanding database and, at times, reported on the full set of data.

2.7.1 Knowledge production

The list of publications below include ones that it seems reasonable to include as probably being linked in some way to this project. Given the difficulties explained above, the list was compiled by considering date of publication and/or submission, subject matter and authors, and funding acknowledgements. In terms of dates, the BHF awarded the funding to Dr Child in December 1993 and, in practice, the project did not seem to start until the middle of the year. Therefore, when some publications from 1994 were examined (Hewett et al., 1994; Lonnqvist et al., 1994; and Kielty et al., 1994) they would have been included on some criteria but were excluded as their submission and/or publication date was too early and in at least some cases they had been included in the references in the proposal for this project as papers in press.

The list of included papers is given below, followed by some account of the reason for their inclusion and the knowledge produced. In the bibliometrics analysis contained in Table 2-1, the first two papers are counted as direct outputs from the project, the third and fourth as indirect outputs (but the fourth is not included in the citation analysis although it has received over 40 citations) and the fifth was not tracked by the Web of Science and so could not be included in the citation analysis. It is noticeable that the bibliometrics analysis would have shown a much higher average citation rate had the analysis focused on Dr Child's previous project funded by the BHF.

- Kielty, C.M., T. Rantamaki, A.H. Child, A. Shuttleworth and L. Peltonen, 'Cysteine-to-arginine Point Mutation in a 'Hybrid' Eight-cysteine Domain of FBN1: Consequences for Fibrillin Aggregation and Microfibril Assembly', *Journal of Cell Sci*ence, Vol. 108, No. 3, 1995, pp. 1317–1323.
- Rantamaki, T., M. Raghunath, L. Karttunen, L. Lonnqvist, A.H. Child and L. Peltonen, 'Prenatal Diagnosis of Marfan Syndrome: Identification of a Fibrillin-1 Mutation in Chorionic Villus Sample', *Prenatal Diagnosis,* Vol. 15, 1995, pp. 1317–1323.
- Child, A.H., 'Marfan Syndrome - Current Medical and Genetic Knowledge: How to Treat and When', *Journal of Cardiac Surger*y, Vol. 12, Suppl. 2, 1997, pp. 131–136.
- Park, E.S., E.A. Putnam, D. Chitayat, A.H. Child and D.M. Milewicz, 'Clustering of FBN2 Mutations in Patients with Congenital Contractural Arachnodactyly Indicates an Important Role of the Domains Encoded by Exons

24 through 34 during Human Development', *American Journal of Medical Genetics*, Vol. 78, No. 4, 1998, pp. 350–355.

- Comeglio, P., P. Johnson, G. Arno, G. Brice, A. Evans, J. Aragon-Martin, F.P. Da Silva, A. Kiotsekoglou and A.H. Child, 'The Importance of Mutation Detection in Marfan Syndrome and Marfan-related Disorders: Report of 193 FBN1 Mutations', *Human Mutation,* Vol. 28, No. 9, 2007, p. 928.

The paper by Kielty et al. (1995) describes the progress made in terms of using cell cultures to investigate the effects of a known mutation from a single patient on fibrillin expression and deposition and the consequences in terms of microfibril assembly and organisation. This paper was further described in the processes section above because it helped to illustrate the complexities of the analysis being attempted in this study.

Rantamaki et al. (1995) is included in the list a result of meeting various criteria although its subject matter is slightly different from that of most other papers. It describes how the team had earlier identified a 366-base pair deletion of fibrillin messenger RNA (mRNA) in a three-generation family with MFS from the UK. An affected female in the family, together with her husband, sought prenatal diagnosis. This was performed by extracting a sample of tissue from the developing placenta and testing for the suspected deletion. To the authors' knowledge this was, 'the first instance of prenatal diagnosis of MFS by identification of a known mutation' (Rantamaki et al., 1995). The difficulties of using the information in counselling are also described.

Child (1997) is a paper based on a general presentation given in 1996 to a surgical conference describing the then current medical and genetic knowledge about MFS. While it drew on a wide range of knowledge, it specifically acknowledged BHF funding and included an account of the paper above, ie Rantamaki et al. (1995).

Park et al. (1998) describes investigations of mutations in the gene that encodes for fibrillin-2 (*FBN2*), which cause CCA, and shows that the mutations cluster in a limited region of the gene – the same region in which mutations in the gene that encodes for fibrillin-1 result in the most severe, congenital forms of MFS.

Comeglio et al. (2007) is regarded by Dr Child as her team's best paper from this stream of research, as it brings together the data from years of work identifying mutations in the gene that encodes for fibrillin-1. Overall 193 mutations were identified in this study and the article describes how the identification of a mutation allows for early diagnosis, prognosis, genetic counselling, preventive management of carriers and reassurance for unaffected relatives. The article also makes clear that while the genetic screening is now part of the service from the NHS, it is still part of a research activity because each new mutation identified strengthens the overall picture: 'In our experience it is paramount for the scientific community to be able to observe as many *FBN1* mutations as possible, since the correlation of mutations already reported with phenotypes will be invaluable in terms of confirmation of causative role, counselling and long-term clinical follow-up' (Comeglio et al., 2007).

Table 2-1and Figure 2-1 show the results of bibliometric analysis for the case study grant.

Table 2-1 Publication output and impact of directly related publications[1]

Number of journal articles:	2
Number of articles included in citation analysis:	2
Total number of citations (all papers):	23
Aggregate relative citation impact:	0.28 (Class II)
Self-citations:	30%

Distribution of publications across relative citation impact classes:

	Class I (uncited)	Class II (>0 and <0.8 citations)	Class III (0.8 to 1.2 citations)	Class IV (1.2 to 2.0 citations)	Class V (>2.0 citations)
Number of publications		2			
Proportion of total output		100%			

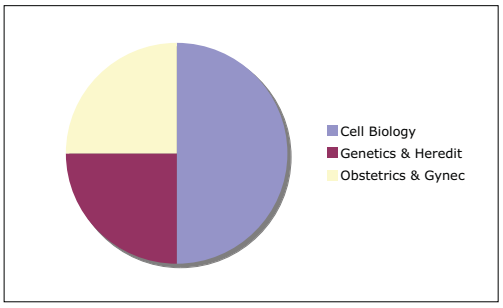
Most highly cited publication[2]:	Kielty, C.M., T. Rantamaki, A.H. Child, A. Shuttleworth and L. Peltonen, 'Cysteine-to-arginine Point Mutation in a 'Hybrid' Eight-cysteine Domain of FBN1: Consequences for Fibrillin Aggregation and Microfibril Assembly', *Journal of Cell Sci*ence, Vol. 108, No. 3, 1995, pp. 1317–1323
Times cited:	13

[1] In addition, two publications were indirectly linked to this grant. One of these publications indexed in Web of Science received 19 citations in total, giving a relative citation impact of 1.30. Its relative citation impact class was IV, and it had a self-citation rate of 0%.

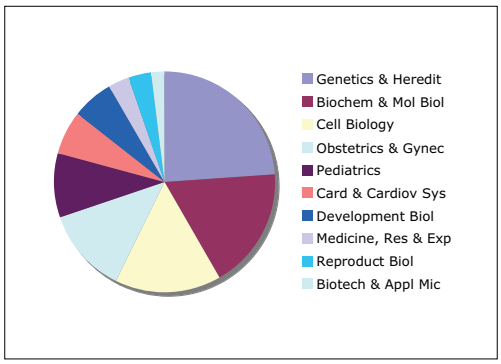
[2] Citation count for most cited paper extracted April 2009

Figure 2-1 Knowledge diffusion: fields of research of output (a), fields of citing papers (b) and countries of citing papers (c)

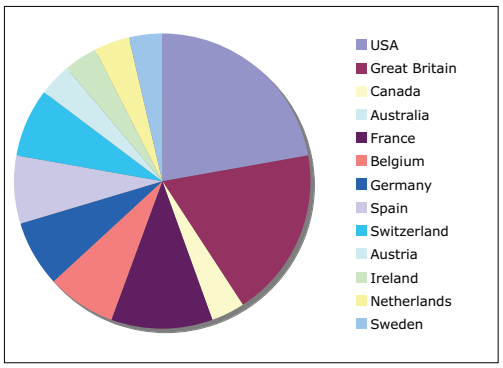
(a)



(b)



(c)



The work described in the 2007 article is primarily linked to the studies undertaken at the Sonalee laboratory (described below) at St George's from 1999 to 2006. The inclusion, however, of Johnson as second author suggests that the paper indeed incorporates the full list of mutations identified by Dr Child's team during this stream of research that started with the BHF-funded project.

There are, perhaps, questions as to why there was not more reporting on the mutations as they were identified in the 1990s. It seems that a view was taken that it might be best to

hold off until a reasonably large number of mutations had been collected before reporting on them.

2.7.2 Benefits to future research and research use

While it is complicated to trace all the follow-on research that was informed by this BHF-funded project, the project is seen as a key bridge that led on to substantial further research. The continuation of this project at the Heart Science Centre had made good progress in identifying *FBN1* mutations. At times Dr Child found it very difficult to raise funds to conduct her research, but she had been a key member of the Marfan Trust since its inception and, following a large bequest, it was able to expand its operations and begin to fund raise on a bigger scale. By 1998 the Marfan Trust was able to play the leading part in the establishment at St George's of the Sonalee laboratory for Child and her team. This was the first laboratory in the UK dedicated to conducting research and clinical services for the spectrum of disorders associated with fibrillin-1 (St George's Hospital Medical School, 2009). Additional funding came from various sources, including the BHF, which provided an equipment grant. It was the work of this group that was described most fully in Comeglio et al. (2007).

In 1995 a French team led by Dr Catherine Boileau, a member of the international collaboration, established the Universal Mutation Database for *FBN1*, and Dr Child's team has been one of the major contributors of mutation data. A series of major publications have incorporated data from the Sonalee laboratory and included Drs Child and Comeglio as authors (for example Collod-Beroud, 2003, and Faivre, 2007). According to the Chair of the Marfan Trust, the UK, through the Sonalee laboratory, has 'contributed the second largest number of mutations of any country in the world' (Tippin, 2008). Even in the 2007 article the authors stress that the complexities are such that it is still difficult to use the results for individual prognosis, despite being able to use the data to 'show that the location of a mutation in the exon 24–32 region is associated with a severe prognosis, not only in newborns but at all ages' (Faivre et al., 2007).

Potentially breakthrough research is being conducted in the United States by Professor Hal Dietz and his team using a Marfan mouse; the most promising of the treatments tested on mice is now being trialled in humans. The database of MFS patients and *FBN1* mutations build up by Dr Child over many years, including during the period covered by the specific grant covered by this case study, means that she is playing a leading role in a trial in the UK funded by the British Heart Foundation and the Marfan Trust, with additional support from the National Institute of Health Research and the NHS (Royal Brompton and Harefield Trust). It has long been known that antihypertensive drugs are an important therapy for patients with MFS, but the particular drug now being trialled is Irbesartan. Given the extent of her database, Child is able to contribute patients with different known phenotypes and mutations and so generate data about the specific patients that are responding best to the therapy.

In their research Drs Child and Sarfarazi found that a number of patients with MFS suffered from glaucoma. While various other researchers have also been working in this field, investigations of her data led on to Child working with the Moorfields Eye Hospital and Sarfarazi on a series of studies that have resulted in a stream of well-cited publications

describing progress in searching for genes linked to glaucoma (for example, Monemi et al., 2005, and Rezaie et al., 2002, which have been cited 102 and 240 times, respectively).

2.8 Interface B – dissemination

The findings from the mutation mapping were presented by Dr Child and the research fellows at various conferences, including conferences that Child helped organise, such as the Fourth International Marfan Symposium/Centennial Marfan Conference at Davos, Switzerland, in 1996 and the Fifth International Marfan Symposium at Helsinki, 1998. An abstract from the conference in Davos in 1996 lists all three research fellows as co-authors (Shabbeer et al., 1996).

As Medical Advisor of the Marfan Association (UK) and Medical Director of the Marfan Trust, Dr Child has been involved in a very large range of dissemination activities. These obviously draw on considerably more than just her own research, but the findings from her stream of work make a contribution to what is presented. In turn, the findings from the specific research included in this case study make a small, but possibly key, contribution to her stream of research in this field. Child's activities have included organising an annual spring one-day symposium for patients, families and interested professionals at St George's Hospital. Child has overseen the production by the Marfan Trust and the Marfan Association (UK) of a series of leaflets for patients and clinicians on various topics related to the diverse problems associated with MFS. These have been made available to other Marfan organisations in other countries and translated into many languages.

2.9 Stage 4 – secondary outputs

The Sonalee laboratory was the first in the UK to offer service screening for MFS patients (St George's Hospital Medical School, 2009). The work of the Sonalee laboratory was important in both showing it was possible to provide a screening service and in developing ways to reduce the costs of providing such screening. It is likely that this made an important contribution to the decision by the NHS that genetic screening for MFS could be provided nationally by the NHS, even if it is not yet routine (Gold, 2006). It is highly likely, too, that the work of the Sonalee laboratory was behind the decision that the NHS screening centre for MFS for the Greater London Genetic Testing Network should be based at St George's (Child interview, 2007).

To the extent that publications aimed at the clinicians and patients from the Marfan Trust (and similar organisations in other countries) represent official advice from the charity they could be seen as a form of policy document. As noted, these were often written by Dr Child and members of her team and were informed by the international developments in the research, including those conducted by herself and her team. Similarly, in 2002 Child co-edited a revised edition of *The Marfan Syndrome: A Clinical Guide;* published by the British Heart Foundation, and carrying their official logo, this, too, could be considered a type of policy document (Child and Briggs, 2002). It again drew on a wide range of international research but it included Child's own research – for example, Kielty et al. (1995).

While the current clinical trial is for an existing drug, and therefore there is no development of a new product taking place, if the trial is successful it will lead to an expansion of the conditions for which the product can be applied and should lead to a health gain.

2.10 Stage 5 – adoption by practice and the public

In terms of genetic screening for MFS, Dr Child's team led the way in providing this in the UK, although it is now available much more widely. Many general practitioners etc will have little or no experience of dealing with a case of MFS and, as a consequence, a particularly important role has been played by the Marfan Trust, with the leaflets that patients can give to their doctors, and also directly by Child through the hundreds of patients to whom she speaks each year through her roles with the Marfan Trust and the Marfan Association (UK).

This activity started before the specific project that is being considered here and continued after it. Furthermore, much of the advice is based on the international research findings and not at all confined to the findings from the work of Dr Child and colleagues. Therefore, the contribution from this specific study is not great, but again it did provide funding that played a part in enabling Child to continue making her wide-ranging contributions for a number of years; and some of the specific breakthroughs reported above are incorporated in the services now provided, including prenatal diagnosis and counselling. Also, in terms of timing, the mutation analysis conducted in the mid 1990s was directly being fed back, as appropriate, to inform the treatment of the relevant patients and their families. It is an unusual feature of this research that in some cases the findings were informing practice many years before the 2007 article was published showing the cumulative picture.

The strong link with patients has not only proved to be a valuable way of informing patients, and their doctors, about aspects of the condition, but it has also at times assisted the researchers by providing feedback. For example, patient input has assisted the development of patient surveys.

As a result of all the activity there is some evidence of increasing awareness among GPs (Child interview, 2007).

2.11 Stage 6 – final outcomes

There has been a cumulative effect of the international research stream in terms of contributing to improving diagnostic and prognostic tests, risk stratification, raising awareness, and developing and applying effective therapies. Because most families have a unique mutation, the research to identify the mutations is particularly important to allow the provision of an improved service to those families.

Dr Child's team, in their 2007 article, describe the benefits that have arisen from their screening research and service provision. These are quoted at length and then analysed in terms of various categories of benefits. The team claim that: 'the characterisation of the

mutation in a particular family allows preventive management of thoracic aortic aneurysm rupture or dissection through early diagnosis and follow-up of the carriers…Mutations detected in our laboratory have been used for cost-effective family screening, including postnatal diagnosis, thus preventing wasteful resource use in annual follow-up of unaffected members, and permitting concentration of scarce resources on affected members only…Reassurance for at-risk family members who do not carry the familial mutation prevents unnecessary fear and chronic anxiety' (Comeglio et al., 2007).

Many of the health benefits are linked to the diagnostic tests. In particular, as a result of the preventive management, including the prescribing of beta blockers and elective surgery on the aorta, the average age of death has been pushed considerably higher. This has resulted in a substantial health gain for these patients. But, there are also health gains in terms of the reassurance that can be provided to those family members who do not carry the familial mutation.

There could also, in theory, be cost savings in terms of preventing resources being used on unnecessary check-ups on unaffected family members. In practice, such savings are unlikely to be realised but, instead, such developments will mean that the resources can better be targeted at those for whom the need is greatest and with whom the probability of health gain most likely.

The trials with Irbesartan, if successful, hold out the prospect of considerable further health gain.

Finally, the increased life expectancy has mostly been among people of working age, so there have been some broader economic benefits in terms of a number of people remaining active in the workforce for a longer period.

2.12 Summary of case study impacts

Table 2-2 Payback

Payback category	Impacts from case study
Knowledge production	• Two direct peer-reviewed articles • Two indirect peer-reviewed articles • One more recent article
Research targeting and capacity building	• Research played an important role as a bridge that led to a major stream of continuing research • Research helped pave the way for the PI to establish her own (Sonalee) laboratory, the first in the UK dedicated to conducting research and clinical services for fibrillin-1 disorders • Mutations identified in the PI's laboratory make a major contribution to the international Marfan syndrome database • Based on the database built up by the PI, she is currently contributing to a major trial of a promising treatment • Findings from this stream of research led the PI to make important contributions to genetic research on glaucoma
Informing policy and product development	• Work of the Sonalee laboratory in providing a screening service made an important contribution to the NHS decision to provide some genetic screening for Marfan syndrome • The NHS screening centre for MFS in London was based at the PI's institution • The PI and members of her team often drew partly on their own research when writing the publications that provide 'official' advice for clinicians and patients from the Marfan Trust and the BHF • Current trial could be regarded as a form of product development: success would lead to expansion of the conditions for which an existing drug could be applied
Health and health sector benefits	• PI has contributed to the international research that has resulted in improved diagnostic tests etc that inform preventive management that in turn has pushed the average age of death of MFS patients considerably higher, thus leading to substantial health gains for some of the people affected by MFS[2]. • Also health gains from the reassurance provided to family members who do not carry the familial mutation • Potential cost savings through avoiding unnecessary check-ups on unaffected family members; in practice such 'savings' used for better service for others • Potential of considerable further health gain from the current trial
Broader social and economic benefits	• Increased life expectancy has mostly been among people of working age, therefore there have been some broader economic benefits in terms of a number of people remaining active in the workforce for longer

2.13 Additional observations

Some of the circumstances described in this case study are somewhat similar to those examples have been identified in previous payback case studies that have been undertaken on research conducted at what is either officially, or unofficially, the national centre for service provision in a rare condition (Buxton et al., 1999). These have shown rather unusual, but very significant, patterns of adoption of research: if the clinician dealing with a high proportion of cases of a rare condition conducts relevant research and applies their

[2] Dr Child reported an estimate that there were now thought to be 18,000 people affected by MFS in the UK alone.

own findings, then it inevitably makes a substantial impact on the treatments provided for that condition. The case of MFS has some similarities with this situation, but the number of clinicians dealing with patients is greater than with the very rare conditions.

2.14 References

Buxton, M., S. Hanney, T. Packwood, S. Roberts, and P. Youll, Assessing the Benefits from North Thames Research & Development, *HERG Research Report No 25.* Uxbridge: HERG, Brunel University, 1999.

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CHAPTER 3 'Heartstart Scotland' – analysis of the results of a national programme

3.1 Overview of case study grant

Defibrillation involves giving an electric shock in an attempt to restart the heart following cardiac arrest. By the late 1980s the use of defibrillators in out-of-hospital situations was more limited in the United Kingdom (UK) than in the United States, and its adoption had been particularly slow in Scotland. By then automated external defibrillators (AEDs) had become available, and the time needed to train people to use them was much less than for the traditional manual defibrillators. In the late 1980s the Scottish Ambulance Service decided to introduce AEDs into all its frontline vehicles and provide the limited training needed for all vehicle crews. The money for this was raised through an appeal launched jointly with the British Heart Foundation (BHF). This case study is based on a two-year grant (1990–1992) from the BHF to Professor Stuart Cobbe, BHF Walton Chair of Medical Cardiology at the University of Glasgow, to analyse the results of the Heartstart Scotland programme.

The evaluation project resulted in a series of well-cited publications that described how, by establishing the Heartstart Scotland programme, the Scottish Ambulance Service had introduced an effective scheme for out-of-hospital defibrillation. At the end of the two-year evaluation project Cobbe successfully applied for a further grant to continue collecting the data from the ambulance crews and to continue expanding the database of results from all attempts at defibrillation conducted by Scottish ambulance crews. Furthermore, after this extension of the project, Cobbe was subsequently able to include funding to support yet further continued data collection and analysis as part of the programme of work supported through his position as BHF Walton Chair of Medical Cardiology.

This continuous stream of work has resulted in one of the most important databases on out-of-hospital defibrillation in any country – possibly the most significant. The funding for this project represented only a small proportion of the total research funding Cobbe received during this period, as part of a very broad portfolio of research. Nevertheless, the case study shows how the evaluation project has made considerable impact in a range of ways. It has influenced the development of the specific Heartstart Scotland programme in various ways, and here the close links between leading members of the research team and the Scottish Ambulance Service ensured the direct transfer of relevant findings. Furthermore, through the publication, dissemination and promotion of the findings by

other key actors outside of the research team, the evaluation findings have also provided strong evidence that helped encourage the wider adoption of AEDs in ambulance services in the UK and elsewhere, especially Europe. This influence on both the decisions to introduce AEDs and the details of how the schemes should operate is further demonstrated by the widespread citing of the key papers in a range of guidelines and training documents from many organisations in Europe and the United States, as well as from the leading international professional body in this field, ie the International Liaison Committee on Resuscitation (ILCOR). While it is impossible to quantify the health gain from this specific project, it has clearly made an important contribution to the health gain that has resulted from the expansion of out-of-hospital resuscitation.

3.2 Introduction to the case study

3.2.1 Overview

Defibrillation involves giving an electric shock to stop the heart fibrillating and thereby re-establish a proper rhythm that allows the heart to start pumping blood again. Out-of-hospital defibrillation was shown to be feasible as early as the 1960s in Belfast (Pantridge and Geddes, 1967) but this early initiative involved doctors travelling in ambulances and applying manual defibrillators. A pioneering scheme involving ambulance crews applying manual defibrillators was successfully introduced in Brighton in 1971 under the leadership of consultant cardiologist Dr Douglas Chamberlain and reported in a series of articles (White et al., 1973; Briggs et al., 1976; and MacKintosh et al., 1978).

In addition to the pioneering scheme at Brighton, there were a few other schemes, for example in the Oxford Ambulance Service NHS Trust, but generally introduction of the manual defibrillators was limited in the UK. This was partly because of an official decision not to support the development of such schemes (Department of Health and Social Security, 1976). Even when this was reversed (Department of Health and Social Security, 1984), initially no additional resources were made available for such schemes and the cost of the month-long training that each ambulance crew required meant the introduction of prehospital resuscitation was slow (Cobbe et al., 1991). Introduction was particularly slow in Scotland. In contrast, in the United States, a larger number of successful out-of-hospital schemes were developed using manual defibrillator machines (Eisenburg et al., 1982).

Automated external defibrillators were originally developed in the United States but initially there was resistance to using them. The AEDs are battery-powered devices with built-in monitoring performed by standard self-adhesive electrocardiogram electrodes for patients who have a spontaneous heart rhythm or by larger (12cm diameter) self-adhesive defibrillation pads if cardiac arrest has occurred. Again a pioneering role was played by Dr Chamberlain, who ensured their introduction into the Brighton ambulance service as early as 1980 (Jaggarao et al., 1982). As shown in the United States, a key feature of the new AEDs was the dramatic reduction in the time needed to train people to use them compared with the manual defibrillators. A clinical trial in the United States reported in 1987 that: 'Automatic external defibrillators appear to have advantages over standard defibrillators in training, skill retention, and faster operation. Such devices can make early

defibrillation available for a much larger portion of the population. They are a major innovation for the prehospital care of cardiac arrest patients' (Cummins et al., 1987).

In the late 1980s, the Scottish Ambulance Service decided to introduce a type of AED called Laerdal Heartstart 2000 into all its frontline vehicles. To facilitate this it instituted a programme of training in defibrillation only for all ambulance crew rather than adopting the slower and more expensive option of training ambulance crews according to the full guidelines of the National Health Service (NHS) Training Authority, which covered other procedures as well as defibrillation (Cobbe et al., 1991, and Rowley et al., 1987). There was no NHS funding for the purchase of the defibrillators and, therefore, a fundraising appeal, Heartstart Scotland, was launched jointly with the BHF. The aim was to raise £2.25 million to equip all 407 ambulances and operational support vehicles with defibrillators and provide the associated training and consumables.

As part of the initiative in Scotland, a protocol was developed to guide the ambulance crew through the various steps they had to follow. These steps included verifying cardiac arrest and then attaching the pads and pressing the analyse button on the defibrillator. If a treatable rhythm was present it resulted in automatic charging of the defibrillator, but the ambulance crew had to press the shock button to deliver the shock (Cobbe et al., 1991).

The training could be delivered by ambulance training officers without direct medical supervision, but the protocol, standing orders and training scheme had been medically approved. The eight-hour training session included four hours of revision in basic cardiopulmonary resuscitation plus four hours of practical tuition in the use of the defibrillator (Cobbe et al., 1991).

The AEDs were introduced in October 1988, and Scotland became the first country in the world to equip every emergency ambulance with a defibrillator (Colquhoun et al., 2004). An evaluation sub-committee was established by the Scottish Ambulance Service under the chairmanship of Professor Stuart Cobbe, BHF Walton Chair of Medical Cardiology at the University of Glasgow. Cobbe was medically trained and, having been a senior registrar at Oxford, also had a year's research fellowship at the University of Heidelberg. He returned to Oxford as BHF Clinical Reader in Cardiovascular Medicine before being appointed to the position of BHF Walton Chair of Medical Cardiology at Glasgow in 1985 at the age of 37 years. By the late 1980s he had already secured a considerable amount of funding for projects ranging from basic science to major clinical trials. These included a grant in 1989 from Bristol-Myers Squibb for £12.5 million to lead the six-year West of Scotland Coronary Prevention Study (WOSCOPS) – a high profile trial of statins that was to result in various highly cited publications, including one (Shepherd et al., 1995) that has been cited more than 4,000 times.

This case study is based on a two-year grant (1990–1992) from the BHF to Professor Cobbe to analyse the results of the Heartstart Scotland programme.

3.2.2 The case study approach

For this case study the principal investigator (PI), one other member of the research team, and Dr Douglas Chamberlain were interviewed. Documentary and bibliometric analysis was conducted of: various articles from the research team describing the original research; various articles from the stream of research that followed on from the original funding; and

a large number of guidelines and other policy statements identified by extensive searches (using the Science Citation Index and Google) of citations received by the articles. Neither the original application nor the final report was available from the BHF, but the PI provided a copy of his full curriculum vitae.

3.3 Stage 0 – topic identification

Professor Cobbe's interest in analysing the performance of the Heartstart Scotland programme arose from a mixture of his clinical interest in this field, his involvement in encouraging the particular Heartstart Scotland initiative and his recognition of the scientific possibilities for a thorough evaluation presented by the way in which the Scottish Ambulance Service had taken the opportunities provided by the new technology to go from being behind the field to playing a leading role in this area.

3.3.1 Clinical interest

When interviewed, Professor Cobbe explained that he had been asked to help the local ambulance service, which was training its paramedics in cardiac rhythm recognition and the use of manual defibrillators, as part of his role at Oxford. When these defibrillators were used the operators had to interpret the rhythm themselves and decide whether or not to deliver the shock. Therefore, it was necessary to train them to a reasonably high level. As a result of his interest in this area Cobbe recognised that because the majority of deaths in this area occurred outside the hospital the only way to address that was, 'to improve resuscitation of patients who suffer cardiac arrest in the community' (Cobbe interview, 2008).

On his arrival in Scotland, Cobbe was surprised and disappointed that there were no similar schemes in operation. When he talked to the ambulance service about this he found that they were willing to consider starting a programme of paramedic training, but the scale of the operation necessary to train all staff was daunting. Automated external defibrillators provided a way forward because the training requirements were so much more modest, and hence the Scottish Ambulance Service made the decision to go ahead with the Heartstart Scotland initiative to try to raise the money and introduce the scheme. According to Cobbe: 'The key thing about resuscitation is not really how clever the operator is in interpreting the cardiac rhythm, but can he get there fast enough? It is far better to have 100 individuals who are simply trained to operate the automated external defibrillators than 10 highly skilled individuals who can operate a manual defibrillator, and that created a lot of interest' (Cobbe interview, 2008).

3.3.2 Active involvement in the service improvement

Given the role he had played in encouraging this development within the ambulance service, and with his experience in this field, Professor Cobbe was invited to chair the evaluation committee for the programme. He said, 'I was in a unique position to be able to document the results of the programme…[and]…in a position to give a degree of externality to the assessment of the results, which I think gave it a great deal of credibility' (Cobbe interview, 2008). Cobbe worked closely with David Carrington, who was the Chief Ambulance Officer.

3.3.3 Scientific possibilities for a thorough evaluation

Professor Cobbe, given his position as Chair of the evaluation committee and head of a university research team, was able to organise the initial data collection about resuscitation attempts using the AEDs. He soon realised that to make the most of the opportunities it would be desirable to extend the scope of the evaluation and recruit a specific research assistant to work on the project full-time. He decided to collaborate with Carrington and Ian Ford, Professor of Statistics at Glasgow Medical School, to apply for funding to conduct a thorough analysis of the national programme. There had not previously been a reported study of the widespread introduction of AEDs into an ambulance service anything like the size of the one in Scotland (Cobbe et al., 1991).

3.4 Interface A – project specification and selection

Professor Cobbe, Carrington and Professor Ford therefore worked together to submit a proposal to the BHF for a thorough analysis of the national scheme. As noted, although Cobbe had already begun to oversee the collection of data, the proposal was for sufficient funding to allow them not only to establish a database but also to undertake evaluations based on it. In some ways it was on the margins of being an audit and a research project: if, according to Cobbe, the information gathered had been used solely to provide an account of the outcomes that would have been an audit, but 'it was useful for more than that to investigate and describe outcomes that were not generally recognised' (Cobbe interview, 2008).

Given the key role played by the BHF in the Heartstart Scotland initiative, and given that Cobbe was already a BHF chair, the BHF was the obvious organisation to approach for funding for this project. The archive of this application is not available, therefore it is impossible to provide many details about the specific proposal and selection process.

3.5 Stage 1 – inputs to research

3.5.1 Facilitators

The BHF provided £36,365 for a two-year study from 1990 to 1992. As is clear from Appendix A at the end of this document, this represented a very small proportion (perhaps <1%) of Cobbe's research funding that overlapped with the period 1989–1993, and none of the other projects were directly in the same field, apart from the 1992–1994 grant from the Scottish Home and Health Department (SHHD), which was to continue building the database and analysing the results. As discussed below, this SHHD grant can be seen as a continuation of the original BHF grant.

3.5.2 Knowledge and expertise

Professor Cobbe, as described above, had a wide range of research experience but the resuscitation field was an area where his knowledge and expertise was primarily as a clinician and he was chairing the evaluation committee for the Heartstart Scotland initiative. Nevertheless, in the Heartstart Scotland project, Cobbe's basic research in

electrophysiology (the study of the electric pulses in the heart) gave him a particularly good understanding of the mechanisms responsible for cardiac arrest.

The knowledge and expertise of Professor Cobbe was complemented by that of Carrington who, as Chief Ambulance Officer, played a key role in the Heartstart Scotland programme. The continuing collaboration with him was 'essential' for the success of the project, according to Cobbe. When Carrington moved to a new post, Dr Andrew Marsden, Medical Director of the Scottish Ambulance Service, became strongly involved in the project. The analysis of the data also needed a strong statistical input, which was provided at the proposal stage by Professor Ford. The researcher recruited to work full-time on the project, Kirsty Dalziel, was also a statistician. As discussed later in the processes section, she was able to suggest various improvements in the data collection methods to help the analysis. She felt her suggestions probably had particular influence because Ian Ford, one of the senior members of the team, was a statistician (Dalziel interview, 2008). As is clear from Appendix A, Cobbe had many research commitments running simultaneously in addition to his clinical responsibilities and asked Dr Martin Sedgwick, one of his registrars, to take a closer interest in the Heartstart Scotland project.

3.5.3 Techniques

Broadly the techniques adopted involved gathering, and then analysing, data from various sources, and to some extent these techniques evolved as parts of the research developed. One source was patient records, and because the record linkage systems in Scotland were more developed at that time than in England this also helped facilitate the thoroughness of the analysis.

3.5.4 Samples/study recruits

Data were gathered about all patients in Scotland who suffered cardiac arrest and were attended by an ambulance.

3.5.5 Continuing projects

When the BHF-funded project ended in 1992, the SHHD funding began for essentially a continuation of the project. The data collection continued in a seamless fashion, and the analysis continued on the ever-expanding database that contained all the information on patients going back to the start of the programme in 1988. It is therefore difficult to see the project as being confined to the original BHF grant, especially as Dalziel continued performing exactly the same role as she had been doing under the BHF funding. Furthermore, all the data from October 1988 to June 1994 were brought together and analysed in the last article in which Dalziel participated as the full-time researcher (Cobbe et al., 1996).

After the end of SHHD funding, Professor Cobbe was able to obtain further funding for a research assistant to continue organising the data collection and maintenance of the database. He was able to get the funding for the post rolled into the core funding he received as a BHF chair. In this way two further research assistants have been funded and, according to Cobbe, 'effectively for 18 years there has been a full-time research assistant employed, supported almost all the time by the British Heart Foundation *for this project*…[we have] now got getting on for 40,000 individuals in this database' (Cobbe interview, 2008, emphasis added). As far as Cobbe is concerned, it has been one

continuing project and the analysis in more recent articles still includes the data gathered in the initial phase of the programme. We analyse below how such a continuing stream of work is best addressed in a way compatible with the projects being analysed in other case studies.

3.6 Stage 2 – research process

A range of skills were required to obtain and analyse the data used in the evaluation. The information came from various sources including: the form completed by all ambulance crew performing cardiac monitoring or attempting resuscitation; details of the resuscitation obtained from the memory module fitted into the defibrillator; and a follow-up of the records of patients in whom defibrillation was attempted (Cobbe et al., 1991). The processes continued to evolve following the appointment of Dalziel as the research assistant in 1990 because, as a statistician, she was able to suggest ways to build on the original data collection form. Furthermore, because of the importance of the crews' cooperation in collecting the data, Dalziel spent a considerable time liaising with ambulance staff – including going out with them on emergency calls – and redesigned the form both to meet the concerns of staff and to increase the efficiency of the forms by removing some items that were proving obsolete or redundant and adding additional items that would enhance the analysis (Dalziel interview, 2008). During the project Dalziel went round to various ambulance stations providing feedback on the findings that were emerging; this was seen as an important way to maintain the active cooperation of the ambulance staff.

A further factor that impinged on the design of the data collection and reporting was that a group of experts in the field of resuscitation met at Utstein Abbey in Norway and produced recommendations for standardised reporting (Cummins et al., 1991). The initial data collection in the Heartstart Scotland project occurred prior to this, but the data collection was revised to conform more closely with the 'Utstein style' for subsequent analyses (Sedgwick et al., 1993)

The memory module of the defibrillator recorded the electrocardiographic rhythms at the time of analysis, the timings and energy of shocks given, and the electrocardiogram following the shock (Cobbe et al., 1991). All the rhythm strips were analysed and classified by Dr Sedgwick; instances where an apparent misdiagnosis had occurred were reviewed by Professor Cobbe and discussed with the technical staff of the manufacturer before final classification (Sedgwick et al., 1992). This detailed level of clinical input helped ensure the quality and usefulness of the database.

3.7 Stage 3 – primary outputs from research

3.7.1 Knowledge

The considerable number of publications linked to this project are listed below, and the standard bibliometric analysis conducted for all case studies is shown in Table 3-1, with the data extracted from the Web of Science (WoS). It is a little difficult to be precise about the boundaries of this project because, as noted above, the analysis of the Heartstart Scotland programme started before the BHF-funded project began and it has continued ever since then.[1] However, to be reasonably compatible with other case studies it seems appropriate to include the first six publications as being directly from the project, and these are the ones included in the bibliometric analysis. The next 11 are viewed as being indirectly from the project. The last three of these 11 include team members as authors, although they were from projects led from outside the Glasgow team, which were separately funded, but drew on the database in some ways. The bibliometric analysis shows that taken together the six directly relevant publications are in the top citation category and have been cited more than twice as often as the average for papers of a similar age in the same journals. Furthermore, Figure 1.1 (c), which lists the locations of the authors citing these papers, indicates the broad international reach of the papers.

Papers directly linked to the project

- Cobbe, S.M., M.J. Redmond, J.M. Watson, J. Hollingsworth and D.J. Carrington, '"Heartstart Scotland" – Initial Experience of a National Scheme for Out of Hospital Defibrillation', *British Medical Journal*, Vol. 302, 1991, pp. 1517–1520.
- Sedgwick, M.L., J. Watson, K. Dalziel, D.J. Carrington and S.M. Cobbe, 'Efficacy of Out Of Hospital Defibrillation by Ambulance Technicians Using Automated External Defibrillators: the Heartstart Scotland Project', *Resuscitation*, Vol. 24, 1992, pp. 73–87.
- Sedgwick, M.L., K. Dalziel, A. Watson, D.J. Carrington and S.M. Cobbe, 'Performance of an Established System of First Responder Out-Of-Hospital Defibrillation. The Results of the Second Year of the Heartstart Scotland Project in the "Utstein Style"', *Resuscitation*, Vol. 26, 1993, pp. 75–88.
- Sedgwick, M.L., K. Dalziel, J. Watson, D.J. Carrington and S.M. Cobbe, 'The Causative Rhythm in Out-Of-Hospital Cardiac Arrests Witnessed by the Emergency Medical Services in the Heartstart Scotland Project', *Resuscitation*, Vol. 27, 1994, pp. 55–59.

[1] One reviewer would, quite reasonably, have liked the case study to provide a more discrete account of items such as the key research questions, data collected, outcomes etc. This was more difficult to do within the structure of the case study, especially given there was some early evolution of the exact data collected and since then the data have been continuously fed into an ever-expanding database with a series of publications presenting the data after different periods. Perhaps the article based on the first five and a half years of the study (Cobbe et al., 1996) provides the most comprehensive account. A brief account of this is given below, and it shows that 14.6% of patients on whom resuscitation was attempted were admitted to hospital.

- Marsden, A.K., G.A. Ng, K. Dalziel and S.M. Cobbe, 'When is it Futile for Ambulance Personnel to Initiate Cardiopulmonary Resuscitation?', *British Medical Journal*, Vol. 311, 1995, pp. 49–51.
- Cobbe, S.M., K. Dalziel, I. Ford and A.K. Marsden, 'Survival of 1476 Patients Initially Resuscitated From Out of Hospital Cardiac Arrest', *British Medical Journal*, Vol. 312, 1996, pp. 1633–1637.

Papers indirectly linked to the project

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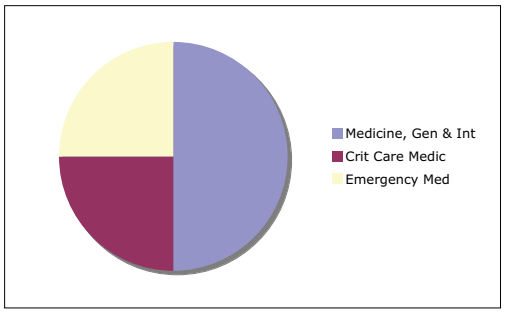
Table 3-1 Publication output and impact of directly related publications

Number of journal articles:	6				
Number of articles included in citation analysis:	6				
Total number of citations (all papers):	287				
Aggregate relative citation impact:	2.26 (Class V)				
Self-citations:	13%				
Distribution of publications across relative citation impact classes:					
	Class I (uncited)	Class II (>0 and <0.8 citations)	Class III (0.8 to 1.2 citations)	Class IV (1.2 to 2.0 citations)	Class V (>2.0 citations)
Number of publications		1		2	3
Proportion of total output		17%		33%	50%
Most highly cited publication[1]:	Sedgwick, M.L., K. Dalziel, A. Watson, D.J. Carrington and S.M. Cobbe, 'Performance of an Established System of First Responder Out-Of-Hospital Defibrillation. The Results of the Second Year of the Heartstart Scotland Project in the "Utstein Style"', *Resuscitation*, Vol. 26, No. 1, 1993, pp. 75–88				
Times cited:	92				

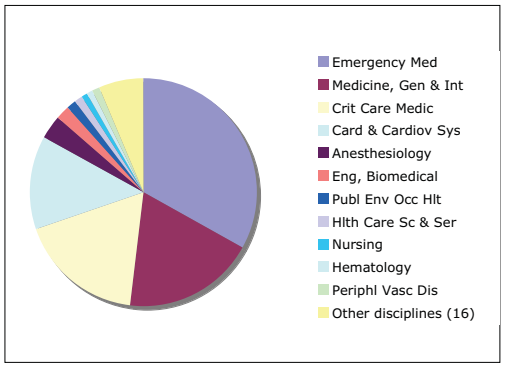
[1] Citation count extracted April 2009

Figure 3-1 Knowledge diffusion: fields of research of output (a), fields of citing papers (b) and countries of citing papers (c)

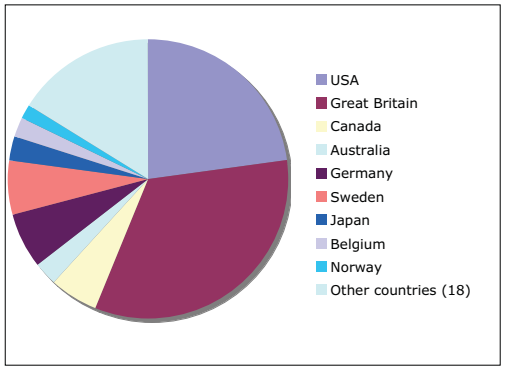
(a)



(b)



(c)



The brief account below of some of the main direct papers highlights the key knowledge produced by the project.

Cobbe et al. (1991) described the data collection that occurred before the BHF-funded project started, but it is included here because some of the analysis took place during the period when the BHF-funded evaluation was underway and after Dalziel had been appointed. Furthermore, there was no other project funding for this data collection with which it could be associated. In Cobbe et al. (1991), the phased establishment of the Heartstart Scotland programme was described. The main findings are that of the 1,111 cardiac arrests recorded; defibrillation was indicated and undertaken in 602 cases. A

spontaneous pulse was present on arrival at hospital in 30% of the defibrillated patients and 75 (12.5%) were subsequently discharged alive. The likelihood of survival was inversely related to the delay from onset of cardiac arrest to the time of first shock. If ventricular fibrillation occurred after the arrival of the ambulance, survival to discharge was 33%. The paper concluded that: 'an effective scheme for out of hospital defibrillation can be introduced rapidly, and with limited training implications and costs, by the use of automated external defibrillators in ambulances' (Cobbe et al., 1991).

Sedgwick et al. (1993) assessed the performance of the second year of the Heartstart Scotland programme and as far as possible used the 'Utstein style'. The results show that 174 (10%) of the 1,676 patients whose arrests were traced in the year were discharged from hospital alive. The main part of the analysis draws on the detailed data gathered to identify characteristics of those who survived. If the cardiac arrest was witnessed by ambulance staff (because they had been called when the patient was unwell) and required defibrillation then survival to discharge was 39%. Survival of bystander witnessed arrests was increased from 7% to 15% with bystander cardiopulmonary resuscitation (CPR).

Cobbe et al. (1996) drew on the data from the first six years of the Heartstart programme, from 1988 to 1994, to determine the short- and long-term outcomes of the 1,476 patients admitted to hospital after successful resuscitation. In that period there were 10,081 attempted resuscitations; 680 (46%) of the 1,476 patients admitted alive to hospital were discharged alive and their estimated four-year survival after discharge was 68%. The analysis concentrated on attempting to identify categories of patients at high risk of subsequent cardiac arrest who might benefit from further cardiological evaluation. Relating back to the importance of working with the ambulance crew to ensure cooperation in data collection, the acknowledgments in the article refer to the skill and enthusiasm of the technicians and paramedics who 'undertook the initial resuscitations and completed the report forms' (Cobbe et al., 1996).

The importance of the knowledge produced in the 1996 article is highlighted by a cumulative meta-analysis of the effectiveness of defibrillator-capable emergency medical services for out-of-hospital cardiac arrest (Nichol et al., 1999). This study, conducted by a Canadian team, identified 37 articles that met their inclusion criteria. The total number of cardiac arrests reported in these 37 studies was 33,124, almost a third of which came from Cobbe et al (1996), which was more than twice as large as the next study.[2]

Marsden et al. (1995) reported on whether patients with pre-hospital cardiac arrest in whom ambulance resuscitation attempts would be futile and therefore, 'distressing both for staff and for relatives' could be identified. A detailed review was conducted of the AED rhythm strips of patients in whom no shock was advised. The article concludes: 'On the basis that it would be inappropriate to initiate vigorous resuscitation in patients who can be identified as "dead" and beyond help an algorithm was prepared to guide ambulance personnel' (Marsden et al., 1995).

[2] As noted by one reviewer, and highlighted in the meta-analysis by Nichol et al (1999), in a full analysis it is also relevant to consider studies that, unlike the ones reported here, compare different systems of managing out-of-hospital cardiac arrest. Of those, the most robust studies are probably the ones from the Ontario Prehospital Advanced Life Support (OPALS) Study, for example Stiell et al. (1999).

Those papers listed as indirectly linked to the project cover are discussed below as part of the further research.

3.7.2 Benefits to future research and research use

Capacity building and career development

Dalziel received her master of science (MSc) in statistics and clinical medicine based on the work undertaken on this project. Although her career moves then took her out of the medical field, her range of posts was highlighted in a publication from the Royal Statistical Society as a successful example of the flexible careers statisticians can pursue. This stream of research has been important to Professor Cobbe as an area he wished to continue but has not been his most important field of research.

Targeting further research

The original project was producing such important and useful data that, as we have seen, Professor Cobbe sought to continue funding the data collection and analysis. Initially this was done through the further grant from the SHHD, which we have classified as part of the original research project because it was so closely linked to it.

Subsequently the research has continued through the BHF funding for Professor Cobbe's position as BHF chair. Cobbe and his colleagues have used the continuing database to explore topics ranging from the use of paramedic skills in resuscitation (Adams et al., 1997), sex differences in outcome from cardiac arrest and resuscitation attempts (Pell et al., 2000) and the effect of reducing ambulance response times on deaths from out-of-hospital cardiac arrest (Pell et al., 2001) to the debate about public access to defibrillators (Pell et al., 2002). In most of these continuing papers a key role has been played by Professor Jill Pell, a public health specialist at Glasgow University.

In addition, researchers from Edinburgh led on several projects for which they received separate funding (from the BHF and the Chief Scientist's Office of the Scottish Executive) but which drew on the Heartstart database, and Professor Cobbe was a co-author. These studies examined chronic memory impairment after cardiac arrest (Grubb et al., 1996), ways of predicting such cognitive dysfunction (Grubb et al., 2007) and factors affecting survival rate after cardiac arrest at home (Lyon et al., 2004). As noted in the list of publications, the papers from these studies could also possibly be considered to be indirect outputs from the original project.

3.8 Interface B – dissemination

Professor Cobbe, Dr Sedgwick, David Carrington and Kirsty Dalziel made a series of oral and/or poster presentations in the early 1990s at conferences such as the European Resuscitation Council (ERC). As an example, at the first CPR Congress of the ERC, held at Brighton, UK, in 1992, Cobbe presented the main findings at a plenary session and the team had three posters on various aspects of the work that were all written up into papers.

The findings from the continuing stream of work have also been drawn upon by Professor Cobbe in a series of named lectures:

- Professor R.W.F. Campbell Memorial Lecture, Scottish Cardiac Society, 1999, 'Clots or Sparks – the Key to Sudden Cardiac Death?'
- Professor R.W.F. Campbell Memorial Lecture, British Cardiac Society, 2003, 'Sudden Cardiac Death – the Shocking Truth'
- Evan Jones Memorial Lecture, King's College, London, 2004, 'What Can We Do About Sudden Cardiac Death?'.

Dalziel's presentations to the ambulance crews, and at the training centre, were mainly aimed at showing the crews the results, and value, of the time they spent completing the forms.

Presenting the findings to the Scottish Ambulance Service at an organisational level was greatly facilitated by the fact that key members of the research team were leading members of the service (initially David Carrington and later Dr Marsden). Furthermore, Professor Cobbe chaired the evaluation sub-committee for the Heartstart Programme, and since 1992 has chaired the Professional Advisory Group to the Scottish Ambulance Service. Particularly within Scotland, therefore, Cobbe could very effectively operate as a translator, or 'product champion', for the findings from the evaluation.

3.9 Stage 4 – secondary outputs

Overall, the evaluation project has had a widespread impact on a range of policies. It influenced the development of the specific Heartstart Scotland programme in various ways but has also influenced the policy of many ambulance services to adopt defibrillators in ambulances and influenced guidelines and training documents from many organisations in Europe and the United States as well as ILCOR.

In relation to the Heartstart Scotland programme itself, the continuing evaluation could be regarded as a formative evaluation because the findings have been fed directly into the management of the ambulance service at various levels. Professor Cobbe noted that, as he was chairing the Professional Advisory Group, sometimes the insights from the research 'will have informed our discussions with the service through that forum' (Cobbe interview, 2008). Not only could the research inform the way the management considered issues, but it could also provide evidence to reinforce current policy of the programme. An example of both points arose in the very sensitive area of when it was futile to initiate resuscitation. As described in the article by Marsden et al. (1995), a study conducted using the database provided evidence for the Scottish Ambulance Service to draw up a guideline, in the form of an algorithm, to govern the actions of ambulance staff confronted with a patient in whom attempts at resuscitation may be futile. The study also provided reassurance to staff using the AED.

When, in the mid 1990s, the time came to replace the defibrillators originally purchased through public subscription, the Scottish Office decided to provide the funding. Professor Cobbe observed, 'They might have done it anyway, but the case was very much stronger because we had robust outcome data and in many areas of the health service, in those days at least, robust outcome data were not readily available' (Cobbe interview,

2008). This quotation raises the issue of the counterfactual, ie what would have happened without the research.

The case for saying that the evaluation probably made a considerable impact on the Heartstart Scotland initiative itself is strengthened by examining the impact that the evaluation made elsewhere, albeit largely through a completely different route. The impact made in Scotland was probably due in large part to the direct involvement of key members of the research team (Professor Cobbe, David Carrington and Dr Marsden) in the Scottish Ambulance Service. In contrast, most of the impact that the evaluation made in the UK and internationally was probably more caused by the nature of the high-quality articles, the advocacy of key figures such as Dr Chamberlain, who used the data from the evaluation directly to promote the adoption of defibrillators, and the incorporation of the findings into guidelines by various committees (often involving Chamberlain).

Many of the ambulance services elsewhere in the UK eventually took the decision to purchase AEDs. It is quite likely that the stream of research played some part in informing at least some of these decisions. As noted above, Dr Chamberlain is a leading figure in this field; therefore, it is worth quoting his interview evidence at length. He said that while at this distance it was difficult to remember precise incidents, he was clear that Heartstart Scotland made a big impact, and this was based not just on the initiative itself but on the papers describing the findings from the evaluation by Professor Cobbe and his team. Chamberlain said, 'But the big thing about Heartstart Scotland is that this was the very first time that a decision was made at a national basis to put AEDs on every ambulance and that provided a tremendous boost to persuading other authorities that this was the way to go. And I remember quoting Heartstart Scotland over and over again to people encouraging them saying, "Look, they have done it in Scotland, they have shown that the amount of training required was very modest and they showed that lives could be saved and that it was entirely feasible." So that was a very, very important development, one that was talked about a lot' (Chamberlain interview, 2009).

Dr Chamberlain thought that, while he himself was impressed by the Heartstart Scotland initiative, in terms of being able to use it to persuade other people it was necessary to have the evaluation and that Professor Cobbe and his colleagues did a very careful evaluation. Chamberlain played an important role in the creation of the ERC and became Editor of the journal *Resuscitation*, which became the official journal of the ERC (Baskett, 2007). Chamberlain stated, 'In Europe I used Heartstart Scotland very often to persuade people of the ease with which you can provide pre-hospital resuscitation' (Chamberlain interview, 2009). As an illustration of the longevity of the impact, Chamberlain reported that he had referred to the evidence from Heartstart Scotland during a visit in early 2009 to Chile, where he was attempting to encourage them to adopt AEDs in ambulances on a national basis (Chamberlain interview, 2009).

The evaluation of Heartstart Scotland also made a substantial, and almost immediate, impact on the guidelines of various bodies, starting in 1992 with the *Guidelines for Advanced Life Support* from a working party of the ERC chaired by Dr Chamberlain (Chamberlain, et al., 1992). These guidelines state: 'The *second principle* underlying the new guidelines is the paramount importance of minimum delay in the administration of defibrillating shocks. Thus the role of chest compression during advanced life support has

also been modified to afford greater opportunity for electrical defibrillation as early as possible in the course of the arrest. This is also unconventional and calls for justification'. The main evidence cited to support this major aspect of the guidelines is Cobbe et al. (1991).

Some of the early guidelines informed by this stream of research have been updated or replaced, but an example of one that, though published in 1995, is still current comes from the American Association for Respiratory Care (AARC). One of the AARC's clinical practice guidelines, *Defibrillation During Resuscitation,* lists various process issues that should be included in assessments of the use of defibrillators (AARC, 1995). They include 'response time', for which Sedgwick et al. (1993) is the only reference, and 'first-responder response times', for which that article is one of two references.

The European guidelines were amended on various occasions. 'The 1998 European Resuscitation Guidelines for Adult Advanced Life Support' were published almost simultaneously in two journals (Robertson et al., *Resuscitation* and *BMJ,* 1998). They cited three articles from Professor Cobbe's stream of work: Cobbe et al. (1991) and Sedgwick et al. (1993 and 1994). The guidelines use Sedgwick et al. (1994) as one of four sources for the early explanation that the most common primary arrhythmia at the onset of cardiac arrest is ventricular fibrillation (VF) or pulseless ventricular tachycardia (VT). The guideline goes on to make a key point that is supported by just two sources, one of which is Cobbe et al. (1991): 'The *only* interventions that have been shown unequivocally to improve long term survival are basic life support and defibrillation. VF is a readily treatable rhythm, but the chances of successful defibrillation decline substantially with the passage of each minute'.

For most of the 1990s, ILCOR encouraged liaison between different research groups. In 2000 the resuscitation councils of Australia, Europe, New Zealand, Latin America and Southern Africa, along with the American Heart Association and the Heart and Stroke Association of Canada, all came together to produce a set of international guidelines (ILCOR, 2000). In the part on AEDs, Sedgwick et al. (1992) is cited in support of six different points.

Different organisations continued to produce resuscitation guidelines and these often cited the stream of work relating to Heartstart Scotland. Examples include guidelines from the American College of Occupational and Environmental Medicine (ACOEM, 2001) and the Australian Resuscitation Council (Wassertheil, 2006). An editorial introducing the latter guidelines again cites Sedgwick et al. (1993) as one of five sources to support the key statement that, 'Defibrillation of ventricular fibrillation and ventricular tachycardia is still recognized as the single most efficacious procedure in survival from sudden cardiac arrest' (Wassertheil, 2006).

The stream of research has also been used in formal documents for a wide variety of education and training programmes in resuscitation. In the ILCOR advisory statement, 'Education in Resuscitation', Sedgwick et al. (1993) is again cited prominently as evidence to support the value of bystander CPR (Chamberlain and Hazinski, 2003). The research is also cited in various more localised continuing education programmes; for example, Sedgwick et al. (1992) is cited as one of two sources to support some specific advice about using AEDs in the *Journal of the Pharmacy Society of Wisconsin* (Dobb, 2006).

Another important role played by Dr Chamberlain in the UK was in relation to the Joint Royal Colleges Ambulances Liaison Committee. This committee developed a model reporting form that it was hoped would be widely used to record incidents of the use of AEDs in a standardised way. The experience of the evaluation of Heartstart Scotland was again drawn upon in doing this (Chamberlain interview, 2009).

The ERC also set up a manufacturers' group so that issues in common could be discussed, and Dr Chamberlain again thought it likely that evidence from the evaluation of the Heartstart Scotland programme would have come up in the discussions (Chamberlain interview, 2009).

3.10 Stage 5 – adoption by practice and public

In terms of adoption of the findings by practitioners (in this case mostly ambulance crew) and the public, it is again relevant to look at the impact within Scotland and then elsewhere. Within Scotland, the data from the evaluation were available at ambulance station level and so it was possible to draw attention to areas that were not performing so well, but it is not known if the service was able to do much about that. Some findings were being incorporated into messages that the ambulance crew were getting through their training centre. There is evidence from outside Scotland that it can sometimes be difficult to maintain the momentum over a number of years and achieve the same rate of survival from out-of-hospital cardiac arrest that had been achieved in the initial years following the introduction of innovative schemes (Fletcher et al., 2008). There are likely to be various reasons for this, but it could be argued that the continuing audit and evaluation project associated with the Heartstart Scotland programme has probably helped to maintain the commitment of ambulance crews in Scotland to applying the AEDs on the maximum number of occasions appropriate and to completing the information forms.

Findings from the evaluation of the Heartstart Scotland programme about the importance of calling the ambulances as soon as possible and the role of bystander CPR were messages that were being promoted to the public in response to this and other evidence. Attempting to engage the public with such messages was a continuing part of the Heartstart Scotland initiative, and Professor Cobbe chaired the BHF Heartstart Scotland Committee from 1994 to 2001.

In terms of the rest of the UK, and elsewhere, it is very likely that the findings from the evaluation will have had some impact on the operation of ambulance services in addition to any policy decisions to introduce the schemes. This is again because of the quality and importance of the stream of publications about Heartstart Scotland, the reputation of Professor Cobbe, the promotion of the findings by 'product champions' such as Dr Chamberlain, and the impact of the findings on guidelines and training documents. It would be impossible to measure the extent of the impact compared to that from other sources.

Across the UK it is claimed that, 'Initiatives to train the public in CPR techniques have proved popular and have made an important contribution to improved survival rates' (Colquhoun et al., 2004). It is impossible, however, to know how much, if at all, the adoption of these ideas should be attributed to the specific evaluation of the Heartstart

Scotland programme. As noted above, that stream of research has been drawn upon to support guidelines stressing the value of bystander CPR, although the major evidence on this comes from the United States, especially the 'chain-of-survival' concept associated particularly with the long-standing initiative in Seattle (Cummins et al., 1991). This concept highlights the importance of a series of activities if the chance of survival after cardiac arrest is to be increased. These include: early recognition of symptoms/cardiac arrest and activation of emergency services, early CPR, early defibrillation and early advanced care.

3.11 Stage 6 – final outcomes

Any attempt to assess the benefits of the evaluation of the Heartstart Scotland programme to Scotland would probably need to start with an assessment of the benefits of the programme itself, but accounts above do show how important the continuing evaluation has been to inform the detailed operation of the programme and publicise its success. As we saw above, the evaluation of the first year, during which there was a phased introduction of the programme, saw 75 patients discharged alive (Cobbe et al., 1991). In the second year 174 patients were discharged alive (Sedgwick et al., 1993). In the evaluation of the period from the start of the initiative on 1 October 1988 up until 30 June 1994, a total of 680 patients were discharged and an estimate of the four-year survival after discharge was 68% (Cobbe et al., 1996). Writing some years later, Dr Marsden claimed that figures from the database suggested that over the years there had been almost 1,800 long-term survivors, which worked out at about 150 survivors a year (Marsden, 2004). Most of these would not have survived without out-of-hospital defibrillation. Whereas most of this health gain is clearly a result of the Heartstart Scotland programme itself, rather than the evaluation project, the evidence above indicates that the evaluation has made an important contribution.

As noted, the chain-of-survival approach from the United States emphasises the importance of a range of activities to maximise the chances of survival. Similarly, the Heartstart Scotland programme attempted to do more than introduce the defibrillators. An analysis of the impact of the Heartstart Scotland initiative in its first year in north Glasgow, one of the most deprived areas of Scotland, indicated that the number of survivors seemed to be smaller than the average across Scotland and that greater attention to the wider range of activities would be beneficial (Leslie et al., 1996).

When comparisons are made between Scotland and elsewhere in the UK, however, the benefits from the early introduction of the Heartstart Scotland programme are indicated. Dunn et al. (2000) analysed cardiac arrest survival rates figures at a regional level in the UK, which probably obscured the impact of the small number of local pioneering initiatives, such as at Brighton, to introduce AEDs in ambulances in England and Wales. This paper pointed out that the Heartstart Scotland programme was completed before similar initiatives in England and Wales and went on to suggest: 'It is thus possible that the lower case fatality proportion in Scotland reflects better response times and actions by ambulance crews, relative to their colleagues in the south of England' (Dunn et al., 2000).

The evidence in previous sections of this case study indicates that the evaluation of the Heartstart Scotland programme helped encourage the spread of AEDs elsewhere in the UK, and more widely, through its publications, impact on guidelines and training, and its usefulness to key advocates of the introduction of defibrillators such as Dr Chamberlain. It therefore seems reasonable to suggest that the evaluation of Heartstart Scotland could be linked with at least a small proportion of the health gains associated with the more widespread introduction of defibrillators. One heroic estimate of the total health gain in the UK from community resuscitation – of which defibrillation by ambulance staff would be an important element – put the annual figure in the UK at 5,300 quality-adjusted life years (QALYs) (Buxton et al., 2008). This seems broadly compatible with the figure for Scotland. A small number of the long-term survivors will have been able to return to work, and thus continue to contribute broader economic benefits.

3.12 Payback

The payback framework describes the process from research to several categories of impact. The categories allow us to compare across different case studies. Table 3-2 shows, in point form and by impact category, some of the impacts, described more fully above, that have emerged from this grant.

Table 3-2 Payback

Payback category	Impacts from case study
Knowledge production	• Six direct peer-reviewed articles • 11 indirect peer-reviewed articles
Research targeting and capacity building	• One MSc degree • PI built on the original project in a stream of further work • Other researchers have worked with the research team to conduct studies using the database
Informing policy and product development	• Informed management decisions of the Heartstart Scotland programme in a formative way • Probably had some influence on the decision to renew the AEDs in Scotland from Scottish Office funds • Informed guidelines and training documents at many levels, including guidelines from ILCOR (the major international collaboration in resuscitation), the European level; and many national, sub-national and professional bodies • Almost certainly contributed to the decisions of some ambulance services in the UK and elsewhere to adopt AEDs • Probably informed some discussions with manufacturers
Health and health sector benefits	• Made an important contribution to the increased survival rate following out-of-hospital cardiac arrest, both in Scotland and elsewhere, but it is impossible to quantify the contribution of the specific project
Broader social and economic benefits	• It is possible that a few people have been able to return to work who might not otherwise have done so

3.13 Additional observations

The quality and associated credibility of the evaluation of the Heartstart Scotland programme is indicated by various factors, including the comments from Dr Chamberlain, the location of the journal articles and the high level of citations received by the articles. It has also been noted that Professor Cobbe conducted a very wide range of research, going

from basic research (including on the electrophysiology of the heart) through very major clinical trials to health services research such as this evaluation of the Heartstart Scotland programme. According to Cobbe, there is considerable pressure on medical academics these days to specialise on a narrower range of research (Cobbe interview, 2008). However, the evidence from this case study indicates that considerable impact can be made by researchers who conduct a wide range of research. This echoes an observation in an earlier study of the considerable impact made by the portfolio diabetes of research conducted by Professor, now Sir, George Alberti (Hanney et al., 2006). In that study it was noted that researchers who conduct a wide spectrum of basic and clinical research can sometimes successfully use the understandings gained from one area to inform and enrich the work they do elsewhere.

3.14 References

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APPENDIX

Appendix A: Grants held by Professor Stuart Cobbe overlapping with 1989–1995

Grant: Annual chair funding **Period:** Annual **Total:** £30,000–50,000 **Funding:** BHF

Grant: Junior Research Fellowship **Period:** 1987–1989 **Total:** £47,683 **Project title:** Risk Factors for Sudden Cardiac Death in Hypertensive Left Ventricular Hypertrophy **Funding:** BHF

Grant: Contribution to the RITA study **Period:** 1988–1992 **Funding:** BHF

Grant: Junior Research Fellowship **Period:** 1989–1991 **Total:** £48,272 **Project title:** Electrophysiological Changes in Experimental Cardiac Failure: The Effects of Mechano-Electrical Feedback **Funding:** BHF

Grant: Junior Research Fellowship **Period:** 1989–1991 **Total:** £53,990 **Project title:** 'The Pathophysiological Role of Endogenous Opioid Peptides in Myocardial Ischaemia and Cardiac Failure' **Funding:** BHF

Grant: "Heartstart Scotland" – Analysis of the Results of a National Programme **Period:** 1990–1992 **Total:** £36,365 **Funding:** BHF

Grant: Junior Research Fellowship **Period:** 1992–1994 **Total:** £65,660 **Project title:** The Effects of Acute Hypotension on Coronary Vascular Reserve in Hypertensive Left Ventricular Hypertrophy **Funding:** BHF/Friends' Provident

Grant: Junior Research Fellowship **Period:** 1992–1994 **Total:** £69,036 **Project title:** Calcium Handling in Non-Selectively Skinned Cardiac Muscle in Two Models of Heart Failure **Funding:** BHF

Grant: MONICA coronary registration project **Period:** 1984–1990 **Total:** £541,718 **Funding:** Chief Scientist's Office, Scottish Home and Health Department

Grant: Collaborative studies on electrophysiology of isolated myocytes **Period:** 1987–1989 **Total:** £32,000 **Funding:** Chief Scientist's Office, Scottish Home and Health Department

Grant: Role of Potassium Channels in the Increased Susceptibility of Hypertrophied Hearts to Myocardial Ischaemia **Total:** £49,935 **Funding:** Chief Scientist's Office, Scottish Home and Health Department

Grant: Audit of the management of out-of-hospital cardiac arrest by the Scottish Ambulance Service **Period:** 1992–1994 **Total:** £42,757 **Funding:** Clinical Resource and Audit Group, Scottish Home and Health Department

Grant: The Electrophysiological Actions of Adenosine and Adenosine Triphosphate on Isolated Cardiac Myocytes and the Effects of Ischaemia **Period:** 1987–1989 **Total:** £32,324 **Funding:** Scottish Hospitals Endowment Research Trust

Grant: Studies of Accumulation of "labelled" Platelets and White Blood Cells in Acute Myocardial Infarction **Period:** 1990–1991 **Total:** £48,081 **Funding:** Wellcome Foundation

Grant: Prospective Study of Captopril in Acute Myocardial Infarction **Period:** 1987–1989 **Total:** £150,000. **Funding:** Squibb

Grant: West of Scotland Coronary Prevention Study **Period:** 1989–1995 **Total:** £12.5 million **Funding:** Bristol-Myers Squibb

Grant: Studies on the new Class III antiarrhythmic agent dofetilide **Period:** 1991–1993 **Total:** £75,000 **Funding:** Pfizer

CHAPTER 4 Haemostatic markers in acute transient ischaemic attacks

4.1 Overview of case study grant

This case study investigates the work conducted through the grant titled 'Hemostatic Markers in Acute Transient Ischemic Attacks', which was funded from 1992 to 1994 by the Heart and Stroke Foundation of Canada (HSFC). Using new methods, the research team proposed to measure various products of clot formation (thrombosis) and breakdown (fibrinolysis) in patients with recent (less than seven days) transient ischaemic attacks (TIAs) to determine how levels of these markers change with time. The research team hypothesised that these markers would help identify patients with a very high risk of stroke or heart attack, incidence and death, according to their pathophysiological state, in a simple, non-invasive manner. The general aim of the study was to evaluate several haemostatic parameters reflecting clot formation, the breaking down of the clot and platelet activity in the context of acute reversible ischaemia and asymptomatic cervical atherosclerosis. This study was intended to identify a subgroup of high-risk patients who may ultimately present different prognoses and different responses to either established or new therapeutic measures in stroke prevention. Such information was required for the design of large-scale therapeutic trials based on the prevention of thrombus formation or the promotion of thrombus breakdown. In addition, it was believed that these types of markers could also help to identify when carotid arterectomy, a relatively dangerous surgery, was necessary.

This work was led by Dr Robert Côté, a clinician who, at the time, was an associate professor in the Departments of Neurology and Neurosurgery and Medicine at McGill University in Montreal. It was a multi-centre study based in Montreal, with a portion of the work being conducted in Alberta. Ultimately the study concluded that there was no association between the marker levels studied, or fluctuation of the marker levels, and clinical outcomes.

4.2 Introduction to case study

A TIA, often referred to colloquially as a 'mini-stroke', is caused by changes in the blood supply to a particular area of the brain, which results in brief neurologic dysfunction that persists, by definition, for less than 24 hours. If symptoms persist then it is categorised as a

stroke. Accumulation of thrombus (a clot) in vessels affected by atherosclerosis ('hardening' of the arteries) is believed to play a prominent role in the development of TIAs and stroke (Beers, 2003).

In the early 1990s, there were few treatment or prevention options for patients with TIA or stroke. One interesting area that was spurning a lot of new literature at the time was carotid surgery, which is also known as carotid endarterectomy (CE). There were differing results regarding the efficacy of this procedure, and researchers were interested in knowing if it was better to operate on patients who had had a TIA, or a minor stroke, to prevent them from having another stroke than to use medical treatment. In efforts to evaluate the benefit of these operations, the North American Symptomatic Carotid Endarterectomy Trial (NASCET) was initiated, receiving funding from the National Institutes of Health (NIH) from 1987 to 1992. The study intended to clarify the following questions: 1) How efficacious is CE compared with medical care alone? 2) Which patients should be offered CE? 3) What is the acceptable complication rate? 4) What bearing do risk factors have on benefit? 5) How durable are the benefits of CE? 6) Do other causes of stroke confound the interpretation of results? This trial, which involved 50 North American centres, included 1,212 randomised patients who had experienced symptoms related to arteriosclerotic stenosis of the carotid artery and who had received medical therapy alone or medical therapy coupled with CE and charted their outcome events as nonfatal and fatal stroke or death from other cause. The initial results, reported in August 1991, demonstrated a highly beneficial effect of CE in patients with angiographically confirmed high-grade carotid stenosis, defined as stenosis of 70 to 99 percent of the luminal diameter (NASCET Steering Committee, 1991). The final results of the study concluded that CE in patients with symptomatic moderate carotid stenosis of 50 to 69 percent yielded only a moderate reduction in the risk of stroke. Decisions about treatment for patients in this category were to be based on recognised risk factors, and if CE was to be performed, exceptional surgical skill was recommended. Patients with stenosis of less than 50 percent did not benefit from surgery. Patients with severe stenosis of greater than or equal to 70 percent had a durable benefit from CE at eight years of follow up (Barnett et al., 1998). Dr Côté participated in the NASCET study as the principal investigator (PI) for McGill University.

In the early 1990s, there was also a lot of interest in coagulation and especially hypercoagulation (excessive blood clotting), not only in stroke but also in cardiovascular disease in general. Several publications in the cardiac field, specifically in relation to atrial fibrillation and acute myocardial infarction, indicated that some markers of the haemostasis of coagulation could play a very active role in the acute setting of thrombotic process in both cardiac and cerebrovascular cases. Several mechanisms had been postulated to be involved in the pathogenesis of ischaemic cerebrovascular disease in the setting of extracranial atherosclerosis. One such mechanism was the accumulation of thrombus with either distal propagation or embolisation in the affected blood vessel. At the time, there was no existing simple, reliable biological method for identifying patients in whom this mechanism was operative.

Previous studies investigating stable angina, unstable angina and myocardial infarction had found haemostatic marker elevations (fibrinopeptide A (FPA) and D-dimer) in the hours and days following a symptomatic event (Kruskal et al., 1987, and Théroux et al, 1987). Levels of these markers in acute cardiac conditions (Waters and Lam, 1989) have been

studied to assess their value as a diagnostic aid (Théroux et al., 1987), and to assess thrombolytic therapy (Eisenberg et al. 1986). Several researchers had reported on the measurement of different haemostatic factors in cerebral ischaemia. It was estimated that the risk of infarction in the first year after a TIA was as high as 15 percent (Barnett, 1991). The diagnosis was largely based on history and, because of this, variability was observed in the diagnosis of TIAs, as reported in several clinical trials (Calanchini et al., 1977). These patients were also observed to be at risk of other vascular events, especially cardiac events (Barnett, 1991). Many researchers, including Marra (1983) and Mettinger (1982), had addressed the potential role of haemostatic markers not only as contributors to the pathogenesis of these events but also as possible biological markers that could assist in the diagnosis and definition of subgroups of patients with TIAs with different prognoses or different responses to antithrombotic therapy.

While Dr Côté and his research team were interested in finding novel biomarkers, other researchers in this field were using and exploring traditional risk factors. The largest body of this type of research, known as the Framingham Heart Study, started in 1948 and finished collecting data in 2005. Under the direction of the National Heart Institute (now known as the National Heart, Lung and Blood Institute), the Framingham Heart Study, which later involved the University of Boston, aimed to identify the common factors or characteristics of cardiovascular disease by following a large group of patients over a long period of time. Researchers recruited 5,209 men and women between the ages of 30 and 62 years from the town of Framingham, Massachusetts. Subjects went through detailed medical examinations every two years, and various data regarding their lifestyles were collected. In 1971, a second generation was enrolled into the study, which involved another 5,124 subjects who were the adult children and spouses of the first generation. In 2002, a third generation – involving 4,095 grandchildren of the original cohort – was enrolled in the study. Careful monitoring of the study population has led to the identification of major cardiovascular disease factors such as high blood pressure, high cholesterol, smoking, obesity, diabetes and physical inactivity (Framingham Heart Study home page, 2010).

4.2.1 The case study approach

The case study based on this research grant involved a combination of: three face-to-face interviews with Dr Côté (the PI), Dr Wolfson (a statistician/epidemiologist) and Dr Mackey (a fellow at the time of the research); a review of the PI's curriculum vitae; a review of relevant administrative documents from Heart and Stroke Canada; and documentary analysis of the scientific literature and bibliometric analysis.

4.3 Stage 0 – topic identification

Dr Côté wrote the grant application while he was an associate professor in the Department of Neurology and Neurosurgery and the Department of Medicine at McGill University and an associate physician of Neurology at Montreal General Hospital. The co-applicants were Jacques Leclerc, who was in the Department of Haematology at Montreal General Hospital, and Duncan McIlraith, who was at the University of Alberta Hospital, also in the Department of Neurology. As written in a referral letter for the grant application, Côté

was 'running an extremely good cerebral vascular research unit, regarded by [his colleagues] as one of the best in North America' (Barnett, personal communication, 1991).

Dr Côté had completed his cerebrovascular training in 1982 at the University of Western then worked at the Montreal General Hospital as an assistant physician and clinical neurologist, where he continues to work today as a senior physician. Côté also maintains a position at McGill University as a full professor, where he has been since 1983. This was his first biomarker study. The factors leading the PI to this grant application were threefold:

1. the PI's prior research experience
2. collaborations with colleagues
3. gaps in the body of knowledge about haemostatic markers and limitations of previous studies.

4.3.1 The PI's previous research experience

Dr Côté had completed a clinical fellowship in cerebrovascular disease with Dr Henry J. Barnett, a very prominent stroke researcher, in 1982. Côté described Barnett as a very intelligent, persuasive individual. Côté claimed Barnett was pivotal in the renaming of the Heart Foundation of Canada to the HSFC, thereby including 'stroke' not only in their name but also in their research priorities. He achieved this by maintaining that there were many commonalities between the risks, symptoms and treatments for heart disease and stroke, thus it was natural to group them together (Côté Interview, 2008). A prize awarded through HSFC is now named after Barnett.

After his training with Barnett, Dr Côté moved back to his home town of Montreal, with stroke as his area of interest. Côté also translated what he knew of NASCET into the stroke area with the help of his colleagues in haematology.

4.3.2 Collaborations

Dr Côté arrived in Montreal shortly after completing his research fellowship in cerebrovascular disease. He wanted to broaden his research experiences and sought out keen individuals to work with. At McGill University, where the majority of research focused on basic biomedical studies, he found clinical epidemiologists and biostatisticians who were enthusiastic about collaborating; he described the early 1990s as a time when researchers were starting multidisciplinary collaborations (Côté interview, 2008).

Pairing up with colleagues, Dr Côté found himself working with Jacques Leclerc and Susan Solymoss, both haematologists with special expertise in coagulation. Working together provided them with an opportunity to look at the potential mechanisms of hypercoagulable states. At the time, several markers of coagulation had been identified.

4.3.3 Gaps in the knowledge at the time

Several publications in the cardiac field, especially in relation to atrial fibrillation and acute myocardial infarction, indicated that some markers of the haemostasis could play a very active role in the acute setting of the thrombotic process, whether cardiac or cerebrovascular. Patients with asymptomatic carotid stenosis did and still do pose a big treatment dilemma, in that it is unclear when and if CE is beneficial. The research team

thought that finding a marker would allow them to identify a subgroup of high-risk patients who would most benefit from preventative surgery, as the surgery itself can be dangerous.

The research proposal built on the body of international literature, which largely came from the United States and Europe. Dr Côté believed that many of the existing studies surrounding haemostatic parameters had several limitations. For instance, they did not take into account some of the inherent properties of these markers from a haematological or clinical perspective (section 13 of the application). The studies often included patients with cerebral infarction, which could lead to elevation of markers as a secondary phenomenon through breakdown of the blood–brain barrier and the release of brain thromboplastin (Feinberg et al., 1989). The PI believed that some of the previous studies likely misdiagnosed the type of vascular event (i.e. TIA or cerebral infarct) as they were conducted prior to the availability of computed tomography (CT) and magnetic resonance imaging (MRI). Some bias may also have been present in previous work due to the lack of control groups and the inclusion of subjects in whom haemostasis may have been altered by other factors such as concomitant use of anticoagulation.

In addition, few studies had focused exclusively on haemostatic markers and their significance in the acute and chronic period after a TIA (section 13 of the application. Other studies that had included patients with TIAs had done so with such small numbers that separate analysis for this subgroup of patients was not possible. Thus, no study using sensitive assays available at the time had examined the temporal profile of specific haemostatic parameters in a population of patients presenting exclusively with acute TIAs.

4.4 Interface A – project specification and selection

This study involved 121 patients, who were divided into three subgroups: Group 1: patients who had recently been diagnosed with a TIA but were physically and neurologically intact; Group 2: patients with a history of remote TIA who had been asymptomatic for at least the past year; and Group 3: a control group who had never experienced a TIA. The specific objectives of the study were:

1. to monitor the temporal profile of different haemostatic markers (activated coagulation and fibrinolysis) and platelet reactivity in patients with acute TIAs secondary to atherosclerosis
2. to compare the levels of these haemostatic factors in patients with TIA and neurologically asymptomatic patients with documented cervical atherosclerosis and sex- and age-matched normal controls
3. to assess the sensitivity and specificity of these markers for the diagnosis of TIA.

The research team identified their population of interest as patients admitted to the emergency department or neurology clinic of Montreal General Hospital with a diagnosis of recent TIA (<7 days) who had no functional deficits and who were intact neurologically (n=20, Group 1). A TIA was defined as an episode of focal neurological deficit, believed to be secondary to inadequate blood supply, that is sudden in onset, resolves completely within 24 hours and leaves no residuum. Enrolment was conditional on informed consent.

Initial data recorded included past medical history, risk factors and medications taken. Patients were excluded from the study if they were younger than 50 years of age, had an ischaemic lesion on CT scans, were currently taking anticoagulants, had potential causes of TIA other than atherosclerosis or had other conditions that could affect haemostasis, such as liver disease or congestive heart failure.

The control group (n=65, Group 3) consisted of volunteers who did not have a history of cerebrovascular events or symptoms suggestive of cerebral or retinal ischaemia. The control group was recruited concurrently with the patients with TIA and were matched for age and sex. For comparison, markers were also measured in a group of patients with a history of remote TIA who had been asymptomatic for at least the past year (n=36, Group 2).

To minimise variability, all patients in the TIA group met with the same registered research nurse, Bourque, who took initial blood tests for baseline measurement of FPA, D-dimer and thrombin-antithrombin III (TAT) at least once within the first seven days and one month and three months after the qualifying event. For patients in both the control and remote TIA groups, a single blood sample was drawn for measurement. Complete blood count, prothrombin time, partial thromboplastin time, electrolytes and liver, renal and lipid profiles were undertaken in every patient. Most patients also had an electrocardiogram and, if there was any clinical or electrographic suggestion of a cardioembolic etiology, an echocardiogram was done to further exclude a cardiac source. Patients with TIA also had a CT scan of the brain, which was reviewed by a neuroradiologist to rule out a possible new cerebral infarct, as well as a duplex ultrasound of the cervical arteries to assess the degree of extracranial atherosclerosis.

Patients with TIAs were treated medically or surgically, as judged appropriate by the consultant neurologist. Patients were then followed prospectively. For patients who underwent coagulation therapy, angiography or surgery, only blood samples drawn before the intervention were included in the data analysis. Patients in the TIA group were seen every six months to check vital signs, for blood work and to undergo a neurological examination in the neurology clinic. The occurrence of further vascular events, defined as either a single TIA, multiple TIAs, stroke, myocardial infarction and cardiac or cerebrovascular death, was recorded during the follow-up period. For patients with stroke or myocardial infarction during the study period, only blood samples collected before these further vascular events were included in the data analysis. The follow-up period was short, with an average of only 13 months. Patients received verbal, individualised feedback regarding their results at the final follow-up visit.

The team counted TIAs as an outcome event. Some studies do not do this because the diagnosis of TIA is based on history only and does not entail a lasting neurological or functional deficit. However, the team counted TIAs as a proof of concept, as it was known that TIAs come from the same physiopathology as stroke. The team also used the plaque as a surrogate marker. The research team believed that patients with TIAs would have 'active plaques', which could be reflected by increases in coagulation parameters. Active plaque is an atherosclerotic plaque in either the extracranial or intracerebral arteries that is influenced by certain biological changes in coagulation. This activity is linked to clot formation. These clots can be dislodged from the plaque and then migrate to the brain, where they cause a stroke

Decisions as to which other biological markers to investigate (i.e. FPA, TAT and D-dimer) were based on those already identified in the cardiac literature (this idea was very new in the neurological literature) and those available commercially. The team used commercial kits for the assays.

There were no alterations to the methods initially proposed, as they were found to be appropriate, and the team delivered on the objectives outlined in their proposal. However, there were some challenges with the recruitment of patients and the time frame allowed for follow up, which was constrained to three years by the grant. The team was not able to recruit at the rate that they had anticipated in the application. As stated by the methodologist, 'You don't recruit everyone on the first day of the grant. If it takes you a year to recruit subjects then you have a major challenge. Only a very small number of subjects are available to be followed for the whole timeframe' (Wolfson interview, 2008).

Another challenge was that there were not enough events (strokes) over the two-year follow-up period to allow sufficient power to the study. To help correct for this, the team extended the follow-up period for their sample by another four months.

The PI did not remember any comments received from the peer-review process. He does recall, however, that the composition of review panels at the time did not always include content experts (Côté interview, 2008). Documentation from the Review Committee was unavailable for analysis.

4.5 Stage 1 – inputs to research

4.5.1 Funding

Dr Côté held two grants with the same title, one running from July 1992 to June 1994, which was funded jointly by the Heart and Stroke Foundation of Quebec and the Heart and Stroke Foundation of Alberta (as one of the rare multi-province-funded projects), and another follow-up grant that was funded from July 1994 to June 1996 by the Heart and Stroke Foundation of Quebec. The former is the grant of specific interest in this case study. The Heart and Stroke Foundation of Alberta was involved via the participation of a co-applicant, Duncan M. McIlraith.

In 1991, Dr Côté and his research team applied in response to an open call for a total amount of Can$81,570 over three years or Can$27,640 per year commencing in 1992. In the team's first attempt to secure finances, they were granted funding for two years and received Can$55,280,[1] which paid for two nurse coordinators (one at Montreal General Hospital and one at the University of Alberta Hospital in Edmonton), materials and supplies (largely blood tests) and MRI. The PI mentioned that he felt lucky to have received funding from the Heart and Stroke Foundation, which, he says, was 'very supportive'. He also claimed that it was very difficult in those days to get clinical research in the area of stroke funded. Côté said that it was pointless to propose a randomised

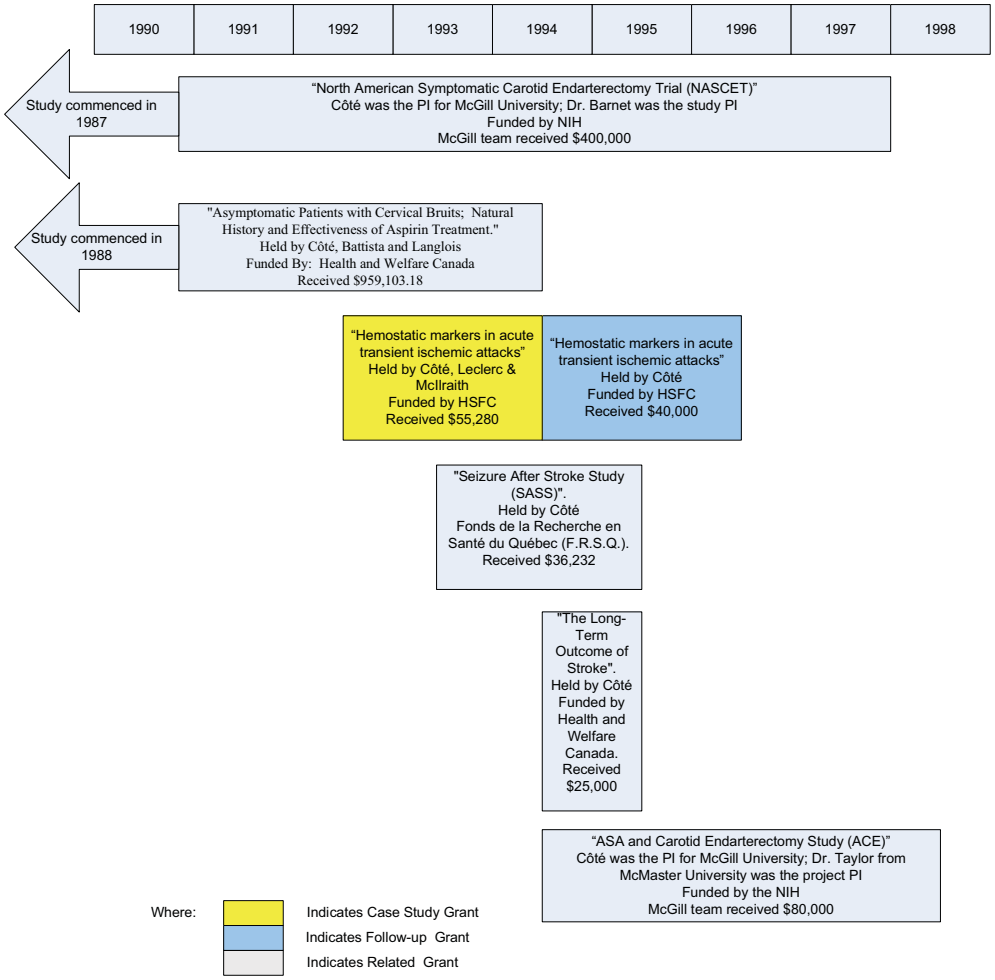
[1] Dr Côté claimed that, at the time, about Can$25,000 per year was the maximum one could receive from the Heart and Stroke Foundation of Quebec for operating grants (Côté interview, 2008).

controlled trial because they were too costly and the funding organisations did not have the means to support them financially.

The PI indicated that he did not have enough money. Although there were other possible sources, such as the Medical Research Council (MRC), he was doubtful that his application would have been funded by the MRC. He also stated that doing research for the pharmaceutical industry often generated generous grants and that, at times, he had applied surpluses from industry funding to support his stroke research.

Dr Côté believes that he may have had more resources had he been in Ontario, Alberta or British Columbia. He says the funding situation in Quebec was, and still is, very difficult. The amounts of grants in Quebec were very small, especially considering the size of the team and their degree of productivity. The PI says it was not easy, but they managed with a lot of good help and positive attitudes. The fellows had their own specific funding or were paid by the university or through extra money from the pharmaceutical industry.

Figure 4-1 Other peer-review grants held by the PI between 1990 and 1996



4.5.2 Facilities

This project was a multi-centre trial that attempted to determine which patients experiencing TIA should undergo a surgical procedure, so patient care and follow up was necessary. This research was conducted at Montreal General Hospital and the University of Alberta Hospital. The specialised clinics and laboratory facilities required for this study were already in operation. As the project largely involved blood work and the statistical analysis of results, there was little need for additional equipment. The medical investigations, including CT, Doppler imaging of the carotid arteries and so on, was being done as part of regular patient care and thus was not covered by funds from the research grant.

4.5.3 Research team

After his clinical cerebrovascular fellowship in London, Ontario, in 1983, Dr Côté returned to McGill University in the mid 1980s. At the time, most of the health research at McGill University was basic biomedical research. Côté brought the team together under his own initiative. He was interested in clinical research, so he initiated these studies and found on site the necessary colleagues, including those from the nursing, biostatistics, epidemiology and neurology departments. The team specifically involved: Jacques Leclerc and Susan Solymoss, who were both thrombosis experts and haematologists by training; Ted Fon, Duncan McIlraith and Ariane Mackey, who were fellows in cerebrovascular disease; Christina Wolfson, a neuroepidemiologist and statistician by training, who worked primarily as a methodologist on this study; Fabrice Rouah, a statistician; Barbara Lage and France Bourque, both clinical co-ordinators; and Dr Côté, a neurologist.

The two clinical co-ordinators were both registered nurses: one at Montreal General Hospital and another at the University of Alberta Hospital. The co-ordinators were responsible for screening the patients, drawing blood samples, collecting data and documenting clinical endpoints

It was through these collaborations and his interest in stroke that Dr Côté found himself working on his first study of biomarkers. Côté recalled that they were great colleagues, who were nice to work with and willing to give their time. He stated that this matters more than anything else. He stressed that they were a great group together and had the right balance of expertise to do the research effectively.

Dr Wolfson said that working with Dr Côté was and is one of the better collaborations she has been involved in as a methodologist (Wolfson interview, 2008). It was a very integrated and dynamic group. She said that people's opinions were respected even when they were making suggestions across disciplinary boundaries. The team members felt comfortable about asking questions. Wolfson said her participation in this project was unpaid extra work that she was doing because she liked the group.

4.5.4 Other facilitators

The reagents used to identify some of the biomarkers associated with plaque growth (typically antibodies within the blood) were very expensive, but the research team was able to get the kits at a very good price through the haematologists. This was because the haematologists worked in big laboratories, often with the same companies who furnish

other equipment for patient care, and they would frequently give big rebates for using the experimental markers.

4.6 Stage 3 – primary outputs from research

Ultimately, the team was trying to identify people who have a higher risk of a recurrent ischaemic event. They concluded that there was no association between the marker levels studied, or fluctuation of the marker levels, and clinical outcomes. The team also tried to correlate the level of the marker to the degree of carotid stenosis or blockage on ultrasound. No such correlation was identified. That was not surprising given the small sample size and the limited number of clinical events observed.

4.6.1 Knowledge production

The three articles listed below are those identified by the PI as having come from this project. The PI said the first two articles were directly related to the case study grant, while the third was indirectly related.

1. Fon, E.A., A. Mackey, R. Côté, C. Wolfson, D.M. McIlraith, J. Leclerc and F. Bourque, 'Hemostatic Markers in Acute Transient Ischemic Attacks', *Stroke*, Vol. 25, 1994, pp. 282–286.
2. Côté, R., C. Wolfson, S. Solymoss, A. Mackey, J.R. Leclerc, D. Simard, F. Rouah, F. Bourque and B. Léger, 'Hemostatic Markers in Patients at Risk of Cerebral Ischemia', *Stroke,* Vol. 31, August 2000, pp. 1856–1862.
3. Ehrensperger, E., R. Côté and J. Minuk, 'Predictive Value of Soluble Intercellular Adhesion Molecule-1 for Risk of Ischemic Events in Individuals With Cerebrovascular Disease', *Cerebrovascular Diseases*, Vol. 20, 2005, pp. 456–462.

The first paper, written by Fon et al. (1994), describes the results from 121 patients observed from 1989 to 1992. The team determined the levels of three markers in 36 patients who had experienced a TIA within the last seven days at three different time points after the TIA: less than seven days, one month and three months. The research team found that abnormalities of sensitive markers of blood coagulation exist in some patients with acute reversible cerebral ischaemia. The three elevated markers were FPA, TAT and D-dimer. FPA and TAT were elevated in the very acute stage after the TIA but had returned to normal values within one month. They were more like acute phase reactants when compared to D-dimer, which seemed to be the most robust marker. D-dimer was significantly increased in both the acute and subacute phase (less than seven days, one month and three months) in patients after a TIA compared with levels in the remote TIA and control subjects; that it remained chronically elevated indicates that it is a more useful marker. Elevated D-dimer levels throughout the three-month sampling period were thought to suggest a prolonged dynamic state of increased thrombus formation and breakdown. Alternatively, they could signal an elevation of non-thrombus-associated 'soluble fibrin' serving as an independent substrate for proteolysis by plasmin and perhaps reflecting a hypercoagulable state. The design of the study did not allow the team to determine if the so-called hypercoagulable state precedes or is a consequence of the TIA. These findings suggest that there is early transient activation of thrombogenesis and

ongoing fibrinolysis after acute reversible cerebral ischaemia. This work was described by the PI as a pilot study given the small sample size and the short follow-up time.

Dr Côté pursued a second grant with the same title, and the results of this work are presented in the second paper. This research was funded by the HSFC from 1994 to 1996 and received Can$20,000 per year. The team used a similar method as in their 1992 study and followed a larger study population from July 1993 to September 1996. The objective was to use markers to identify neurologically intact individuals who were at an increased risk of ischaemic events. The main differences between the two projects were:

- The team added two new markers that they had identified through the literature.
- Another patient population was added. This group involved people who showed asymptomatic carotid stenosis. Thus, there were two populations at risk of stroke but without any neurological damage.
- The sample size was much larger (304 patients) and the follow-up time was longer (an average of almost three years).

Of the 304 subjects, 82 had experienced a recent (within 7 days) TIA, 157 were asymptomatic individuals with a cervical bruit and 65 people were in a control group. The study concluded that prothrombin activation fragment $F_{1.2}$ levels were independent predictors of subsequent cerebral and cardiac ischaemic events in patients with TIAs and asymptomatic individuals with cervical bruits.

The findings from this second grant were more interesting and more conclusive than those of the first study. The results were consistent with the notion of an active role of the coagulation system through upregulation of thrombin in carotid disease progression and in the pathogenesis of ischaemic events in patients at risk (Côté et al., 2000).

The third, indirectly related, paper studied whether soluble intercellular adhesion molecule-1 (sICAM-1) is a predictor of future ischaemic events in high-risk individuals and also whether it is associated with carotid artery stenosis (Ehrensperger et al., 2005). Researchers performed a three-year prospective study of sICAM-1 concentration in three groups: Group 1: subjects with recent (<7 days) ischaemic stroke or TIA, Group 2: asymptomatic subjects with carotid stenosis ≥ 50 percent, and Group 3: asymptomatic individuals with vascular risk factors. Through observations of 275 subjects, the team found that mean sICAM-1 levels were significantly higher in those with recent ischaemic stroke or TIA than in those with risk factors alone. During follow up, ischaemic events occurred almost nine times more frequently in subjects in Group 1 compared with those in Group 3. However, no association between sICAM-1 and carotid artery stenosis was observed. Neither baseline nor subsequent sICAM-1 levels were predictive of the risk of future ischaemic events.

The bibliometric output in Table 4-1 includes only the first two articles mentioned above, which are considered directly related by the PI.

Table 4-1 Publication output and impact[2]

Number of journal articles:	2
Number of articles included in citation analysis:	2
Total number of citations (all papers):	41
Aggregate relative citation impact:	1.02 (Class III)
Self-citations:	2%

Distribution of publications across relative citation impact classes:

	Class I (uncited)	Class II (>0 and < 0.8 citations)	Class III (0.8 to 1.2 citations)	Class IV (1.2 to 2.0 citations)	Class V (> 2.0 citations)
Number of publications			2		
Proportion of total output			100%		

Most highly cited publication[3]:	Fon, E.A., A. Mackey, R. Côté, C. Wolfson, D.M. McIlraith, J. Leclerc and F. Bourque, 'Hemostatic Markers in Acute Transient Ischemic Attacks', Stroke, Vol. 25, 1994, pp. 282–286
Times cited:	29

4.6.2 Dissemination

In addition to publishing in peer-reviewed journals, the research team also disseminated their findings via abstracts, presentations and posters at national and international conferences, such as the annual conferences of the Canadian Congress of Neurological Sciences, American Stroke Association, American Heart Association, and the World Congress of Neurology. These conferences are largely geared towards academic audiences and health professionals such as basic and clinical researchers and physicians. They would include a minority of policy makers. Dr Côté also presented this work to the Department of Neurological Sciences at the University of Western Ontario in 1996 as a keynote speaker. Côté explained that most of the presentations were made by the fellows, with the intent of helping them gain exposure and experience. He feels that the exposure he had early on in his career was very beneficial and wants his trainees to experience the same.

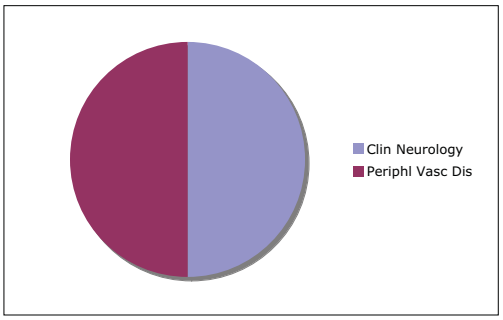
Figure 4-2 illustrates the knowledge diffusion created by the two directly related publications, showing that the United States and Canada were the two countries that most cited this research. The most common fields in which the papers were cited are medicine, pharmacology, clinical neurology and psychiatry.

[2] The one publication that was indirectly linked to this grant was indexed in Web of Science and received two citations in total, giving a relative citation impact of 0.40. Its relative citation impact class was II, and its self-citation rate was zero percent.

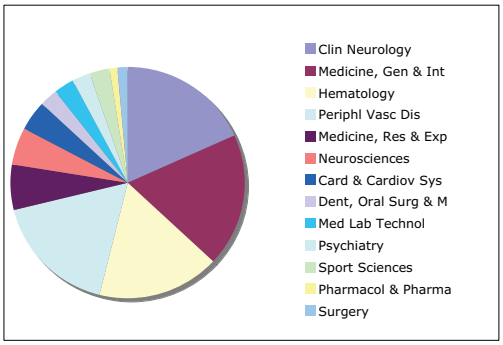
[3] Citation count extracted April 2009.

Figure 4-2 Knowledge diffusion: fields of research of output (a), fields of citing papers (b) and countries of citing papers (c)

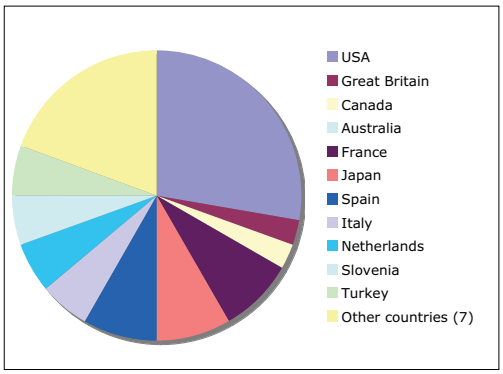
(a)



(b)



(c)



Dr Côté said that he thought his team broke ground in the very specific cerebrovascular niche of patients with TIAs or asymptomatic carotid disease in Canada. He also stated that his team has published more than any other in the area of haemostatic markers and cerebrovascular disease in Canada.

4.6.3 Training and capacity building

Dr Côté does not believe that this study had much influence on his career, because there was no clinical impact. He explains that it was simply an area of interest that he followed.

Côté has since expanded his research to look at biological markers of inflammation, which are closely related to markers of coagulation, after some interesting results from immunological and cardiovascular studies attracted his interest. Thus Côté's research has progressed, and he continues to build on his knowledge of markers and uses many of the same techniques. He says that researchers are now looking at new markers using much more sophisticated equipment to correlate events. He and his team are currently finishing a five-year prospective study of biological markers for high-grade carotid stenosis in about 300 patients, of whom 50 to 80 percent are asymptomatic. One area in the field where there is still uncertainty and controversy is whether surgeons should prophylactically operate in patients with carotid lesions, which are often asymptomatic. During the interview Côté said 'there could be a major impact from a public health point of view if we did so'.

Another current area of interest for Dr Côté is aspirin resistance. Aspirin has been used to treat atherothrombotic vascular events and prevent death in a broad category of high-risk patients. However, aspirin as a therapy is not that effective. About 75 percent of patients with symptomatic atherothrombosis who are taking aspirin will still have a serious vascular event (Côté interview, 2008). The term 'aspirin resistance' has been used to describe several different phenomena. One is the inability of aspirin to protect patients from ischaemic vascular events. The term has also been used to describe an inability of aspirin to produce an anticipated effect on one or more tests of platelet function, such as inhibiting biosynthesis of thromboxane, inhibiting platelet aggregation and causing prolongation of the bleeding time. Neither the precise qualitative and quantitative abnormalities of platelet function that define biochemical aspirin resistance nor their clinical relevance have been established (Antithrombotic Trialists' Collaboration, 2002). Aspirin resistance has been reported in patients after stroke and in those with cardiovascular disease. Côté is currently working with Susan Solymoss to look at platelet aggregation in order to try to determine whether all patients have the same anti-aggregate defect. The research protocol aims to investigate the antiplatelet effect by looking at patients with recent TIAs to see if they can determine the mechanisms involved in platelet aggregation.

Dr Côté has received numerous special honours, awards and recognitions throughout his career, including an appointment as Chair of the Research Planning and Policy Advisory Committee of the HSFC. He also presently serves as a member of the Board of Directors for the HSFC and the Canadian Stroke Network (Centers of Excellence).

Dr Leclerc has since left academia and now works in the pharmaceutical industry for Eli Lilly. He is still very active in the area of stroke research and still publishes articles in the stroke literature.

This project involved two trainees: Edward (Ted) Fon, a doctor of philosophy (PhD) student at the time, and Duncan McIlraith, who was a fellow in cerebrovascular disease. Another member of the research team was Ariane Mackey, a former fellow. All three were involved in work with the clinical parameters, thus gaining experience in assessment of data quality, evaluation of imaging and clinical outcomes, and follow-up procedures. They were not involved in the technical laboratory analysis of the markers, which was undertaken by the haematologists. Fon obtained his PhD while working on this project.

Dr Fon is now an assistant professor of Neurology and Neurosurgery at McGill University at the Montreal Neurological Institute (MNI). He is a clinician scientist with a particular interest in Parkinson's disease. Fon was chief neurology resident at the Montreal Neurological Hospital in 1994. He then completed a clinical and research fellowship in neurogenetics with Dr Guy Rouleau at McGill University, which was followed by four years of training as a postdoctoral research fellow in the laboratory of Dr Robert Edwards at the University of California, San Francisco. He returned to a faculty position at the MNI in 1999. Fon's research now focuses on the molecular events leading to the degeneration of dopamine neurons in Parkinson's disease.

Dr McIlraith is a general neurologist affiliated with Ottawa Hospital. He is also a part-time faculty member in the Ottawa Adult Neurology Residency Training Program.

In her interview, Dr Ariane Mackey said that her experience on this project indirectly assisted her career in research by putting her in contact with other researchers and statisticians. She said she found it very educational in general. She learned much about planning and running a research study. Mackey is now a researcher within the Department of Medicine at Hôpital de l'Enfant-Jésus, Université Laval, and a member of the Centre Hospitalier Affilié Universitaire de Québec. Mackey's principal area of work remains in secondary prevention of stroke (i.e. investigating patients who have a TIA and then a stroke). She is currently participating in several NIH-funded research projects focused on different aspects of stroke. She is also investigating a new medicine for treating high levels of cholesterol, carotid stenosis, acute stroke and neuroprotection/neurological interventions. She continues to work with Dr Côté on another marker study, which is investigating different markers and carotid stenosis. In an unrelated field, Mackey is also participating in an NIH-funded study of the efficacy of treatments for insulin-resistant people (pre-diabetics).

Dr Christina Wolfson is currently Professor and Director for the Division of Clinical Epidemiology at McGill University. She says that participating in this work was important for her at the time because she did not have tenure. She described this project as good evidence to show the university that she was capable of collaborative work. She says her participation did not generate a great number of publications, although she was the first author on a few publications that resulted from her collaboration with Côté. Wolfson and Côté continue to collaborate on projects.

The established team continues to attract neurology residents and fellows who are integrated into the research team. Since forming in 1983, the team has worked with 15–20 fellows, usually for two years each. Many are authors on the papers produced by the team. The majority of the fellows who worked with Dr Côté have stayed in the cardiovascular field, and although some do not continue to conduct research, Côté regards them as stroke experts in their specific areas.

4.6.4 Benefits to future research and research use

Dr Côté claimed that the work of the research team has advanced understanding of coagulation markers. The findings were important for increasing the body of knowledge concerning such biomarkers, even though the findings were partially negative and had little

clinical impact. These findings tell others to invest their time and money in different pursuits.

Dr Côté said that the work he did on haemostatic markers is specific to the areas of cardiology and stroke and is not applicable elsewhere in medicine. Other researchers in the United States and Italy were investigating markers for cardiovascular disease. Two such researchers are neurologists Feinberg and Coull, who were both doing work similar to that of Côté.

New research is showing that the markers Côté and his team were investigating in the 1990s could be related to dementia. Neurological vascular disease can be a cause of vascular dementia and may even be related to Alzheimer's disease. Some researchers have now looked at the same biological markers Côté has studied as risk factors for cognitive decline. Côté has not been involved in this work.

4.7 Stage 4 – secondary outputs

Just recently the team's paper published in 2000 in the journal *Stroke* was quoted in stroke clinical guidelines, being cited in a section on optional coagulation screening tests for patients with TIAs (Easton et al., 2009). Dr Côté said that the fact that this work was quoted nine years after it was first published indicated that it was unique research and that few others have done this type of work.

4.8 Stage 5 – adoption by practice and the public

This research was not adopted by practice and was not relevant for public adoption. The findings had no impact on the daily management of these patients. The team concluded that their findings were not robust enough, as they did not emerge from randomised controlled trials. Further studies with larger samples, as well as multiple randomised controlled trials, would have been required to confirm their findings.

4.9 Stage 6 – broad health and economic outcomes

No documented widespread health gain or cost savings have been associated with the use of these markers. There has been some very good work from the Framingham Group on prognostic markers in addition to traditional risk factors. The proper statistics on patients with hypertension, diabetes and so on shows that the incremental value derived to prognosticate from these markers is very little. To date, no effective markers have been found and excitement has diminished in this field of study. The new trend is moving towards imaging and the use of imaging investigative tools to look at the growth and instability of arteric plaques.

4.10 Payback

The payback framework describes the process from research to several categories of impact. The categories allow us to compare across different case studies. Table 4-2 shows, in point form and by impact category, some of the impacts, described more fully above, that have emerged from this grant.

Table 4-2 Payback

Payback category	Impacts from case study
Knowledge production	• Two directly related peer-reviewed articles • Presentations at various meetings/conferences
Research targeting and capacity building	• Knowledge transfer and training within laboratory to students • One PhD degree obtained
Informing policy and product development	• Inclusion in 2009 guidelines for optimal blood screening of patients with TIA
Health and health sector benefits	• Not applicable
Broader social and economic benefits	• Not applicable

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CHAPTER 5

A follow-up study of heart attack patients

5.1 Overview of case study grant

In 1992, Professor Dobson (the principal investigator (PI)) received a grant in aid of $119,500 over three years from the National Heart Foundation of Australia (NHFA) for a grant titled 'A Follow-up Study of Heart Attack Patients' (grant reference: G9153283). The research project was conducted at the University of Newcastle in collaboration with hospitals in the region (eg John Hunter Hospital) and with clinicians, patients and patient groups.

The project investigated what happened to people after heart attack (or myocardial infarction) or hospital admission for other serious heart diseases, with the aims of identifying risk factors that could be modified and examining how effective different drugs were (known as secondary prevention). The grant allowed the Newcastle Collaborating Centre to conduct a follow-up study to the World Health Organization (WHO) Multinational MONItoring of trends and determinants in CArdiovascular disease (MONICA) project in the lower Hunter region of New South Wales (NSW). The project examined factors that predict infarction, development of congestive heart failure (CHF) or other complications, and death, especially sudden death. The aim was to identify those patient characteristics and secondary preventative activities that are associated with better (or worse) long-term outcome and also to enable comparison of reinfarction and survival rates with the other Australian collaborating centre of the MONICA project in Perth and relate these to differences in medical care and other factors. The follow-up study involved linkage of records and questionnaire responses for more than 7,000 subjects.

The grant was important in providing the seed funding to extend the data collection and analysis being undertaken in the MONICA project beyond 28 days from onset of symptoms in order to examine long-term survival rates and to assess the effect of medical care on reinfarction and coronary death.

The primary outputs from the case study grant were the seven directly attributable publications, with the key findings being that changes in risk factors were influencing changes in mortality and incidence. Among the specific findings it showed the benefit from beta blockers after myocardial infarction and no effect on the risk of recurrent myocardial infarction and death with the use of calcium antagonists. In addition, 32

publications from work that followed were identified as indirectly attributable to the case study grant.

As part of the wider impact of the MONICA project, the case study grant also had an impact on:

- the development of data management skills for the large population dataset
- the expertise in epidemiology in the Hunter region institutions, through the training of doctor of philosophy (PhD) students and a number of clinicians collaborating and acting as authors to the publications from the research
- a range of health-promotion activities
- providing valuable employment for people working on the project within the difficult employment environment of the Hunter region.

5.2 Introduction to case study

5.2.1 Overview

The focus of this grant was to provide a follow-up study to the MONICA[1] project, an international study that was coordinated by the WHO to monitor trends and determinants of cardiovascular disease over a 10-year period that began in 1984. In Australia there were two MONICA collaborating centres in Perth and Newcastle.

Funds for the heart attack study did not cover follow-up beyond 28 days from onset of symptoms. The collaborating centre in Perth, however, received funding from the NHF to conduct a follow-up study to examine long-term survival rates and to assess the effect of medical care on reinfarction and coronary death.

The purpose of this particular grant was for the collaboration centre in Newcastle to conduct a follow-up study in the lower Hunter region of NSW to examine factors that predict infarction (heart attack due to tissue death caused by blockage of the tissue's blood supply), development of CHF or other complications, and death, especially sudden death. The aim was to identify those patient characteristics and secondary preventative activities that are associated with better (or worse) long-term outcomes and also to enable comparison of reinfarction and survival rates in the two centres and relate these to differences in medical care and other factors.

The follow-up study involved linkage of records and questionnaire responses for more than 7,000 subjects.

5.2.2 Understanding the broader research field

Large and sustained downward trends in mortality from ischaemic heart disease (IHD) were apparent in Australia since the later 1960s but the reasons were not fully understood.

[1] The MONICA Project monitored trends in cardiovascular diseases and related these to risk-factor changes across 32 populations in 21 countries. The project ran from 1979 to 1996 and provided a valuable 10-year dataset.

Most coronary deaths continued to occur suddenly and outside hospital, and time delays before a patient with acute symptoms got to a modern coronary care facility remained long.

It was understood that intervention before onset of symptoms has a greater potential for reducing mortality than care of the acute event. Furthermore, a large and possibly increasing proportion of patients who were either dying from IHD or being admitted to hospital with chest pain for possible or definite acute myocardial infarction (AMI) were already 'known to the system', in that they had previously been treated for and/or admitted to hospital with some acute or chronic manifestation of IHD.

It was therefore hypothesised that the potential impact of secondary prevention or 'post-acute' care was considerable. It was thought that any treatment or clinical management strategy that would reduce rates of reinfarction or other complications in those with a previous heart attack could have a major impact on overall morbidity and mortality from IHD in the population as a whole.

5.2.3 The case study approach

The case study based on this research grant involved a combination of: a review of documentation for the grant; one face-to-face interview with the PI on the project (Professor Annette Dobson); interviews with other members of the research team; a review of the PI's curriculum vitae; and documentary analysis of key citing papers, publications and conference abstracts arising from it.

5.3 Stage 0 – topic/issue identification

As identified by Professor Dobson at interview, the project idea was significantly influenced by the Chief Medical Advisor of the NHF at the time and the local environment in the study area of Newcastle in terms of the high cardiovascular disease (CVD) burden. The following examines how the research topic was identified and the three factors that were crucial:

- stems from a larger study
- high CVD rates in the local area
- key mentor/supporter.

5.3.1 Stems from a larger study

It was explained at interviews and confirmed in the documentation that the case study grant built on a large international WHO study that had involved the study sites of Perth and Newcastle. A key aspect of the case study grant was that it was able to leverage from the work, data, research and resources established for MONICA. Dobson said, 'It was simply a small part towards the end of a very large project that was funded by the Heart Foundation from the mid 80s. The Heart Foundation with help from others…was to have two Australian centres in a World Health Organization study to monitor cardiovascular disease in defined populations over a 10-year period…so the original impetus for the

project was just to build on those original MONICA protocols, to take the study out and look at longer term outcomes' (Dobson interview, 2008).

Professor Dobson explained that by the time of this grant in the early 1990s, the research team had a fair idea that in addition to the severe myocardial infarctions (MIs) there were increasing rates of less severe MIs occurring in the Hunter region due to their process of looking at all MIs, including the ones Dobson called borderline (which she explained would now be called acute coronary syndrome (ACS)). It was further explained that the Perth centre was only looking at the 'real hardcore' MIs, whereas the team in Newcastle (Hunter region) could see that while survival rates were getting better, the more mild cases of MIs were increasing. Dobson said, 'We were seeing event rates of severe myocardial infarctions and fatalities coming down, but more of the mild ones increasing, which meant that we were building up in the population people that had impaired coronary function one way or another. The question was what would happen to them in the long term. Would we be getting more infarctions, so it was just a lag.' (Dobson interview, 2008).

Interviews for this case study have also suggested that when the MONICA project was originally started there were no coronary artery bypass procedures taking place in the Hunter region and then during the MONICA project bypasses started to be performed on the 'absolute healthiest best prospects' *(*Dobson interview, 2008), while angioplasty was not being performed for a long time, as compared with in Perth, where angioplasty started to be used during this period. Dobson said, 'So the question was, is it just that are they going to end up with congestive heart failure...you do what you can to patch them up but you get gradual accumulation of deficit' (Dobson interview, 2008).

5.3.2 High CVD rates in the local area

The high rates of CVD in the Newcastle area had already been an influence on Professor Dobson and key members of the team moving into the CVD field. Dobson said, 'And as soon as we looked at what data was available from the Hunter [region] it became very clear that cardiovascular had to be the major thing that we would work on' (Dobson interview, 2008).

More specifically, for the case study grant it was identified as a valuable comparison site to the same work being undertaken in Perth at the time. Dobson said, 'Very quickly we found that we were probably in CVD heartland in the Hunter [region], it was very, very high, and initially we had the idea that we could collaborate or get the repetition in Perth and Newcastle, and it quickly became apparent that the choice was brilliant in the sense that you had in Newcastle a place where CVD was very high. At that stage nobody was looking at Tasmania, which is what we now know is the other very high place [for CVD]' (Dobson interview, 2008).

5.3.3 Key mentor/supporter

Professor Dobson advised at interview that the Chief Medical Advisor of the NHF had an influence on her moving into CVD earlier on in her career and provided key support. She said, 'The reason I came into heart disease was because, when the medical school was started at Newcastle, in the mid 70s, I had just been hired by the maths department and they said you had better go and talk to this medical school [with a lot of projects needing statisticians and the practice at Newcastle University of the departments working together

rather than establish their own separate resources]…and [the Chief Medical Advisor of the National Heart Foundation] had decided that the Professor of Community Medicine was somebody to work with...so by second hand he supported me as well' (Dobson interview, 2008).

Directly relating to the case study grant, the idea of having two centres in Australia and therefore adding Newcastle to the Perth study was inspired and encouraged by the Chief Medical Adviser of the NHF at the time. Dobson said, '[The Chief Medical Advisor of the National Heart Foundation] was interested in the new group that had developed at Newcastle and he encouraged us to do some research on cardiovascular disease…In Perth they were much more sophisticated in terms of their treatment and we had no major hospital in the Hunter [region] when [the Chief Medical Advisor of the National Heart Foundation] was first talking about these things…so the idea of having two centres in Australia was really an inspired piece of work by [the Chief Medical Advisor of the National Heart Foundation]' (Dobson interview, 2008).

5.4 Interface A – project specification and selection

The aim of the study was to use record linkage and to perform an annual follow-up of all heart attack survivors identified in the Newcastle collaboration centre of the MONICA project to determine the extent to which secondary prevention activities were performed and how they influenced re-infarction and death rates (especially sudden death) and the development of CHF and other complications. The hypothesis being tested by the study was that:

- rates of reinfarction, development of CHF and other complications, and coronary death among survivors of heart attack are related to use of secondary prevention procedures and medications and to changes in levels of risk factors
- differences in reinfarction and mortality rates among survivors of heart attack in Newcastle and Perth can be explained by differences in the use of secondary prevention procedures and medication, alteration of levels of risk factors for IHD and severity of initial attacks.

By investigating the extent to which the above-mentioned medical care is actually used on a population basis, and its effectiveness, it was hoped to improve secondary prevention and outcome for victims of heart disease.

Funds for the second half of the MONICA study period to 1993 were provided jointly by the National Health and Medical Research Council (NHMRC) and the NHF in Australia. This funding for the MONICA project did not cover follow-up beyond 28 days from onset of symptoms. However, the Perth group received funding from the NHF to conduct a follow-up study to examine long-term survival rates and to assess the effect of medical care on reinfarction and coronary death. The purpose of the case study grant application for the Newcastle group was to examine a wider range of outcomes (eg especially CHF and sudden death) in relation to patient factors and post-acute care. It was to complement the work in Perth and also to enable the comparison of reinfarction and survival rates in the two centres and relate these to differences in medical care and other factors.

It was noted in the grant application that very little data existed on the factors predicting the development of CHF following AMI and the intention of the study was to examine baseline admission factors and follow-up data to see to what extent prediction could be made of later development of CHF and to examine the predictors of mortality in those who develop CHF either during a first admission or later.

It was also noted in the grant application that the MONICA project provided an ideal vehicle for assessing the extent of use and effectiveness of strategies for secondary prevention and that it would be enhanced in Australia by the opportunities to compare findings from the two centres with different levels of IHD and possibly different levels of medical care.

Results from the MONICA project had shown at that time that mortality from IHD was significantly lower in Perth than in Newcastle and that this difference was not adequately explained by differences in the levels of conventional risk factors like blood cholesterol, blood pressure and cigarette smoking. Rates of MI were also much lower in Perth than Newcastle. There were a number of ways in which the use of secondary prevention agents differed between Perth and Newcastle. For example, the MONICA study had identified that only 40% of cases discharged alive in 1984 to 1985 and 41% in 1989 were on beta blockers at discharge (57% in 1989 were on aspirin) and the percentage of those with a prior history of MI who were on beta blockers on admission for a recurrent event was even lower. The case study grant was therefore also going to examine the extent to which the different use of secondary prevention agents between the two centres contributed to the gradient in rates of infarction and death.

All three reviewers of the grant application rated the track record of the investigators as 'outstanding'. Two of the investigators rated the project as 'excellent' and the third rated it 'reasonable (clearly worth funding)'. The following statements reflect the positive views expressed by each of the reviewers (Grant-in-Aid Assessment Forms, 1991):

- 'This is an attractive proposal, based on the MONICA project…The investigators have established reputations as contributors to knowledge of the epidemiology of heart disease in Australia and, in my view, this proposal represents good value for money.'
- 'This is an extremely important topic. The MONICA centres in Australia present opportunities that should be fully exploited and encouraged. The project is well written, the design is appropriate and the likelihood of gaining new knowledge is high. The funding request is also appropriate (probably a little lean).'
- 'It is clearly a worthwhile study provided that the databases can be adequately linked and that there are no significant gaps in the acquired data.'

In relation to the grant application, the reviewers raised the following issues that they felt needed to be raised in the grant application interview (Grant-in-Aid Application reviewers, 1991):

- 'The follow-up questionnaire would need to include socio-demographic information because a cardiac episode can produce a marked change in a person's lifestyle [and] not necessarily for the better.'

- 'The socio-demographic differences in Newcastle to Perth (eg lower socio-economic status) could result in a lesser response (and thereby gaps in the data), necessitating vigorous pursuit of patients in order to obtain comparable responses to Perth across all socio-demographics – would there be phone follow-up of the mail survey?'
- 'Has the questionnaire been adequately pilot tested? What is the respondent's burden?'
- 'What loss to follow-up is anticipated? Will this affect power considerations?
- 'How will proxy information be handled? This is likely to differ in quality from that obtained from living subjects.'
- 'How will non-respondents be handled? These are likely to differ from respondents with respect to compliance with preventative regimes [sic] and behavioural change.'
- 'The study is said to have sufficient sample sizes to detect relative risks of two or more with 80% power (or relative protection of 0.5 or less). Are effects of this magnitude anticipated?'
- 'Have the investigators given some thought about how to describe (quantitatively) complex treatment regimes [sic] for each patient? How will treatment/response relationships be handled? Record linkage has attracted considerable attention in the statistical literature of late and I would draw the investigators' attention to a recent article which appeared in the Journal of the Royal Statistical Society.'

While the budget was seen as reasonable and there was confidence in the investigators achieving the required outcomes, there were also consistent queries as to whether the budget and the hours allocated to the project by the investigators were sufficient.

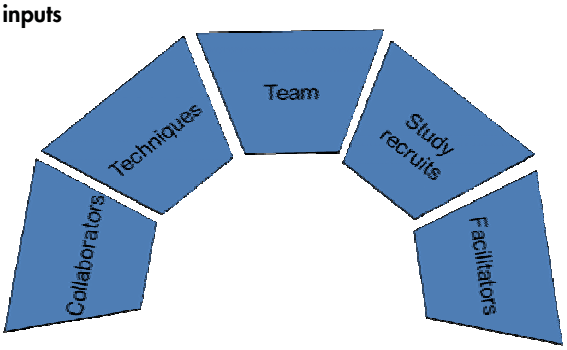
The ballot papers from the interview ranged from 'Good – in front of second rank of project' for three of the reviewers to 'Very good – still first class but not at the top' by one reviewer.

Professor Dobson believed that the issues raised by the reviewers were considered and addressed; however, she did not believe there were any significant impacts or changes made to the project.

5.5 Stage 1 – inputs to research

In this section we outline the key inputs to the research. In summary, Professor Dobson thought that the case study grant evolved around the facilitators, collaborators and techniques in terms of overall quality and that the study recruits (ie study population in the Hunter region) provided the essential and key input. Time and consumables were also identified as inputs. Dobson said, 'Time and consumables for this sort of research are always ongoing petty battles' (Dobson interview, 2008). Figure 5-1 indicates the key inputs specifically to this case study.

Figure 5-1 Key inputs



5.5.1 Facilitators

One of the facilitators for the research was the grant from the NHF. The grant application was for an amount of Aus$152,518 over three years. However, letters on file indicate that as part of across-the-board cuts by the NHF to the amounts originally nominated for all projects, the final grant totalled Aus$119,500.00 over three years (Aus$47,100 for 1992, Aus$34,200 for 1993 and Aus$38,200 for 1994). The grant was to cover the full-time salary of the person performing the role as research assistant, statistician and data manager for the project, part-time (half-time) salary for a clerk and costs associated with printing, postage (including additional letters to those not responding to the initial contact and reply-paid returns), telephone follow-up calls, data entry and maintenance.

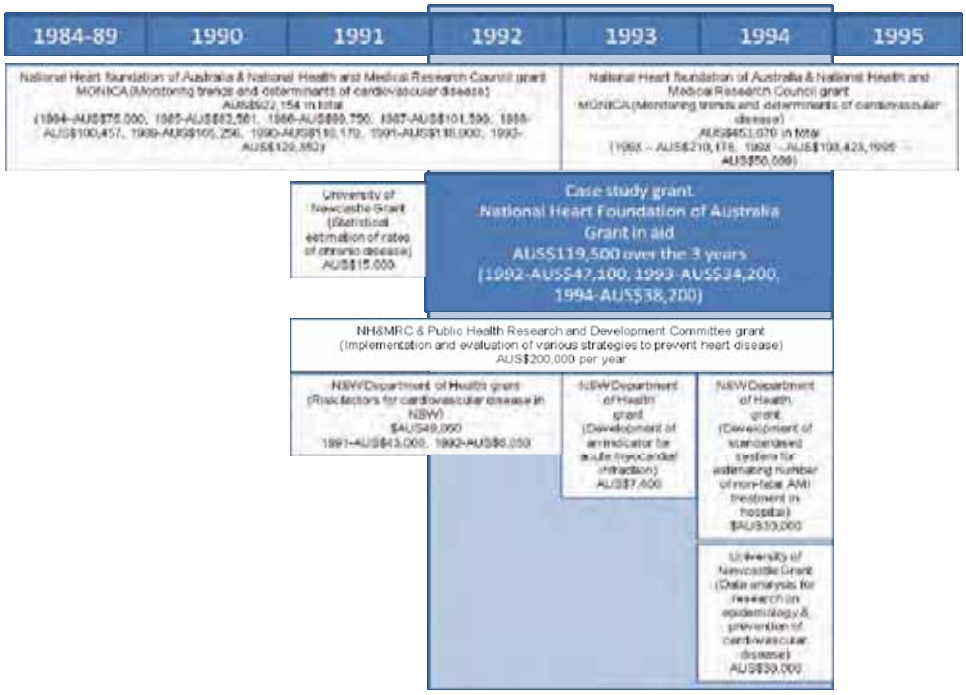
It was argued in the grant application that a full-time statistician/data manager was essential due to the extensive record linkage required in excess of the data-handling requirements of the main MONICA project and therefore the person needed to be familiar with database management and, if possible, to have experience with record linkage. They were also to carry out the data analysis required for the study.

It was also presented in the application that a half-time clerical assistant needed to be funded as the study involved considerable clerical work to identify the more than 7,000 subjects; send questionnaires to all subjects believed to be alive; follow-up with telephone reminders as many as three times; and perform other checking of non-respondents, code replies and arrange for these to be entered into the computer.

There were a number of other funding sources at the same time for related work, including the main MONICA funding (see

Figure 5-2). Dobson said, 'Well we were funded by NHMRC at [these] stages as well, but the Heart Foundation…there is value…there is huge leverage from Heart Foundation funding for cardiovascular (research)…People recognise the Heart Foundation is not rich, but if you have Heart Foundation funding there is credibility. Just like people who do the big multinational pharmaceutical trials, if they have got NHMRC funds, it improves their credibility enormously…and NHMRC funding almost always needs a little bit of seed funding (Dobson interview, 2008).

Figure 5-2 Funding map for the grant; this shows major inputs to the PI's research activity around the period of the case study grant.



Another key facilitator was the extensive collaborations that occurred. As part of the MONICA international project, the team worked particularly closely with other teams in Perth and Auckland. There were also meetings approximately annually with the people from collaborating centres in other countries involved in the MONICA project.

For example, all of the quality control protocols and systems were international. In addition, even though it is now the best part of 15 years since the project stopped collecting data, all of the data have been pooled and are held at the National Public Health Institute of Finland so that anybody who wants the data can obtain them at any stage. A small committee (including Professor Dobson) that now only meets by email still manages the data. This international collaboration (and the associated meetings) was confirmed by Dobson as a key driver in improving the science and influenced the way research was done: 'Oh yes, oh yes, big time, because there were all these quality audits. They were done by Americans who weren't participants in the study. Occasionally we got the top marks and that was a huge celebration for the whole team to do that' (Dobson interview, 2008).

Within the case study project there was key collaboration, confirmed in interviews and documentation, between the University of Newcastle, John Hunter and Royal Newcastle Hospitals and the Hunter Area Health Services. Dobson said, 'We tried to work very closely with them, and John Hunter Hospital has done really well, I think, in having strong clinical epidemiology at all stages…they have never not had it' (Dobson interview, 2008).

There was collaboration with Dr Scott Kinlay, who was listed as an author of one of the main publications from the case study grant. Based at the Cardiovascular Division of

Brigham and Women's Hospital, Boston, Massachusetts, Kinlay was also a Fellow in Cardiovascular Medicine at the Cardiovascular Unit at John Hunter Hospital; he did a PhD with the research team before he went on to do cardiology training at John Hunter Hospital and then in the United States. Another collaborator, also listed as an author to the same main publication from the study, was the biostatistician Lynette L.Y. Lim of the National Centre for Epidemiology and Population Health and the Australian National University in Canberra.

There were also key collaborations with all the clinicians in the hospitals and in the Hunter area (with the exception of one recalcitrant clinician noted by Professor Dobson) and in particular with some young cardiologists at the time, such as Dr James Leitch at the Department of Cardiovascular Medicine, John Hunter Hospital. Leitch is among the authors listed in some of the main publications stemming from the case study grant and associated research activity. Dobson said, 'So in terms of influencing other people, what we were doing was working with the really young gun cardiologists, and the questions in that case were really their questions, and we were able to work with them. So I think that we might have influenced a few young gun cardiologists' (Dobson interview, 2008).

Professor Dobson felt that these clinical collaborations provided the research team with a grounding in clinical relevance, which she felt was very important. The other thing was that the clinical collaborators eased things for the nurses in hospitals, because they were partners in the research.

5.5.2 Study recruits

As explained earlier, the high rate of CVD in the population of the Newcastle area was a key input for the study, making it particularly valuable as a comparison site for the same work being undertaken in Perth.

There was extensive involvement with people in the community and in particular the patients, their families, their medical practitioners, and patient groups and patient advocacy groups, like stroke and rehabilitation groups. Dobson said, 'They responded quite favourably to us. Now somebody was taking notice of them [people in the Hunter region]. Some of the people in the Hunter coal fields around Cessnock and Kurri Kurri are third- and fourth-generation coal miners from the UK. A lot of them are actually from the Scottish border area. They used to love seeing the Australian data compared with the [United Kingdom] data because then they said that all their heart disease was due to their genes and it wasn't their fault. Glasgow was always worse than the Hunter [region]. There weren't many places that were worse than the Hunter [region], but Glasgow was one of them' (Dobson interview, 2008).

5.5.3 Knowledge, expertise and techniques

As acknowledged in the notes of the grant assessors, Professor Dobson as the PI and Professor Richard Heller as the other investigator brought to the project experience, track records and reputation as contributors to knowledge of the epidemiology of heart disease in Australia.

Professor Dobson brought her knowledge and expertise as the Professor of Statistics (initially Professor of Biostatistics) at the University of Newcastle and as the PI since 1983 for the Newcastle Collaborating Centre of the WHO's MONICA Project.

Professor Heller was a physician and had completed his doctorate in CVD with Geoffrey Rose in the United Kingdom. He was recruited to Australia and worked with Professor Dobson on the MONICA project.

As already noted, the expertise, knowledge, systems, processes, relationships and infrastructure from the MONICA project were able to be leveraged for this grant. This included the experience and network of personnel involved in the project, which is explained in more detail in the next section.

5.5.4 Space, equipment and personnel

The space and equipment used were mostly provided by the institutions of Newcastle University and John Hunter Hospital, with requirements being quite limited. There was also the network of nurses and other personnel that was largely funded by the institutions and the MONICA funding.

There was a big team in Newcastle, as indeed there was in Perth, set up so that all possible heart attacks in the defined population could be looked at. There was apparently very little turnover, with some people being involved for the 10 years from the beginning to the end of the MONICA project and this grant.

Firstly, there was a team of nurses at John Hunter Hospital and Royal Newcastle Hospital every day looking for cases and trying to interview them. The nurses would also visit the outer hospitals once or twice a week. Dobson said, 'They were there all the time. They would register and even continued registration during the Newcastle earthquake. I said they could stop. They said they were nurses, so they said, "We can't stop for things like that. We keep going!" They had such a commitment' (Dobson interview, 2008).

There was one person who performed the electrocardiogram (ECG) coding, and in the end she was also performing the coding for Perth and Auckland. This task was described in interviews as one of the most hated tasks.

The other hated task was trawling through by hand in the local office of births, deaths and marriages. The MONICA study protocol only required the follow-up of people up to 28 days, and so one of the things the team had to do for the case study grant was find the relevant deaths, and this was before the national death index was available. The clerical assistant funded part-time under the grant generally had to perform this task.

In addition, as the MONICA project did not link the data to identify reinfarctions over the longer term, the case study grant was to link the data to identify the reinfarctions, check on the people who they did not know were dead or alive and see whether they would have gone on to experience other conditions (in particular CHF). The linking of the data was managed by the grant-funded data manager. There was also the overall programme manager and another statistician who was involved in the project and who went on to take over the management of the Heart and Stroke Register that was established from the MONICA project to continue looking at MIs, ACS and strokes. The register continues to operate to this day at John Hunter Hospital. Dobson said, 'The kind of data they are looking at now sounds so familiar' (Dobson interview, 2008).

There were also students who were involved at different times during the project, with two PhD students specifically noted and listed as authors in publications from the case study

research. One is now a senior statistician and the other a professor; both are based at the School of Medicine and Public Health at the University of Newcastle.

5.6 Stage 2 – research process

In line with the collaborating centre in Perth, the collaborating centre in Newcastle monitored all suspected cases of sudden coronary death and AMI occurring in their study population. This was done by the establishment of a linked file of all subjects registered in the MONICA project prior to and following 1992 during the study period. The study population in Newcastle consisted of residents of the Lower Hunter region of NSW who were aged 25–69 years old. The study population for Perth consisted of residents of the Perth Statistical Division (effectively the Perth Metropolitan Area) aged 35–64 years. For comparisons with Perth, only data for subjects aged 35–64 years at the index event were used.

Patients were registered as having sustained definite MI if there was evidence of either unequivocal serial ECG progression, according to the coding specified under the MONICA project (the Minnesota code (Tunstall-Pedoe et al., 1994)), during the attack or cardiac enzyme levels twice the level normal, as well as specific combinations of symptoms and ECG changes.

In Perth, the 'cold-pursuit' method of event registration was used; that is, cases were identified through hospital separation data and information was collected by retrospective review of medical record. In Newcastle, the 'hot-pursuit' method was used. Study nurses monitored all hospitals in the area, registering every patient likely to meet study criteria. Patients were interviewed while in the hospital to obtain information on symptoms, medical history and smoking status. Cardiac enzyme results were extracted from hospital records and ECGs were copied and subsequently coded. Details of medication use during the event and at discharge were obtained from the medical record. The data were also supplemented by follow-up questionnaires sent to all patients registered who were classified as meeting the criteria.

All death certificates for the cohort were also scrutinised. Details of fatal cases were obtained from the death certificates, post-mortem records and questionnaires sent to doctors, relatives and other informants.

Professor Dobson noted in interview that there were particular challenges in relation to this: 'As far as I can remember, we had a lot of trouble determining whether people were alive or dead. We sent letters to the last known addresses and I don't remember what percentages we got back. But there wasn't a national death index you could look up electronically, and you couldn't do an electronic search of the national electoral rolls, which you do nowadays. A lot of the tracking mechanisms that we use now just weren't available, so I think that we were overly ambitious about the potential to really identify the live cases…and we may have also missed some deaths' (Dobson interview, 2008).

A range of statistical methods were used to explore the different questions being explored, including: estimates of odds ratios and chi-squared tests for differences and linear trends; logistic regression models to estimate adjusted odds ratios and 95% confidence intervals,

the Kaplan-Meier method for estimates of survival rates (and curves drawn); log rank tests (for testing differences between groups); and Cox proportional hazard models, which were fitted to assess the effect of ECG changes and the presence of abnormal enzymes, with adjustment for age, sex and previous AMI, on survival and reinfarction.

5.7 Stage 3 – primary outputs from research

5.7.1 Knowledge production

The main MONICA project was designed to ask if changes in risk factors were influencing the changes in mortality and incidence. It remains the biggest cardiovascular epidemiology study ever undertaken in the world. The study overall, and particularly clearly the Australian data, indicated that changes in risk factors were influencing changes in mortality and incidence. This also included specific publications on specific factors such as passive smoking, where it was found that passive smoking increased the risk of CHD and increased fibrinogen concentration provides a marker of its effect. Professor Dobson said, 'The net result was we pooled the Auckland and Newcastle data and what we were able to show was an effect of passive smoking. We also looked at time to quitting smoking. And the terrific thing about heart disease is if they stop smoking now, within 6 months their risk of cardiovascular disease has gone down quite substantially. And that was shown from this study. And it's quite different from the historical view with lung cancer, where it's 10 or 20 years before your risk starts going down towards that of a non-smoker. In cardiovascular disease, because it has to do with blood viscosity as well as the atherosclerosis, you turn the blood viscosity around really quickly. It's a huge health promotion message' (Dobson interview, 2008).

It was somewhat difficult to separate the knowledge production of the main MONICA study and the specific case study grant. Analysis identified seven articles as directly attributable to the grant research. Professor Dobson specifically identified four main articles from the grant research.

The meeting abstract and article by Leitch et al. (1997 and 1998) in the *Journal of the American College of Cardiology*, the latter of which was abstracted in *Cardiology Review* (2000), showed the benefit from beta blockers after myocardial infarction and no effect on the risk of recurrent myocardial infarction and death with the use of calcium antagonists. Comparisons between beta blockers and calcium antagonists favoured beta blockers because of the beneficial effects of beta blockers and not because of adverse effects of calcium antagonists. The group also showed that while an earlier paper by the research team found that greater use of cardioactive drugs in Perth apparently did not result in improved short-term outcome (within the first 28 days), there were long-term benefits, with a relationship found between the lower use of beta blockers in Newcastle compared to Perth and the incidence of reinfarctions. The article 'Outcome with Calcium Channel Antagonists after Myocardial Infarction: a Community Based Study' (Leitch et al., 1998) has been cited 19 times. Professor Dobson added that these papers were important because they involved the clinicians through their own patients and because the results showing the benefit for beta blockers after MI and no effect on risk of recurrent MI or death from the use of calcium antagonists were consistent with data from randomised controlled trials.

Another main publication identified by Professor Dobson was 'Can ECG Changes Predict Long-term Outcome in Patients Admitted to Hospital for Suspected AMI' (Lim et al., 1997), which has been cited five times. Dobson explained that the ECG coding had to be performed for all of the patients, and so, while it was not a primary interest of the research team, the clinicians involved were looking at particular patterns in their patients and looking at an outcome which was of interest to them. She said, 'That was never one of our primary interests, but the point was that we had all the set-ups so they could use it. And this was good for these guys. So even though it was of secondary interest to the research, it was still an important clinical outcome, because it was working with the local clinicians' (Dobson interview, 2008).

One of the PhD students working on the project worked on a simplified version of ECG coding to provide an epidemiology system that could be performed by nurses reliably and validly. She also did a lot of work on prediction modelling. Dobson said, 'The methods she devised were used by the Australian Institute of Health and Welfare to estimate numbers of heart attacks in Australia. You can do it two ways. You can either multiply the number of deaths, assuming the case fatality is the same, or you can multiply the hospital admissions by a number. And nobody has ever bothered to check the numbers again. So they still use all of her calculations to do that' (Dobson interview, 2008).

'Success of Cardiopulmonary Resuscitation after Heart Attack in Hospital and Outside Hospital' by Heller et al. (1995) was another of the main publications from the case study grant. It reported that there was a higher survival rate after cardiopulmonary resuscitation in hospital compared with outside hospital and that the good long-term prognosis for survivors in both settings suggested that attempts to improve the success of cardiopulmonary resuscitation outside the hospital may be worthwhile. The article has been cited 13 times.

Other publications found to be directly attributable to the case study grant included:

- 'A Self-Administered Quality-of-Life Questionnaire after Acute Myocardial Infarction' (Lim et al., 1993), which has been the most cited of the directly attributed publications (46 times) and found that the questionnaire had good potential as an instrument for assessing quality of life in post-acute MI patients and that it can be successfully self-administered.
- 'The Accuracy of Hospital Records and Death Certificates for Acute Myocardial Infarction' (Boyle et al., 1995), which has been cited 19 times and found that, although the mortality data seemed to be quite accurate, the hospital data alone were not accurate enough to be used to estimate rates or trends of heart attacks, with additional data required in order to determine numbers of non-fatal AMIs accurately.
- 'Medical Care and Case Fatality from Myocardial Infarction and Coronary Death in Newcastle and Perth' (Dobson et al., 1993), which has been cited 23 times and found that case fatality (at 28 days from onset of symptoms) was not significantly different between centres and that greater use of cardioactive drugs in Perth apparently did not result in improved short-term outcomes although potential

long-term benefits could not be judged at that stage (these were later found to be significant).

- 'Relationship Between Risk Factor Trends and Disease Trends' (Dobson, 1994),which has been cited nine times and found that risk factors that are associated with disease in individuals also operate at the aggregate level and that useful results about disease in the population can be obtained as long as adequate steps are taken to avoid selection biases and confounding.
- 'Risk of Primary and Recurrent Acute Myocardial Infarction from Lipoprotein(a) in Men and Women' (Kinlay et al, 1996), which was cited 18 times and sought to examine whether lipoprotein(a) concentrates were risk factors for a first acute and recurrent MI. The paper reported that concentrations may be a marker of vascular or tissue injury or may be associated with other genetic or environmental factors that cause AMI and therefore its measurement could not be recommended at that time for assessment of risk for AMI.

Table 5-1 illustrates the publication output attributed to the case study grant application, its impact and the extent of the knowledge diffusion.

Table 5-1 Publication output and impact of directly related publications[4]

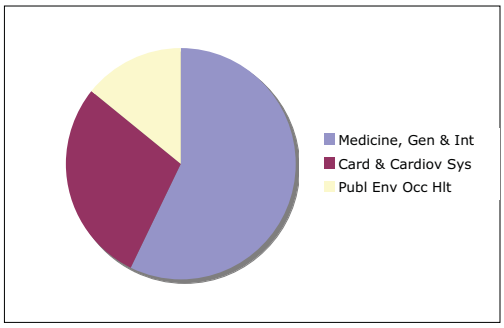
Number of journal articles:	7				
Number of articles included in citation analysis:	7				
Total number of citations (all papers):	132				
Aggregate relative citation impact:	1.24 (Class IV)				
Self-citations:	10%				
Distribution of publications across relative citation impact classes:					
	Class I (uncited)	Class II (>0 and <0.8 citations)	Class III (0.8 to 1.2 citations)	Class IV (1.2 to 2.0 citations)	Class V (>2.0 citations)
Number of publications		4	1	1	1
Proportion of total output		57%	14%	14%	14%
Most highly cited publication[5]:	Chambless, L., U. Keil, A. Dobson, M. Mahonen, K. Kuulasmaa, A.M. Rajakangas, H. Lowel and H. Tunstall-Pedoe for the WHO MONICA Project. 'Population Versus Clinical View of Case Fatality from Acute Coronary Heart Disease: Results from the WHO MONICA Project 1985-1990', *Circulation*, Vol. 96, 1997, pp. 3849–3859				
Times cited:	164				

[4] In addition, 44 publications were indirectly linked to this grant. 32 of these publications were indexed in Web of Science and received 1,050 citations in total, giving a relative citation impact of 2.26. The distribution of publications across relative citation classes from I to V was 4, 12, 3, 7 and 6, while their self-citation rate was 11%.

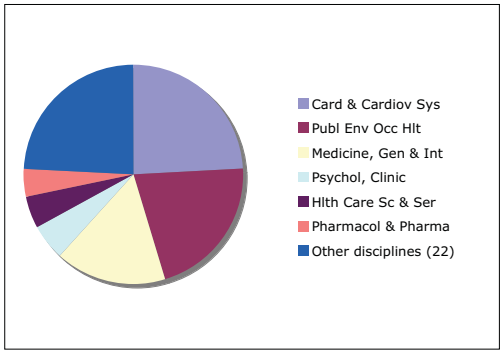
[5] Citation count extracted April 2009.

Figure 5-3 Knowledge diffusion: fields of research of output (a), fields of citing papers (b) and countries of citing papers (c)

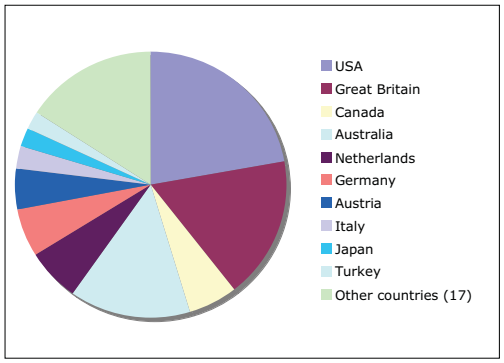
(a)



(b)



(c)



5.7.2 Benefits to future research and research use

Capacity building and career development

It has been difficult to separate the influence and impact of the larger MONICA project and the specific impact of the case study grant.

At interview Professor Dobson indicated a key capacity building outcome was the knowledge and experience obtained on record keeping and data management. This included the wider international influence of the MONICA project and the clinical

involvement as part of the case study grant. She said, 'I think it's made a huge difference in terms of my views about data management' (Dobson interview, 2008).

The most cited of the directly attributed papers mentioned earlier (Lim et al., 1993) found that the questionnaire had good potential as an instrument for assessing quality of life in post-AMI patients and that it can be successfully self-administered. This meant the questionnaire was able to be used with confidence in the research that followed in the project and MONICA study.

The development of the capacity, reputation and historical data in the Hunter region and the associated institutions, such as John Hunter Hospital and the University of Newcastle, in cardiovascular research, the Heart and Stroke Register, epidemiological studies and health promotion continues today. This would largely be attributed to the MONICA project, but the extension of the case study grant has been described in interviews as a valuable contributor.

Professor Dobson explained at interview that many of the research team, including the nurses, project manager, data manager, PhD students and a range of the clinicians, had had no experience with clinical and epidemiology research and were trained from scratch through the MONICA and case study projects.

At a minimum with the changing economic climate in Newcastle (eg with the closing of steel works), it provided gainful employment to people and professional roles that were really important at the time. Dobson said, 'There have been times when the team in Newcastle were the only bread winners. That is still the case and it's much more important in the Australian Longitudinal Study on Women's Health. (Dobson interview, 2008).

An example was one of the PhD students who worked on the case study grant. A quiet person who had finished his last exam for his mathematics degree and came straight into Professor Dobson's office and said he had been working in coal mines for a decade, did not want to go back and so he wanted a job. He was given a job, ended up studying his PhD with Dobson, later worked with Professor Heller at Manchester University in the United Kingdom and eventually ended up as the senior statistician back at the John Hunter Hospital. Dobson said, '[He] would be one of the stars of all of this...from being the coal miner to senior statistician' (Dobson interview, 2008).

Another student completed her PhD on the project, and while it was not the only influence on her career, it was seen as an important part of her early development. She is now a professor at the School of Medicine and Public Health at the University of Newcastle.

Professor Dobson further explained that they were running masters programmes through the University of Newcastle Centre for Clinical Epidemiology and Biostatistics and tried to get as many of the registrars as possible doing advanced training in clinical epidemiology. Dobson said, 'They were our partners in a lot of this, and I still think John Hunter is the best hospital in the country in terms of this clinical epidemiology-oriented research (Dobson interview, 2008).

Similarly, Professor Dobson's career was significantly influenced by the MONICA project, while the case study grant was noted as adding important value and leverage for the second stage of MONICA funding. Dobson went on to be a member of the Advisory Committee

for the National CVD Monitoring Unit at Australian Institute of Health and Welfare since its inception and also Director of the Australian Longitudinal Study on Women's Health.

Targeting future research

The key value from the perspective of Professor Dobson's later research was that it contributed to the development of her track record and expertise in conducting large studies rather than in informing her research agenda and next body of work. For example, the Australian Longitudinal Study on Women's Health at a national level involved recruiting participants, maintaining their interest in the study and linking records from different sources, such as survey data and linkages to Medicare data, to gain insights into various issues in women's health. This is different from MONICA, in which the research nurses went into the hospitals, obtained the medical records and also questioned and measured people participating in the risk factor surveys.

More recently, Professor Dobson is also partly involved in an attempt to set up and run a long-term surveillance of the health of the Australian Defence Force. Like the MONICA project, they have tried to use defence medical records for the study.

However, despite the case study not necessarily informing the research agenda of Professor Dobson, a further 32 publications were identified as following indirectly from the case study grant research, with quite a lot of work on risk factors. Some of the influences on future research from the larger MONICA project and the case study grant research were indicated as being long term and still happening. Dobson said, 'The fact that some of it is still going now in relation to CHF...I thought it was a major thing to say...to me it's completely a new concept that you get the impacts going on for such a huge length of time...it's amazing!' (Dobson interview, 2008).

For example, a few years ago Professor Dobson suggested a study on CHF to an Iranian cardiologist who came to the University of Queensland for his PhD. Drawing on the case study grant research approach, they extracted records on all of the deaths with mention of CHF anywhere on the death certificate, because they knew they were not looking just at the major cause but also the underlying cause. Using data from the MONICA centre in Perth, they subsequently looked at all hospital coding for every one of the MONICA cases to identify the ones who had any sign of heart failure when they had the first MI and determine how many reinfarctions they had later. They also looked at the ones with much less severe disease but who subsequently had admissions for heart failure (ie early onset and late onset). The research found that mortality from CHF is going down in Australia. Dobson regards four papers from this more recent research as directly following on from the case study grant:

- Najafi, F., A. Dobson, M. Hobbs and K. Jamrozik, 'Temporal Trends in the Frequency and Longer-term Outcome of Heart Failure Complicating Myocardial Infarction', *European Journal of Heart Failure*, Vol. 9, 2007, pp. 879–885.
- Najafi, F., A.J. Dobson and K. Jamrozik, 'Is Mortality from Heart Failure Increasing in Australia? An Analysis of Official Data on Mortality for 1997–2003', *Bulletin of the World Health Organisation*, Vol. 84, No. 9, 2006, pp. 722–728.

- Najafi, F., A.J. Dobson and K. Jamrozik, 'Recent Changes in Admissions to Hospital with Heart Failure in Australia', *European Journal of Heart Failure*, Vol. 9, 2007, pp. 228–231.
- Najafi F., K. Jamrozik and A. Dobson, 'Understanding the 'Epidemic of Heart Failure': a Systematic Review of Trends in Determinants of Heart Failure', *European Journal of Heart Failure*, Vol. 11. No. 5, 2009, pp. 472–479.

Dobson said, 'This has all been published in the last year or so. What we've shown in those studies is that in any particular age and sex group congestive heart failure is going down, but the driver is the increasing number of old people...it's only a short matter of time before the cardiologists' claim of more cases of congestive heart failure will actually be true. At the moment we suspect that the rates are not going up, but we suspect that the myocardial infractions and (acute coronary) syndromes are going down, and as a result congestive heart failure is becoming a higher proportion of their work and they have the impression that it's going up. Eventually it will go up, I suspect, because of the ageing population. So it's really interesting, because all of that is what we hypothesised back in this [case study] grant' (Dobson interview, 2008).

Another example provided by Professor Dobson of follow-on research was to do with exploring the seasonal variations in heart attack in Australia. In running the MONICA project and the case study grant research they knew that the nurses were flat out in winter and had less to do in summer and decided to try to account for it in the statistical analysis. Dobson received a small NMHRC grant to do the study and arranged for a postdoctoral fellow to work on it. She said, 'Very quickly [the postdoctoral fellow] got hold of the international data as well and started looking at seasonality, and we know quite a lot about seasonality in blood pressure. He has published extensively on the effect of seasonality using the international data. The seasonal variation in incidence and mortality differs a lot between countries, but the other thing that he found was a Monday effect...an awful lot of heart attacks on Mondays...it's quite a substantial effect [however, Dobson explained that there is some question as to how much is to do with the quality of the data collection in some countries, the fact that people go in on Saturday and Sunday and the hospitals don't do the admission updates until Monday or if it is in fact to do with things like binge drinking on weekends as published by the Augsburg collaborators]' (Dobson interview, 2008).

There was also a subsequent article published on alcohol and the U-shape effect (McElduff and Dobson, 1997). It showed essentially a small amount of alcohol is a good thing and became part of a big body of evidence.

In terms of research today, Professor Dobson explained that the case study research would not be undertaken in the same way as it was during the MONICA era because the diagnostic methods would be different and the questions to be asked would also be different. Dobson added that the National Cardiovascular Monitoring Unit at the Australian Institute of Health and Welfare is the direct descendant of the MONICA project and the case study grant in Australia. She said, 'We had 10 years of research, we knew how to do these things and we knew what the main questions were. Now it is turned over to a semi-government organisation to continue the monitoring but not with

registration – and that is why I am still on that committee, because I think it's important' (Dobson interview, 2008).

5.8 Interface B – dissemination

The key dissemination vehicles for the study's findings were publications, as identified in Section 5.7.1. Although there was very limited dissemination via conferences, there was dissemination through meetings and with collaborators at the national level and extensive dissemination at a local Hunter region level with the clinicians, patients, community and community groups. Professor Dobson provided the following example of dissemination at the local level: 'Cessnock is probably the best example. We were lucky that a new mayor had been elected...she had a much bigger vision...she would encourage us to do things and she would invite us to activities. And then you get people like the school teachers, who were really keen on changing conditions, the local doctors...and then later on some of the Newcastle graduates also went there once Newcastle [University] started producing the medical graduates. We also had an opportunity with one of our research students...who was a cardiologist, and the first visiting cardiologist...he actually went to Cessnock instead of telling the Cessnock people to come to him – that was unusual' (Dobson interview, 2008).

It was explained at interviews that the people, particularly in the coalfields areas, created opportunities for the research and health-promotion team from the university, who, in turn, made sure that they always took those opportunities. Women tended to be particularly supportive. The research and health-promotion teams undertook extensive prevention and sociological work, particularly with schools 'about how you could change the diet, given the diet was a thing determined by the extended family, you almost had to influence the grandmothers to do anything...their Scottish diet...and the thing about never leaving any food...they ate enormous meals. And by this time...a lot of [the miners] were working in the upper Hunter [region] in open-cut mines, just driving back and forwards' (Dobson interview, 2008).

There was also dissemination through the media and particularly through the local media. Professor Dobson advised that she received valuable guidance and advice on her interactions with the media from the Medical Advisor of the NHF at the time. This included focussing on how people can be proactive and what they can do for themselves to gain control of their blood pressure and cholesterol, etc, rather than just relying on medical treatment. Although there were some examples of difficult experiences with the media, Dobson found that it was valuable in generating more interest in the research and the associated health promotion. She said, '[I learnt] that any media interview is a health promotion opportunity and not to get bogged down in things I don't know about' (Dobson interview, 2008).

5.9 Stage 4 – secondary outputs

5.9.1 Policy/product impacts

The main message from Professor Dobson, other interviews and literature review was that the impact of the case study grant data was on health promotion (particularly in the local area) and as part of a larger body of evidence rather than any direct policy or product impacts. For example, later work on passive smoking leading from the MONICA project and the case study grant was used heavily, with one of the recommendations being a smoke-free Sydney Olympics and no smoking in cars with children (which has started to be introduced in different jurisdictions across Australia). Dobson said, 'Oh yeah, we used it [the MONICA and case study data] as hard as we could on all the smoking stuff...[and] we have used the Australian Longitudinal Study on Women's Health for anti-smoking as well...[It was part of a] larger body of evidence, which was an important base for our health promotion work around anti-smoking and Quit, and then also part of the evidence base used around advocacy to ban smoking in public places, etc' (Dobson interview, 2008).

5.10 Stage 5 – adoption by practice and public

As expressed by Professor Dobson, it has been difficult to establish definitively if the outcomes from the case study grant directly impacted on the practices and guidelines used by clinicians and other health professionals in the area beyond the fact that it clearly contributed to a wider body of evidence, including specific clinical trials in relation to the use of beta blockers. Dobson said, 'Because mainly the guidelines come from trials...we don't know how much change actually occurred [directly from the grant study]. It's really, really difficult. And I don't think that one study in this type of work has that sort of direct impact…it's like prevention…they all just add together' (Dobson interview, 2008).

The more direct impacts were with the clinicians working with the research and the health-promotion team and their work with the Hunter community and the local patient and advocacy groups.

As noted earlier, the fact that some of the articles by clinicians using their own patient data are cited as project outputs demonstrates that there were some downstream influences on clinical practice stemming from the project. Dobson said, 'But that was from their own data, their own patients, so it's sort of small-scale influence on practice one would hope for. Often epidemiological studies that have impact [are] where you are working with clinicians' (Dobson interview, 2008).

Professor Dobson believed that the case study grant did have impact through the health-promotion activity on the way that patients became more aware of and responsible for management of their own care. This occurred particularly through patient groups and patient advocacy groups, who she believed were very good at disseminating and translating the results into patient education and changes in behaviour. As a result the research team and students would put a lot of work and time into these groups and their activities and events.

Less clear was the impact of the research and the associated health promotion on influencing public attitude towards medical research. Dobson said, 'It's difficult to know.

The public in general, I suspect not, but [I believe] the people who were closest to those who were impacted in some way through families [would have been influenced]' (Dobson interview, 2008).

Interviews for this case study suggested that the close work with patients and the community did have an impact on the research team and their approach to future work and links to health promotion.

5.11 Stage 6 – final outcomes

The study published in 1999 (Dobson et al., 1999) suggests that MONICA and the case study grant may have had some impact on wider health benefits in the Hunter region. The study specifically found that rates of death and non-fatal MI had declined, but there was an increase in hospital admissions for prolonged chest pain. Reductions in cigarette smoking, diastolic blood pressure and total cholesterol and increased use of aspirin fully explained the 3.3% (95% confidence interval [CI] 2.4, 4.2) average annual reduction in rates of major coronary events for men and the 4.1% (95% CI 2.7, 5.5) reduction for women. In contrast, increased use of aspirin, beta blockers, fibrinolytic therapy and angiotensin-converting enzyme inhibitors explains less than half of the 8.9% (95% CI 5.9, 11.8) and 6.9% (95% CI 2.7, 10.9) average annual reduction in rates of case fatality in hospital for men and women, respectively. These trends suggested a decline in severity of CHD consistent with reductions in risk-factor levels and improved acute medical treatment.

It is difficult to attribute wider economic benefits to MONICA and specifically the case study research, other than the creation of the new area of employment in epidemiology studies and related activities identified earlier in this paper within the changing and difficult employment environment of the Hunter region and the possible impact on the awareness and attitudes to CVD in the Hunter area.

5.12 Summary of case study impacts

The payback framework describes the process from research to several categories of impact. The categories allow us to compare across different case studies. Table 5-2 shows, in point form and by impact category, some of the impacts, described more fully above, that have emerged from this grant.

Table 5-2 Payback

Payback category	Impacts from case study
Knowledge production	• Clinically relevant outcome data after MI linked to the wider MONICA study and seven peer-reviewed papers

Benefits to future research and research use	• Training of PhD students and a number of clinicians/cardiologists collaborating and as authors to publications from the research
	• Expanded population data for future research studies
	• As part of the wider MONICA impacts:
	  • Development of expertise in epidemiology at John Hunter and helped establish its research credentials in this area
	  • Development of further research questions used to establish Professor Dobson's career and the careers of her students and collaborators
	  • Data management skills for large population data sets
	  • AIHW surveillance (including involvement in committees)

Policy/products impacts	• As part of the wider MONICA impacts it has:
	  • informed health promotion, particularly in the local Hunter region
	  • indirectly and as part of a wider body of evidence led to influencing tobacco control (passive smoking) and health messages on alcohol drinking
	  • led to training of clinicians and other health professionals

Health and health sector benefits	• As part of the wider MONICA impacts it has supported:
	  • patient and patient advocacy groups and their rehabilitation and health promotion activities
	  • a decline in severity of coronary heart disease in the Hunter region

Broader social and economic benefits	• As part of the wider MONICA impacts it has supported: • creation of new area of employment for the Hunter area and the possible impact on the awareness and attitudes to cardiovascular disease

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CHAPTER 6 Coronary lesions and vasoactivity

6.1 Overview of case study grant

The grant of interest to this case study, titled 'Coronary Lesions and Vasoactivity' was funded from July 1990 to June 1992 for a total amount of Can$69,000 by the Heart and Stroke Foundation of Canada (HSFC) and was a renewal of previous funding. The research team used mature, spawning steelhead trout as a model to examine how coronary vasoactivity is modified by intimal proliferation of vascular smooth muscle. The research team felt it interesting to use a fish model, as omega-3 fatty acids had been linked with improved vascular health, yet migratory salmon, which naturally possess a high level of omega-3 fatty acids, also naturally develop severe coronary lesions. The team wanted to determine the specific consequences of myointimal proliferation on vasoactivity and to examine whether coronary lesions that develop in the presence of naturally high levels of omega-3 fatty acids produce unusual coronary vasoactivity. Through this work, the team tested the impact of different vasodilators and vasoconstrictors on the ability of the vessels within fish hearts to contract and relax and concluded that the lesions had no significant effect on the vessels' response to vasoactive substances.

This work was led by Dr Anthony Farrell, a professor of biological sciences at Simon Fraser University.

6.2 Introduction to case study

Interruptions of coronary blood flow from atherosclerosis can lead to myocardial infarction and potentially to death. The reduction in coronary flow associated with myocardial infarction is precipitated by either severe occlusion or arterial vasospasm. Vascular injury and atherosclerosis increase the probability of vascular spasm, which can then close the main coronary artery and precipitate a heart attack. However, the underlying mechanisms behind vascular tone in the coronary artery were unknown.

Diets rich in fish and fish oils (and therefore omega-3 fatty acids) have been associated with a reduced risk of cardiovascular disease and atherosclerosis (Phillipson et al., 1985). Salmon have high levels of omega-3 fatty acids, yet migratory salmonids have a natural propensity to spontaneous and continuous formation of lesions in their main coronary artery (Farrell et al., 1990). Mature, spawning steelhead trout generally have moderate to severe lesions along almost 95 percent of the length of the main coronary artery. Thus, any

section of the coronary artery, called a vascular ring throughout this report, would more than likely contain lesions. These lesions are multifocal myointimal proliferations that resemble the early stages of coronary atherosclerosis in mammals (House & Benditt, 1981). In humans, such lesions represent a diseased state. In salmon, however, whether this is a diseased state at a population prevalence typically greater than 90 percent has been questioned. Nevertheless, coronary lesions are at their most severe when salmon are making their most athletic efforts during their spawning migration up river.

At the time of this proposal, the only studies of vasoactive mechanisms in the coronary artery of fish had been performed in the laboratory of the principal investigator (PI). Previous work conducted by a member of the research team, Susan Small, under the supervision of Dr Farrell, had evaluated what was currently known about coronary arteriosclerotic lesions in salmonids and presented the following findings. Vascular injury to the coronary artery, as a result of the bulbus arteriosus being excessively distended, was believed to be an initiating mechanism for formation of coronary lesions, possibly explaining why severe lesions are restricted primarily to the main coronary artery. The study presented evidence that coronary arteriosclerosis in salmonids develops in immature fish well before maturation and progresses with age. Growth and growth rate were implicated in progression of lesions. Dietary factors, especially polyunsaturated fatty acids (and their metabolites), can significantly stimulate vascular smooth muscle proliferation in the coronary artery of salmon, but a possible link with the progression of coronary lesions had yet to be studied. Whether coronary lesions negatively impact on blood flow to the salmon heart had not been studied properly. Nevertheless, the blood supply to the heart was concluded to have functional importance when salmon exercise, and the coronary flow reserve[1] may be reached when fish swim under mild hypoxic conditions. The paper thus concluded that if coronary arterial lesions adversely affected coronary blood flow, the selective effects would be most prominent in years when upstream migration conditions are particularly severe (Farrell, 2002).

This basic science project, titled 'Coronary Lesions and Vasoactivity', was funded from 1990 to 1992 by the HSFC and examined how coronary vasoactivity is modified by intimal proliferation of vascular smooth muscle.[2] This grant was a renewal and the proposed studies were thus an extension of those in progress.

The work performed leading up to this application provided preliminary evidence for certain vasoactive mechanisms that may be significantly affected by coronary lesions and/or omega-3 fatty acids. The work proposed in the application in 1990 was meant to follow up on these findings and examine the mechanisms in greater detail. In-vivo studies were performed to confirm that the in-vitro mechanisms would have in-vivo significance (Farrell, 1990). Coronary flow for the in-vivo experiments was monitored during drug injections. The selection of drugs was based on the data obtained from the in-vitro studies.

[1] The coronary reserve represents the capacity of the coronary circulation to dilate following an increase in myocardial metabolic demands and can be expressed by the difference between the hyperaemic flow and the resting flow curve.

[2] Intimal smooth muscle cells protect against plaque rupture and therefore the thrombotic consequences of atherosclerosis (Warrell et al., 2005).

The high prevalence of coronary lesions in salmon was viewed as an asset in studying the vascular rings, in that most vascular rings would contain lesions. In addition, the coronary artery of a fish is relatively long and undivided, so several ring preparations could be made from one animal. The proliferation of intimal vascular smooth muscle found in trout lesions is organised in a similar manner to that in mammalian lesions, except that the lesions are devoid of lipid and calcium deposits. This meant that researchers could ascertain for the first time how intimal vascular smooth muscle affects vasoactivity without the confounding influence of the other lesion components typically found in mammalian arteriosclerosis.

The main objective of this research was to establish the detailed consequences of myointimal proliferation on vasoactivity. Specifically, the team wanted to better understand how mechanisms for controlling the diameter of the arteriosclerotic coronary artery are affected by coronary lesions in the presence of high omega-3 polyunsaturated fatty acids. The team also examined whether coronary lesions that develop in the presence of naturally high levels of fatty acids produce unusual coronary vasoactivity.

6.2.1 The case study approach

The findings presented in this case study are based on a combination of: three face-to-face interviews, including Dr Farrell (the PI), Dr Small (a former M.Sc. student who conducted the preliminary studies under Farrell's supervision) and Mr Johansen (the technician at the time); a review of the PI's curriculum vitae; a review of relevant and available administrative documents from the HSFC; and documentary analysis of the scientific literature and bibliometric analysis.

6.3 Stage 0 – topic/issue identification

At the time of the grant proposal, Dr Farrell was an associate professor in the Department of Biological Sciences at Simon Fraser University in British Columbia. He has a doctoral degree (PhD; 1979 – Zoology) from the University of British Columbia.

The idea for this research originated from the following three factors:

1. The PI's previous research and the scientific literature
2. Belief in the benefits of non-mammalian models
3. Gaps in the knowledge base.

6.3.1 Findings in the scientific literature

The idea of studying fish and lesions originated from an article Dr Farrell had read in *Science*, which was written by Robert L. Van Citters and Nolan W. Watson, both from the Department of Physiology and Biophysics and Regional Primate Research Center at the University of Washington School of Medicine in Seattle (Van Citters and Watson, 1968). Published in 1968, the article claimed that 'coronary degeneration was absent in young trout taken in fresh water and rare in immature fish at sea, but the incidence and severity were sharply greater in migrating fish and almost uniformly present in spawning fish. Several fish taken after they had re-entered salt water after spawning had no lesions; lesions in fish taken during their second spawning migration were not cumulative. These facts

suggest that the process is reversible,' thereby meaning that the lesions did regress by some natural process.

Upon reading the article, Dr Farrell phoned Van Citters, who was then the former Dean of Medicine at Washington University, to determine what else he knew. Farrell also asked Van Citters' thoughts about some of the data he had been collecting and his understanding of fish physiology and lesions. The two continued to keep in touch until recently, sharing data and discussing ideas.

6.3.2 Belief in non-mammalian models

Dr Farrell believes that fish can be viewed as the most basic model of the vertebrate circulatory system, including the coronary circulation. The coronary circulatory system seen in mammals is more complex. Many functional similarities exist at the qualitative level in the cardiac physiology of fish and mammals, despite the overt differences in gross anatomy and maximum performance. These similarities include intrinsic, humoral and neural controls of cardiac function (Farrell et al., 1984; Farrell, 1985; and Farrell and Graham, 1986). Farrell suggested that the fish model is like a Model T Ford, while a human model is more like a Lexus. The fundamentals are the same – that is, both have four wheels and a driver's seat – but the Lexus has many more nuances to make it a faster, more comfortable vehicle. He believes that for anyone to understand and be able to repair or diagnose a Lexus, they should first understand the basics, and this is the conceptual value he sees in understanding the mechanisms of fish physiology.

During our interview, Dr Farrell said that it was his stubbornness and insistence that the biomedical world can learn from non-mammalian models that led him to pursue this research (Farrell interview, 2008). There is a vast array of non-mammalian models, and Farrell chose his fish models because he thought there was a lot to be gained from them and he 'wanted to prove it', stating it was a 'personal challenge'. Farrell wrote in his 1990 application that his 'career goal [was] to promote and perform research on cardiovascular systems in lower vertebrates' (Farrell, 1990). He still feels that the scientific community has much to learn from these models, but people have to agree to fund this type of work.

6.3.3 Gaps in knowledge

The aetiology of coronary lesions in salmonids was not well understood. Maturation, sex hormones, high levels of blood cholesterol and low-density lipoprotein (LDL) and diet had been implicated as factors that promote development of lesions (Farrell and Graham, 1986; Eaton et al., 1984; and House et al., 1979), however a primary factor had not been established. A clear correlation exists between growth and lesion progression in wild and cultured Atlantic salmon, with lesions accumulating more rapidly with an accelerated growth rate. The PI believed that the growth-related formation of lesions in salmonids was reflective of an ongoing response to vascular injury. This is of relevance to the grant proposal, as coronary spasm is enhanced by vascular injury in mammals (Ku, 1982).

6.4 Interface A – Project specification and selection

As described in the application, this study set out to examine how coronary vasoactivity is modified by intimal proliferation of vascular smooth muscle (Application, 1990). Mature,

spawning steelhead trout were used as a model because they naturally develop severe myointimal lesions. As these lesions are devoid of cholesterol deposits, the team believed they could ascertain for the first time how intimal vascular smooth muscle affects vasoactivity without the confounding influence of the other lesion components typically found in mammalian arteriosclerosis.

Rainbow trout were used as a natural control because they possess few coronary lesions and have lower levels of omega-3 fatty acids and higher levels of omega-6 fatty acids than steelhead trout. The research team's proposed study represented the first concerted effort to investigate the fish coronary artery at a comparative level. These studies were intended to provide valuable basic information on coronary vasoactivity mechanisms to the biomedical community, including new information on the interactions between arteriosclerosis, omega-3 fatty acids and control of the coronary artery. This project was a pioneering investigation using fish to provide insight in relation to the question 'Do coronary lesions that develop in the presence of naturally high levels of omega-3 fatty acids produce abnormal coronary vasoactivity?' The study was both timely and relevant given interest in the involvement of atherosclerosis in coronary vasoactivity and the therapeutic benefits of omega-3 fatty acids.

The primary experimental approach was to measure isometric tension produced by isolated vessel rings from the coronary artery:

1. to establish the specific consequences of myointimal proliferation on vasoactivity (this is important given the central dogma that vascular repair following injury inevitably involves myointimal proliferation)
2. to examine whether coronary lesions that develop in the presence of naturally high level of omega-3 fatty acids produce unusual coronary activity.

The experimental approach to measure isometric tension produced by isolated vessel rings was deemed adequate for studies of vasomotion because it is sensitive and less costly in terms of expensive drugs (the organ bath requires only small additions of the drugs). The vascular ring technique was already in use in Dr Farrell's laboratory. Jeff Johansen said that Farrell was an expert in these techniques when he came to the laboratory in 1990 and that he learned the methods from him.

Susan Small had initially learned the technique of measuring isometric tension in isolated vessel rings after being trained in 1986 in Los Angeles by a researcher[3] who was performing studies on rabbit arteries. After learning and applying the procedure to rabbit arteries, Small then had to scale down the technique for use on the smaller coronary artery of fish. This involved very fine dissections, specialised surgical techniques and manipulation of tissues. She recalled that it was very finicky work.

One series of experiments examined whether endothelium-derived relaxation factors (EDRFs) were involved in modulating vasoactivity via the actions of acetylcholine and purinergic agents such as adenosine-5'-triphosphate (ATP) and adenosine diphosphate (ADP). Attention was focussed on whether or not lesions attenuated the effects of EDRFs. Comparisons were made between paired control rings in which the endothelium was

[3] Neither Small or Farrell could recall the name of the individual who trained Small.

present and rings with the endothelium removed to determine whether the presence of coronary lesions attenuates the effects of EDRFs.

A second series of experiments involving vascular rings was intended to examine the vasoactive responses to various prostanoids, leukotrienes and thromboxanes. The interest in these autacoids was twofold. First, based on the data obtained to that point, autacoids such as prostaglandin PGF_{2A} and endothelin exerted more powerful effects and therefore were possibly more important than other humoral and neural agents previously tested by the research team. Second, the biosynthesis of these autacoids is altered considerably by omega-3 fatty acids, as eicosapentanoate displaces arachidonic acid in the biosynthetic pathway. The team thus expected to observe differences in the vasoactive effects of these autacoids between steelhead trout and rainbow trout. Cumulative dose–response curves were generated and compared for the following conditions: in the presence of agonist alone, after precontraction and after pretreatment with methylene blue.

The team recognised the limitations involved in extrapolating in-vitro studies to the whole animal. It was for this reason that they devised a second experimental approach to test for in-vivo effects of selected compounds (acetylcholine, adenosine, ATP and ADP) on the rate of coronary blood flow. Coronary blood flow was to be measured using Doppler flow probes after injection of drugs selected on the basis of the in-vitro work. This aspect of the work was to allow the research team to confirm that in-vitro effects do have in-vivo relevance. Coronary blood flow had never previously been measured in vivo in fish.

Criticisms from the grant review committee revolved around questions regarding the relationship between lesions observed in fish and lesions seen in higher mammals. There were no concerns about the budget or ethics. One reviewer said 'the applicant has chosen an interesting model to examine a particularly important phenomenon. He has considerable experience working with the techniques and the experiments should contribute important new knowledge regarding the pathophysiology of coronary lesion as well as their vascular reactivity' (Heart and Stroke Foundation of Canada, 1990).

Dr Farrell recalled that the rebuttal process at the HSFC was fantastic, saying that 'it put a human element into grant writing'. The process allows researchers an opportunity to explain something that may have been misunderstood in the original proposal. Researchers are able to answer questions to explain whether a minor mistake led the reviewer astray or whether something fundamental needs to be reconsidered. The former can be the difference between funding being awarded or not. Dr Farrell claimed that the rebuttal letter gave him a sense of what the reviewers needed to know or may not have understood. However, Dr Farrell also recalled feeling, from the comments of the review committee about this grant, and throughout his career, that the reviewers may have had difficulty understanding the relevance of the fish model to research into human disease.

6.5 Stage 1 – Inputs to research

6.5.1 Funding

The team said that they ran this project on a minimal budget, which was deliberately frugal because of the challenges faced when obtaining funding for research using a non-

conventional, non-mammalian model. Dr Farrell recognised that it was risky for the HSFC to fund as it was not typical mammalian research. Farrell therefore did not want to ask for too much and survived by scaling down their original intentions. Johansen, who was a technician in the laboratory, referred to this work as 'groundbreaking' at the time, continuing to explain that this work had a lot of merit and that is why they were funded.

This two-year grant, funded by the Heart and Stroke Foundation, was a renewal. The amounts requested, as per the grant application are shown in Table 6-1 and the costs in Table 6-2.

Table 6-1 Funding Amouts Requested

Year	Amount (Can$)
July 1990–June 1991	49,150
July 1991–June 1992	49,650
Total funding requested	98,800

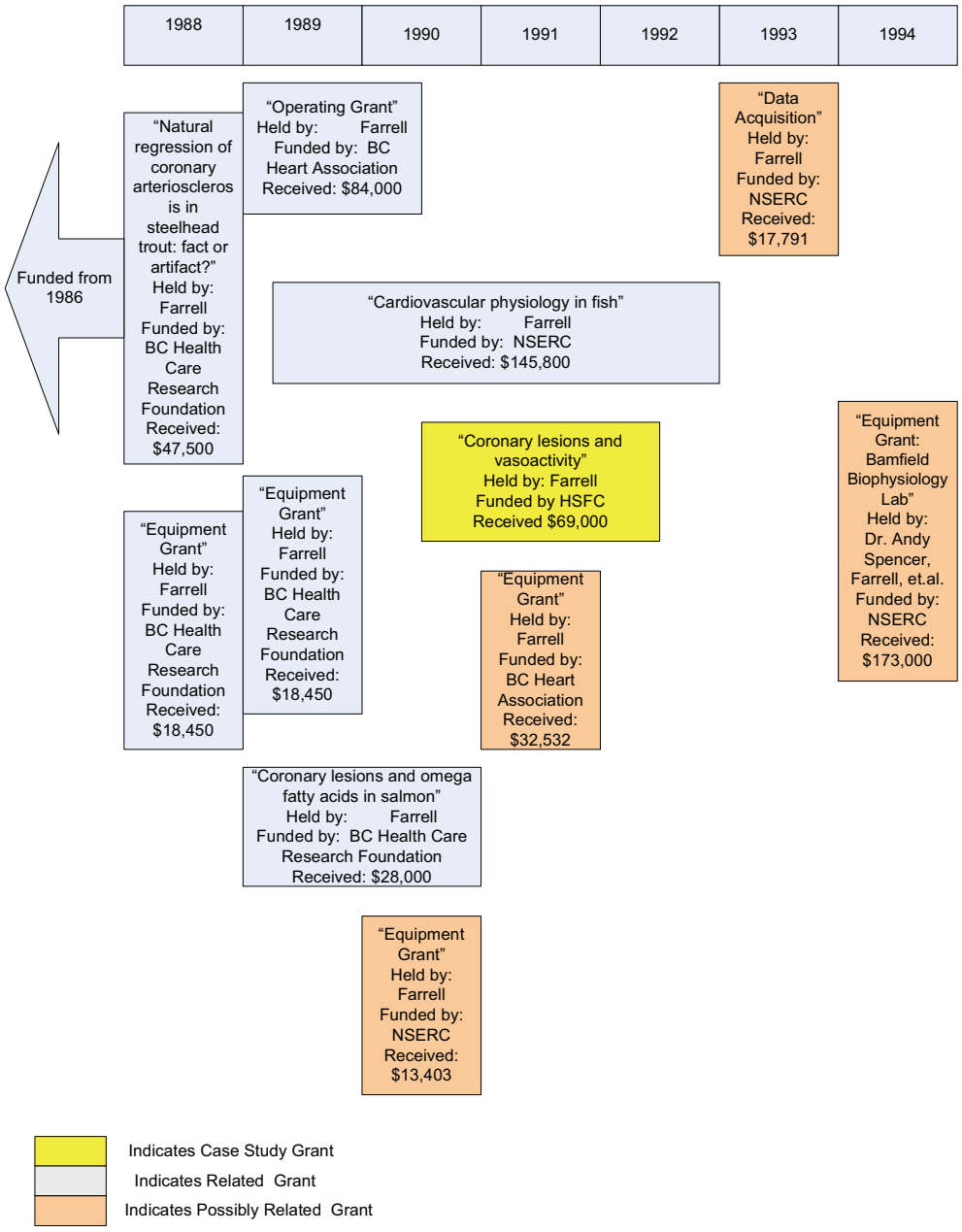
Table 6-2 Costs

Summary	1990–1991 (Can$)	1991–1992 (Can$)
Technician (salary and benefits)	31,500	33,075
Equipment	4,450	1,875
Experimental animals (150 steelhead trout per annum and 50 rainbow trout per annum)	1,700	1,700
Materials and supplies	7,600	9,100
Other (included costs for two publications per annum, office supplies and one conference per annum)	3,900	3,900
Total	**49,150**	**49,650**

The team did not receive the amounts requested: their budget was reduced to approximately Can$46,000 for the first year and Can$23,000 for the second year. Dr Farrell said that he was grateful for the funding he did receive and that 'without this funding [he and his team] would not have been able to afford this research' (Farrell interview, 2008). With this grant money, the team bought a Gould isometric force transducer, one organ chamber, micrometer and stand, and two pressure transducers. Materials and supplies included vasoactive agents, dissecting instruments and general supplies such as pipettes, gases and computer supplies.

Dr Farrell was also funded by the Natural Sciences and Engineering Research Council of Canada (NSERC) and the BC Health Care Research Foundation, although it was made explicit in the grant application, and again by the PI during our interview, that no overlap existed between the projects and that only funding he received from the HSFC was used to meet the objectives identified in the application for the case study grant (Farrell interview, 2008). Figure 6-1 represents the related funding Dr Farrell had in his laboratory over a period of time plus and minus a two-year window surrounding the case study grant. It should be noted that Dr Farrell had other funding, not related to cardiovascular disease, during this time. The data for the figure comes from the PI's curriculum vitae and the HSFC and Canadian Institutes of Health Research (CIHR) databases.

Figure 6-1 Related funding from 1988 to 1994.[4]



6.5.2 Facilities

Dr Farrell said that he had been head hunted by Simon Fraser University (SFU) in the late 1980s, where he continued to work for 20 years until he moved to the University of British Columbia (UBC) (Farrell interview, 2008). Farrell claimed that the space and

[4]Grants titled 'Matching Grant', 'Supplemental Grant', 'Research Grant' and 'Operating Grant' (other than the Operating Grants funded from the BC Heart Association and the BC Health Care Research Foundation) were not included in the chart, although it is recognised that these funds could have also helped related projects. Refer to Appendix 1 for a full list of all funding within the PI's laboratory from 1988 to 1994.

facilities were fantastic and that SFU was 'very generous and very supportive of an ambitious young buck'. He was able to find whatever he needed and did not have in his own laboratory via his colleagues. He did not have to share laboratory space and spoke of the importance of the Alcan Aquatic Research Facility, which allowed him to work with the large salmonids.

6.5.3 Collaborations

Dr Farrell referred to Glen Tibbits as a collaborator and mentor. He provided guidance to the PI on his grant applications, as Tibbits was well funded by the HSFC. As a colleague at SFU, Tibbits was one of the few people with whom Dr Farrell could discuss the details of his projects.

Another key collaboration was with the Robertson Creek Hatchery in Port Alberni, British Columbia. The team received 150 mature steelhead trout per annum for free from the hatchery via an ongoing agreement between the manager of the hatchery and the Provincial Fisheries and Wildlife Department. Farrell's team had to pay a cost of Can$750 to have the fish transported from Port Alberni to where they were held at SFU's aquatic facility. The team also used 50 rainbow trout per annum at a cost of Can$20 each, including purchase, transportation and maintenance.

In addition to the collaboration with Robertson Fish Hatchery, the team also obtained fish from the First Nations people, who would catch fish for the team when allowed to keep a portion of the catch. Both of these collaborations saved the team thousands of dollars by supplying the animals free of charge.

6.5.4 Research team

Reviewers referred to Dr Farrell as 'an extremely knowledgeable and productive investigator' (Scientific Review Committee Report, 1990). At the time of the proposal Farrell had published 63 full papers in peer-reviewed scientific journals, along with three abstracts. Farrell referred to himself as a very dexterous and good surgeon. He also claims to be able to visualise things surgically and that these specialised skills allowed him to conduct this research.

Susan Small completed her M.Sc. with Dr. Farrell in 1988. At the time of the case study grant, she was pursuing a Master of Science in Communicative Disorders, specializing in Audiology, which she completed in 1991. She completed her PhD in 2007 and is currently an Assistant Professor in the School of Audiology and Speech Sciences at the University of British Columbia. Dr. Farrell referred to Small as a very bright student.

Jeff Johansen was a full-time technician. He had previously graduated from SFU with a Bachelor of Science in biology and worked in Dr Farrell's laboratory for three years after graduation. At the time of the grant submission, Johansen had produced four papers and three abstracts. Farrell described Jeff as his right-hand person, who ran the laboratory and who continued on with the experiments after Small had left for the period 1988 to 1992. The research team also included Bang Q. Gong, who was finishing his PhD and was partially funded by the grant from BC Health Care Research Foundation to study a related project on how lesions develop in salmon.

Dr Farrell acknowledged his colleagues and teammates as critical to his successes.

6.5.5 Other facilitators/barriers

The PI indicated that the main barrier to doing his work and promoting his research is a lack of connection with clinical scientists and health researchers. Dr Farrell mentioned that he was isolated as a fish physiologist at SFU and says he did not know how to make the necessary bridges or network outside of his field other than his connections with Van Citters, who was interested in his work, and Tibbits, a close colleague. He believes that he would have no difficulties convincing clinical researchers of the validity and relevance of his fish models had he been working in a more clinical environment rather than a biological environment.

6.6 Stage 3 – Primary outputs from research

The long-term goals for this research team were to determine whether coronary lesions in salmonids are primarily a response to vascular injury and whether the naturally high levels of omega-3 fatty acids limit the intimal proliferation of vascular smooth muscle.

The team showed that the lesions had no significant effect on the ability of the heart vessels to contract and relax. In other words, in a basic atherosclerosis model, the contractility did not change.

The team did not complete the in-vitro piece with this grant funding as originally proposed. Dr Farrell said that the proposed methods should have been adequate but that the team had underestimated certain challenges. The in-vitro application was to perfuse or measure the coronary blood flow, which required a surgical technical precision beyond that of most members of the team. Farrell's team later accomplished these studies through subsequent grants and, to date, members of Farrell's subsequent research team are the only people who have ever measured coronary blood flow in unanaesthetised fish.

The primary outputs from the project can be broken down into three categories: knowledge production, benefits to future research and benefits to the researchers. These are all immediate outputs of the research.

6.6.1 Knowledge production

In original discussions with the PI, Dr Farrell identified the following publications as directly related to the case study grant and HSFC funding:

1. Gong, B.Q. and A.P. Farrell, 'A Method of Culturing Coronary Artery Explants for Measuring Vascular Smooth Muscle Proliferation in Rainbow Trout', *Canadian Journal of Zoology*, Vol. 73, 1995, pp. 623–631.
2. Farrell, A.P. and J.A. Johansen, 'Vasoactivity of the Coronary Artery of Rainbow Trout, Steelhead Trout and Dogfish: Lack of Support for Non-Prostanoid Endothelium-Derived Relaxation Factors', *Canadian Journal of Zoology*, Vol. 73, 1995, pp. 1899–1911.
3. Gong, B.Q., A.P. Farrell., A. Kiessling and D. Higgs, 'Coronary Vascular Smooth Muscle Responses to Swimming Challenges in Juvenile Salmonid Fish', *Canadian Journal of Fisheries and Aquatic Sciences*, Vol. 53, 1996, pp. 368–371.
4. Gong, B., R. Townley and A.P. Farrell, 'Effects of Polyunsaturated Fatty Acids and Some of Their Metabolites on Mitotic Activity of Vascular Smooth Muscle

Explants from the Coronary Artery of Rainbow Trout (*Oncorhynchus mykiss*)', *Canadian Journal of Zoology*, Vol. 75, 1997, pp. 80–86.

The first paper described the method for culturing coronary artery explants for measuring vascular smooth muscle growth in rainbow trout (Gong et al, 1995). A standardised method of in-vitro [3H]-thymidine incorporation during the S phase of cell division was developed to study vascular smooth muscle proliferation in the coronary artery of rainbow trout. The team established a reliable medium system, an optimum dose of [3H]-thymidine and optimum incubation conditions. Incorporation of the radiolabel into vascular smooth muscle nuclei was confirmed with autoradiography. To test the sensitivity of the assay system, the coronary artery of anaesthetised rainbow trout was surgically exposed and gently rubbed. Following a one-, two-, or three-day recovery period, fish were sacrificed and the coronary artery explants cultured with [3H]-thymidine. Gentle rubbing of the coronary artery in vivo resulted in a significant increase in [3H]-thymidine incorporation into the coronary artery explant compared with sham-operated and untreated control groups of fish. Peak incorporation of [3H]-thymidine occurred at day 2 in the treated group, when incorporation was three times that in the sham-operated group. The team believed the technique had potential application in the study of coronary arteriosclerosis in salmonids, in which vascular smooth muscle proliferation is a primary event. Farrell claimed that at the time of publication there was not much interest in this paper, however science has now evolved to show the same outcome in humans.

The team then surveyed and compared vasoactive responses of isolated coronary vessels from steelhead trout, rainbow trout and spiny dogfish (Farrell and Johansen, 1995). The purpose of the investigation was twofold: to identify vasoactive controls that were possibly mediated by the vascular endothelium and to highlight the possible consequences on vasoactivity of the coronary lesions known to be present in the main coronary artery of salmonids but not dogfish. The team tested various substances and found that acetylcholine, adenosine, ATP, ADP and prostaglandin $F_2\alpha$ typically produced contractions. Use of endothelial removal techniques and antagonists failed to reveal any relaxations that might involve the endothelium. Thrombin and bradykinin had no vasoactivity. Serotonin, prostaglandin I_2 and prostaglandin E_2 produced relaxations that were not mediated by the endothelium. The powerful relaxations observed with prostaglandin I_2 and prostaglandin E_2 and the powerful contractions observed with prostaglandin $F_2\alpha$ suggested a major role of prostanoids in coronary vasoactivity in fish. These prostanoid-mediated mechanisms, in addition to powerful contractions demonstrated with endothelin 1, point to an important role for the endothelium. No major qualitative or quantitative differences in vasoactivity could be related to differences in coronary lesion severity.

In the third paper, the team reported using [^{3}H]-thymidine to measure the mitotic activity of medial vascular smooth muscle (VSM) from salmonid coronary arteries and test the hypothesis that periodic anaerobic swimming induces greater VSM mitotic activity than sustained aerobic swimming (Gong et al., 1996). Juvenile chinook salmon were swum either at a submaximal speed of 1.5 body lengths per second for eight months alone or with a combination of forced swims to exhaustion (critical swimming speed) on alternate days in addition to a continuous low speed of 0.5 body lengths per second for three months. Fish swum at a continuous low speed (0.5 body lengths per second) for three or

eight months acted as controls. Rainbow trout were forced to burst swim to exhaustion twice daily for three days. Mitotic activity in coronary arterial explants was measured at the end of each of the regimens using [^{3}H]-thymidine. Compared with controls, the combination of exhaustive swimming on alternate days and continuous submaximal swimming at 1.5 body lengths per second stimulated coronary VSM mitosis. Neither submaximal swimming alone nor burst swimming stimulated coronary VSM mitosis. The team concluded that some but not all forms of periodic anaerobic swimming induce coronary VSM mitosis, with the duration and number of swimming events likely to be important considerations.

The fourth paper then examined the effects of polyunsaturated fatty acids and some of their metabolites on incorporation of [3H]-thymidine into VSM explants from the coronary artery of rainbow trout at a concentration of 120pM eicosapentaenoic acid (EPA; 20:5w3) arachidonic acid and (AA; 20:4ω6) and eicosatrienoic acid (ETA; 20:3ω6) (Gong, Townley and Farrell, 1997). Through their experiments, the team concluded that dietary modulation of polyunsaturated fatty acids (PUFAs) in salmonids could have significant effects on coronary vascular smooth muscle mitosis through incorporation of polyunsaturated fatty acids into cell membranes and the production of eicosanoids.

At the sign-off stage, the PI indicated that the second article listed above was a direct output from the case study grant (Gong et al, 1995), while the other three were produced on a shoestring budget from other grants after the BC Health Care Research Foundation and HSFC grants were terminated (Farrell and Johansen, 1995; Gong et al., 1996; Gong, Townley and Farrell, 1997). However, they were clearly part of a programme of understanding why lesions develop in salmon coronaries and what consequences they have. The PI then recommended that we also include the following articles, indicating the fifth and sixth articles as resulting from the first phase of the HSFC funding. The eighth article listed below describes the subsequent in-vivo work that was completed after the HFSC terminated its funding. Due to time commitments, these articles have not been reviewed or included in this case study:

1. Small, S.A., C. MacDonald and A.P. Farrell, 'Vascular Reactivity of the Coronary Artery in Rainbow Trout (*Oncorhynchus mykiss*)', *American Journal of Physiology*, Vol. 258, 1990, pp. R1402–R1410.
2. Small, S.A. and A.P. Farrell, 'Vascular Reactivity of the Coronary Artery in Steelhead Trout (*Oncorhynchus mykiss*)', *Comparative Biochemical Physiology*, Vol. C 97, 1990, pp. 59–63.
3. Farrell, A.P. and J.A. Johansen, 'Reevaluation of Regression of Coronary Arteriosclerotic Lesions in Repeat-Spawning Steelhead Trout', *Arteriosclerosis, Thrombosis, and Vascular Biology*, Vol. 12, 1992, pp. 1171–1175.
4. Axelsson, M. and A. P. Farrell, 'Coronary Blood Flow *In Vivo* in the Coho Salmon (*Oncorhynchus kisutch*)', *American Journal of Physiology*, Vol. 264, 1993, pp. R963–R971.

Bibliometrics were conducted using the original list of publications identified by the PI as directly related to the case study grant. The results of this analysis are presented below in the Table 6-3.

Table 6-3 Publication output and impact[5]

Number of journal articles:	4
Number of articles included in citation analysis:	4
Total number of citations (all papers):	39
Aggregate relative citation impact:	0.94 (Class III)
Self-citations:	8%

Distribution of publications across relative citation impact classes:

	Class I (uncited)	Class II (>0 and < 0.8 citations)	Class III (0.8 to 1.2 citations)	Class IV (1.2 to 2.0 citations)	Class V (> 2.0 citations)
Number of publications		3			1
Proportion of total output		75%			25%

Most highly cited publication[6]**:**	Farrell, A. P. and J. A. Johansen, 'Vasoactivity of the Coronary Artery of Rainbow Trout, Steelhead Trout and Dogfish: Lack of Support for Non-Prostanoid Endothelium-Derived Relaxation Factors', *Canadian Journal of Zoology*. Vol. 73, 1995, pp. 1899–1911.
Times cited:	27

6.6.2 Dissemination

The team disseminated their findings via publications in the peer-reviewed literature, presentations, international meetings and poster sessions. The PI said that he thinks that publications are pivotal because they are more permanent (Farrell Interview, 2008). Although some people feel that oral presentations are more powerful, Dr Farrell said that he is a good narrator but does not give the same talk twice. He feels that his publications are the most effective method of dissemination.[7]

A review of Dr Farrell's curriculum vitae indicates that he was then (and continues to be) an international player, presenting findings at various meetings and symposiums, including a presentation at the Zoology Department at the University of Canterbury in New Zealand, the Vertebrate Gas Transport Cascade Symposium in Brazil in 1991, the Zoophysiology Department at Goteborg University in Sweden, the American Physiology Society, the Canadian Physiology Society and the Society of Experimental Biologists in the UK, to name a few. In May 1993, Farrell was a keynote speaker to the Canadian Society of Zoologists. His main audience at these meetings was biologists and human physiologists (primarily university-based researchers). Health practitioners would have been at some of these meetings but were not the primary audience. In 1992, Farrell gave a presentation at

[5] In addition, the PI identified 12 publications that were indirectly linked to this grant. Eleven of these publications were indexed in Web of Science and analysed. They received 253 citations in total, giving a relative citation impact of 1.04. Six of these publications had a relative citation impact in Class II, four were in Class IV and one in Class V, while their self-citation rate was 32 percent.

[6] Citation count extracted April 2009.

[7] To date Farrell has published nearly 300 refereed publication and co-edited 18 books. His citation rate is about 500 per annum.

Dalhousie University, where he was speaking mainly to clinicians about coronary atherosclerosis in fish.

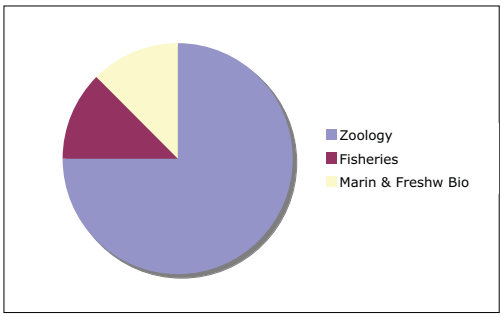
Dr Farrell also disseminates his research by maintaining an open-door policy to his laboratory and has conducted tours through his work areas. He often speaks to high-school students and is always willing to engage the public in response to requests. He also feels that his research experiences feed into his teaching at the undergraduate and graduate level. He continues to pass on the concepts and nuances of his fish model to all interested audiences.

Dr Small mentioned in her interview that Dr Farrell always made dissemination a priority (Small interview, 2008). During her master's degree, Small published three papers, of which she was first author on two and listed as a research assistant on the third.

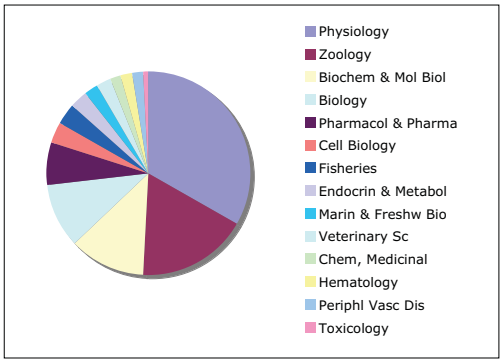
The bibliometric analysis also examined knowledge diffusion. The results are presented in Figure 6-2. As Dr Farrell indicated, most of his papers are published in zoological journals. The majority of citations are from others working in the field of physiology. Researchers in many countries are citing Farrell's papers, suggesting that the knowledge produced is relevant and transferable internationally.

Figure 6-2 Knowledge diffusion: fields of research of output (a), fields of citing papers (b) and countries of citing papers (c)

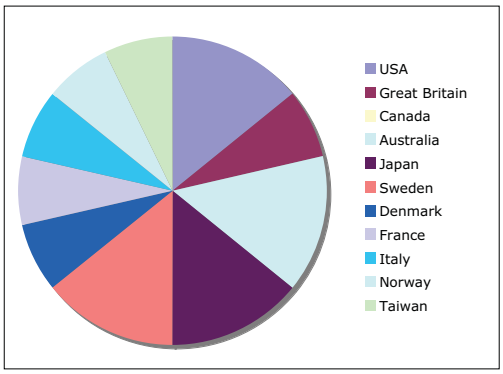
(a)



(b)



(c)



6.6.3 Training and capacity building

Dr Farrell claimed that this grant gave him the confidence to pursue new research directions. With the equipment purchased via this funding, the team was able to continue comparative cardiovascular research after the grant from the HSFC was not renewed.

Dr Farrell says this funding helped him to obtain the notoriety he now has and was instrumental in setting off his career studying cardiorespiratory physiology of fish. He says he opportunistically still works in the area of coronary vasoactivity of fish (that is, when he can build this area into other projects with minimal costs). He considers himself the world

expert in non-mammalian coronary systems. His current research interests lie in integrative and comparative animal physiology, including cardiorespiratory dynamics, myocardial oxygen supply, coronary physiology and pathology, blood flow regulation, hypoxia and anoxia tolerance. He also continues to study salmon migratory passage, exercise, handling stress and recovery, sustainable aquaculture and aquatic toxicology.

Dr Farrell presently has a research chair at the UBC, which is shared between the Department of Zoology and the Faculty of Land and Food Systems. The latter, formerly the Faculty of Agriculture, houses Farrell's research laboratory and team. He also has a research laboratory at the former West Vancouver Department of Fisheries and Oceans marine laboratory, which is now co-run with UBC. This space provides running seawater and freshwater, which allows Farrell to undertake experiments that he cannot do on campus because of inadequate aquatic facilities for fish.

Dr Farrell is a very prominent researcher in the field of fish physiology, as evident by his recognition by the community and recent awards:

- 2009 – Fry Medal for outstanding contributions to knowledge and understanding in Zoology from the Canadian Society of Zoologists
- 2006 – Award of Excellence for Fish Physiology from the American Fisheries Society
- 2005 – Award of Excellence for Fisheries Management from the American Fisheries Society
- 2002 – Murray A. Newman Award for Research and Conservation from the Vancouver Aquarium Marine Science Centre
- 2001 – Honorary degree from Gothenburg University, Sweden.

Dr Farrell professes to be very excited about his work and the people he works with. He says people want to work in his laboratory and attributes some of his visibility to this grant, referring to it as a 'snowball effect', meaning that once you train one good person, there are two wanting to participate in projects, then three and so on. He referred to Michael Axelsson, who he supervised from 1990 to 1991 as an example of this. Axelsson worked with Farrell on a study called 'Coronary and cardiac physiology', which carried on with the in-vivo studies not finished before the case study grant was terminated. Axelsson is currently a faculty member of the Zoophysiology Department at the University of Goteborg in Sweden.

Outside of the students Farrell has supervised or taught, however, he claims to have little formalised outreach, although one must consider the numerous presentations and discussions he has given as a mode of outreach. There are also the more distal effects of the career progressions of his students and the impacts they have on science and further trainees. Farrell stated 'It's important to me that my students graduate and succeed', and many go on to be successful clinicians. Gong, a member of the research team and author on three of the papers previously described, obtained his PhD while working with Farrell on this project. Gong returned to his homeland China to assume a directorship of a biomedical laboratory after spending a period developing new cardiovascular drugs for a pharmaceutical company in the USA.

Dr Small, who was supervised by Dr Farrell prior to this grant during her master's degree, said that Farrell was supportive of his students, allowing them to be first author on their papers. He promoted and encouraged them to present their findings. Although she has left the field, she claims that her participation in this grant, and her other work with Dr Farrell, taught her many of the important skills required of a researcher, which she continues to draw on, such as project design, statistics and linking basic research to application. She also mentioned that her perspective is unique as a result of her degrees in zoology, and because of this experience, she has participated in some animal audiology studies and been a reviewer for animal journals. For instance, researchers in clinical neurophysiology are running hearing studies in dogs by adapting the techniques used on humans and sought Small's advice on their papers since she had the necessary expertise.

Jeff Johansen currently works on aboriginal fisheries strategies as a manager with the Department of Fisheries and Oceans. He is involved in building relationships with aboriginal peoples and integrating local indigenous knowledge with mainstream science in the management of fishing-related activities. Johansen states that Dr Farrell was very supportive of him and his career and was as much a mentor as an employer. Several years after working on the research project, Johansen returned to Simon Fraser University as a laboratory instructor and worked with Dr Farrell in teaching several of his undergraduate courses. Johansen remarked that the cutting edge research, the dynamic nature of the laboratory and the challenges they faced prepared him for future opportunities in his career, and many of the skills learned in the day-to-day management of the laboratory are ones that have been refined and that he continues to use in his current federal government position.

This research did not lead to new collaborations, because Van Citters was approaching retirement. In part, Farrell attributes his inability to bridge his work and collaborate with clinicians to the competitive nature of the clinical world, in which researchers are more cautious about sharing ideas and discussing projects openly.

6.6.4 Benefits to future research and research use

One of the follow-ups to this research intended to understand how omega-3 fatty acids impact on the development of lesions. The HSFC did not renew these projects, but the team continued to pursue these experiments and completed them in the following years. With the hypothesis that lesions were developed through vascular injury, they later found that omega-3 fatty acids do suppress development of lesions. They demonstrated that a protective mechanism exists but is overridden by whatever causes the lesions. To this day, no one knows exactly why the lesions develop or what their consequences are.

The technique is still being used today. Dr Farrell claimed that the fish model was viable because all blood vessels, whether in humans or fish, have a similar structure. Atherosclerosis starts proliferation of vascular smooth muscle in humans, and this also happens in salmonids. As lesions have been shown to regress in fish, Farrell believes that huge gains are to be made by pursuing this research. By understanding what causes the lesions to regress in fish, researchers could gain insight into why they do not in humans.

The PI and his team continue to face challenges with funding agencies and their application of fish models. Dr Farrell is a strong believer in the learning potential to be

gained from exploring non-mammalian models, but he says it is very difficult to persuade the funding organisations of their value and the value of very basic research in non-mammalian systems, because basic research is further removed from tangible outputs and outcomes. A good example of this is Farrell's discovery that trout hearts grow almost entirely by hyperplasia (Farrell et al., 1988). In post-neonatal mammals, this cardiac growth is largely hypertrophic, and this has spurned a whole area of research trying to stimulate new cardiac tissue growth in mammalian hearts, ignoring the fact that fish do it naturally. This year, 20 years later, a 'biomedical' study using the model of zebrafish showed that anaemia stimulates hyperplastic cardiac growth in this fish. Due to difficulties in obtaining funds, Farrell has moved away from this area of research, although he remains open to an opportunity by which he can continue this line of investigation.

This research has led to some impacts outside the human cardiovascular field, as some of Dr Farrell's subsequent research looked at sustainable aquaculture. Using his vast knowledge of fish physiology and lesion development in salmonids, Farrell and his team, with the support of feed companies, have been assisting fish farmers by helping them understand how to create large, nutritional fish while minimising the cardiovascular complications known to occur in larger fish. He recently co-authored a publication in the *Proceedings of the National Academy of Science* on how dietary manipulations of fish feeds can make farming of fish more sustainable and still provide a good source of omega-3 fatty acids (Naylor et al., 2009). Farrell says this work was an obvious spin-off from his research of the early 1990s. His work on sustainable aquaculture has been facilitated with interest and financial support from feed companies.

Dr Farrell has also helped the British Columbian and federal governments pass regulations for fish culture to keep fish physiologically healthy and minimise environmental impact. He linked these activities back to his earlier research by saying 'you build a basic knowledge base and then you can choose how to apply it. By and large people come to me because they know my expertise [in fish physiology and health]'.

6.7 Stage 4 – Secondary outputs

The findings of this research have not been included in any clinical guidelines, although they do support the importance of including omega-3 fatty acids in the human diet.

6.8 Stage 5 – Adoption by practice and the public

This research had no impact on any policies or drug development and it did not inform or change practice within the health service or the practice of doctors or public health officials.

6.9 Stage 6 – Broad economic and health outcomes

Dr Farrell's subsequent research has contributed to the literature, which makes it clear that the types of diet that farmed salmon receive makes them a safe source of dietary input of omega-3 fatty acid for humans. His detailed analysis was recently published in the *Journal*

of World Aquaculture Society in an article titled 'Towards Improved Public Confidence in Farmed Fish Quality: A Canadian Perspective' (Farrell et al., 2010).

6.10 Payback

The payback framework describes the process from research to several categories of impact. The categories allow us to compare across different case studies. Table 6-4 shows, in point form and by impact category, some of the impacts, described more fully above, that have emerged from this grant.

Table 6-4 Payback

Payback category	Impacts from case study
Knowledge production	• Four related peer-reviewed articles • Various presentations to various audiences
Research targeting and capacity building	• Training of highly qualified personnel, including one master of science student, one doctor of philosophy student and one research assistant • Knowledge transfer within laboratory to students of all levels • Refinement of vascular ring technique • Techniques taught
Informing policy and product development	• Not applicable
Health and health sector benefits	• Confirmation of earlier evidence that diets rich in omega-3 fatty acids are good for cardiovascular health
Broader social and economic benefits	• Advice to consumers about sources of dietary omega-3 fatty acids

6.11 References

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Appendix A: All funding from 1988–1994

Grant: Equipment Grant **Awarded:** 1994 **Period:** 1994 **Total:** Can$173,000 **Funding:** Natural Sciences and Engineering Research Council of Canada (NSERC) **Project Title:** Bamfield Biophysiology Laboratory **Involvement:** Joint Investigator **Collaboration:** one of many co-investigators; PI: Dr Andy Spencer

Grant: Equipment Grant **Awarded:** 1993 **Period:** 1993–1994 **Total:** Can$17,791 **Funding:** NSERC **Project Title:** Data acquisition system **Involvement:** PI

Contract: Research Grant **Awarded:** 1993 **Period:** 1993–1994 **Total:** Can$15,000 **Funding:** Fisheries and Oceans Canada (DFO), Green Plan **Project Title:** Reproductive Measures in Fish **Involvement:** Joint Investigator **Collaboration:** PI Dr Ed Donaldson (DFO)

Grant: Research Grant **Awarded:** 1993 **Period:** 1993–1994 **Total:** Can$10,000 **Funding:** Environment Canada **Project Title:** Fraser River Action Plan – Reproductive Measures in Fish **Involvement:** PI

Grant: Research Grant **Awarded:** 1993 **Period:** 1993 **Total:** Can$267,000 **Funding:** BC Centre of Excellence in Environmental Research Programs **Involvement:** PI **Collaboration:** Program leader, involving 25 faculty; 'wind-down' funding for nine months

Grant: Research Grant **Awarded:** 1993 **Period:** 1993 **Total:** Can$53,000 **Funding:** Environment Canada **Project Title:** Fraser River Action Plan – Fraser River Benthos **Involvement:** PI

Grant: Matching Fund **Awarded:** 1993 **Period:** 1993 **Total:** Can$5,000 **Funding:** Departmental Matching Funds for Equipment **Involvement:** PI

Grant: Supplement Grant **Awarded:** 1993 **Period:** 1993 **Total:** Can$3,900 **Funding:** Tri-Council Secretariat **Involvement:** PI

Grant: Research Grant **Awarded**: 1992 **Period:** 1992–1995 **Total:** Can$67,500 **Funding:** Environment Canada **Project Title:** Fraser River Action Plan – Biological Database for the Fraser River **Involvement:** PI: John Richardson, UBC, and with Bill Neill, UBC

Grant: Research Grant **Awarded:** 1992 **Period:** 1992 **Total:** Can$10,000 **Funding:** Tri Council Secretariat – Green Plan Development **Involvement:** PI

Grant: Research Grant **Awarded:** 1992 **Period:** 1992 **Total:** Can$8,000 **Funding:** President's Research Grant **Project Title:** Tuna Research **Involvement:** PI

Grant: Research Grant **Awarded:** 1992 **Period:** 1992 **Total:** Can$10,000 **Funding:** President's Research Grant **Project Title:** Development Funding **Involvement:** PI

Grant: Research Grant **Awarded:** 1991 **Period:** 1991–1992 **Total:** Can$650,000 **Funding:** BC Centre of Excellence in Environmental Research Programs **Involvement:** PI **Collaboration:** Co-program leader, involving 25 faculty; funding for 18 months

Grant: Operating Grant – Cardiac Physiology in Fish **Awarded:** 1990 **Period:** 1990–1992 **Total:** Can$145,800 **Funding:** NSERC **Involvement:** PI

Grant: Equipment Grant **Awarded:** 1991 **Period:** 1991 **Total:** Can$13,403 **Funding:** NSERC **Involvement:** PI

Grant: Equipment Grant **Awarded:** 1991 **Period:** 1991 **Total:** Can$32,532 **Funding:** BC Health Care Research Foundation – Equipment Grant **Involvement:** PI

Grant: Operating Grant **Awarded:** 1989 **Period:** 1989–1990 **Total:** Can$84,000 **Funding:** HSFC **Involvement:** PI

Grant: Coronary Lesions and Vasoactivity **Awarded:** 1990 **Period:** 1990–1992 **Total:** Can$69,000 **Funding:** Medical Research Council **Involvement:** PI

Grant: Conference Grant **Awarded:** 1990 **Period:** 1990 **Total:** Can$5,500 **Funding:** NSERC **Involvement:** PI

Grant: Research Grant – Coronary Lesions and Omega Fatty Acids in Salmon **Awarded:** 1989 **Period:** 1989–1990 **Total:** Can$28,000 **Funding:** BC Health Care Research Foundation **Involvement:** PI

Grant: Research Grant **Awarded:** 1989 **Period:** 1989 **Total:** Can$6,846 **Funding:** NSERC International Collaborative Research **Involvement:** PI

Grant: Research Grant **Awarded:** 1989 **Period:** 1989 **Total:** Can$2,000 **Funding:** Royal Society of New Zealand **Involvement:** PI

Grant: Operating Grant – Natural Regression of Coronary Arteriosclerosis in Steelhead Trout: Fact or Artefact? **Awarded:** 1986 **Period:** 1986–1988 **Total:** Can$47,500 **Funding:** BC Health Care Research Foundation **Involvement:** PI

Grant: Operating Grant **Awarded:** 1987 **Period:** 1987–1989 **Total:** Can$102,000 **Funding:** NSERC **Involvement:** PI

Grant: Equipment Grant **Awarded:** 1988 **Period:** 1988 **Total:** Can$18,450 **Funding:** BC Health Care Research Foundation **Involvement:** PI

CHAPTER 7 The organisation and nature of formed elements within the paracellular clefts of capillary endothelia

7.1 Overview of case study grant

This British Heart Foundation (BHF)-funded grant titled 'The Organisation and Nature of Formed Elements within the Paracellular Clefts of Capillary Endothelia' supported basic research into how capillaries control the transport of various substances (eg water, nutrients, hormones, drugs and chemical messengers) to and from body tissues. The project team was interested in understanding how the structure, organisation and chemical properties of capillary walls are involved in allowing some substances to pass between blood and tissues and in restricting others (ie in selective exchange). The study found that spaces ('paracellular clefts') between cells lining the inside of capillary walls have a regularly spaced matrix of glycoprotein-based molecular filaments spanning them. It was thought that this matrix is likely to act as a substance filter and that the glycoprotein filaments also help localise and stabilise the space. The study also contributed to: the training and career development of a research assistant on the project, who gained her doctor of philosophy (PhD) through the grant; the introduction of new equipment into the principal investigator's (PI's) department; helped to attract follow-on funding for other research in the PI's institution, and also influenced studies in other research organisations.

7.2 Introduction to case study

This case study examines the evolution and impacts of a BHF–funded basic research grant (1989–1992). The PI, Professor Anthony Firth, was then Chair of the Department of Anatomy and Cell Biology at St Mary's Hospital in London.

Professor Firth began his career as an anatomy lecturer at King's College, London (1972–1978). He then became a senior lecturer and reader in anatomy at St George's Hospital Medical School (University of London, 1978–1987) and joined St Mary's Hospital Medical School (Imperial College) in 1987. He remained in a research role until the mid 1990s, when he moved into educational management and medical teaching positions at Imperial College.

The grant we investigated supported research into how capillaries control the transport of various substances (eg water, nutrients, hormones, drugs and chemical messengers) to and from body tissues. More specifically, Professor Firth was interested in understanding how the structure of capillary walls is involved in allowing some substances to pass between blood and tissues and in restricting others (ie in selective exchange).

A properly functioning mechanism of substance exchange (sometimes called chemical communication) is important for a healthy state and is often compromised in disease. Understanding what determines and controls appropriate substance exchange is a prerequisite for the 'effective management of circulatory changes in a wide range of situations, including inflammation, and a varied selection of common and life-threatening disorders' (Firth curriculum vitae, 2008).

Blood flows from the heart to the arteries, which narrow into arterioles and then into the capillaries that permeate tissues. Capillaries then widen to become venules, which then widen to become veins. In general, arteries bring oxygenated blood to the tissues; veins bring deoxygenated blood back to the heart. Capillaries are the smallest blood vessels in the body and have an important role in the delivery of beneficial substances to tissues and the elimination and disposal of harmful substances from tissues.

By the time of the grant, it was known that the endothelium – a thin layer of cells lining the inside of capillary walls – has an important role in controlling substance exchange[1], but it was unclear how it performs this role. Some scientists believed that substance transport occurs through endothelial cells: via intracellular vesicles (ie small sacs) that carry compounds from blood to tissues by moving them through the inside of the cells that make up the capillary walls. Other scientists believed that substances move through small spaces between the endothelial cells (ie paracellular spaces, clefts, openings and splits) to get in or out of tissues. These paracellular spaces were known to have wider (non-junctional) and narrower (tight junction) areas within them (eg Ward, Bauman and Firth, 1988). Professor Firth supported the latter hypothesis, but there was no evidence of how the structure and composition of the paracellular clefts determine which substances are permitted and restricted in relation to entry or exit.

Professor Firth decided to address this evidence gap. With BHF support, he examined the structure and organisation of paracellular clefts in capillaries from the rodent heart, brain and gut. These capillaries have greatly differing permeability properties – ie they allow different substances to pass in and out and at different rates. By comparing the paracellular spaces in the capillaries from different organs, Firth hoped to find evidence of a link between the structure, organisation and chemical composition of the clefts and the differing permeability properties of capillaries. He felt that understanding the capillary transport mechanism was important for more applied research into how chemical communication is regulated in health, how it is altered in disease and what the implications are for treatment (Grant application, 1989).

[1] An endothelium is a layer of cells that lines the inside of certain body cavities, such as blood vessels. The capillary endothelium is a thin single layer of cells. Water and some solutes pass through by diffusion and enter tissues. Waste products (eg carbon dioxide and urea) diffuse back into the blood and are carried for removal from the body.

This study fit in well with the central theme of his research group: ie transport mechanisms across the interface between blood and tissues. It was also in line with Professor Firth's key expertise in structural anatomy.

Below, we elaborate on how the BHF-funded research study was incubated, explain how it evolved and examine its various impacts (ie paybacks). To avoid confusion with other projects Professor Firth conducted, we refer to the study as the 'cleft structure project'.

The key sources of information for this case study were interviews with the PI; interviews with people he collaborated with at various stages in his career, who had insights into the impacts of the cleft structure project; an interview with an external researcher whose work the study influenced; archival documents such as the grant application, interim and final progress reports; Professor Firth's curriculum vitae; relevant scientific literature (publications); and bibliometric data.

Unfortunately, the only secondary investigator on the grant could not be contacted. Communications with Professor Firth (and with other heads of laboratories she was at for various stages in her career) suggested that she left science in 2001. Bibliometric analyses were not fruitful for locating and contacting her.

7.3 Stage 0 – topic/issue identification

There were three key interrelated influences on Professor Firth's identification of the research topic and decision to pursue the study. These were:

- PI's prior research, including 'accidental' findings
- scientific curiosity
- identified gaps in scientific understanding.

We elaborate on each in turn below.

7.3.1 PI's prior research, including 'accidental findings'

Throughout his career, Professor Firth pursued an interest in the relationships between capillary structure and permeability characteristics using rodent placenta, heart, gut and brain tissue, as models for research.

Much of his early work was on the placenta. Supported by a Medical Research Council (MRC) grant (1979–1982), Professor Firth started his career by investigating protein transport across capillary epithelia[2] in the guinea pig placenta[3]. He was interested in understanding how the immunoglobulin G (IgG) protein (which confers protection against infections to an unborn foetus) is transported from the mother to the foetus. The research revealed very little on IgG transport, but unexpectedly – by accident rather than intentional research design – provided interesting insights on certain structural aspects of

[2] An epithelium is a layer of cells which lines the outside of certain body cavities, such as blood vessels.

[3] Placentas vary widely across species, but the guinea pig and human placenta are structurally very similar. Both have one layer of epithelium, and one layer of endothelium.

capillary permeability to proteins. It showed that some proteins (depending on size) pass into the paracellular clefts of capillary endothelia and that others do not. These findings were the underpinning of Firth's engagements in capillary endothelial research. He said, 'It was almost a natural progression, following the path of least resistance' (Firth interview, 2008).

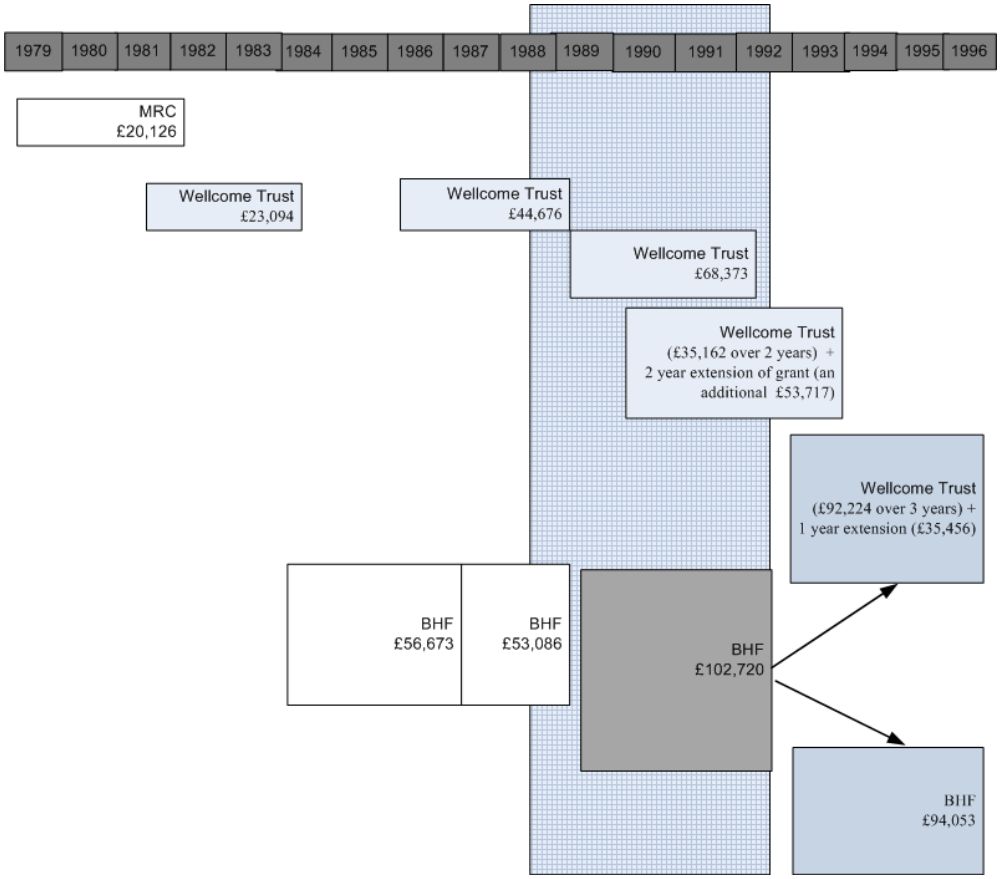
Although he continued to pursue placental work, Professor Firth also developed an interest in cardiovascular anatomy. While a researcher at St George's Hospital (1978–1987), he frequently communicated with a cardiovascular physiology student, who influenced Firth's interest in myocardial (heart) capillary work on rodent hearts (a better understood research model than the placenta). Firth also frequently communicated with cardiovascular research experts at various conferences. This included a physiologist at St Mary's Hospital with whom Firth would later on collaborate informally after joining the hospital.

In the 1984–1989 period, with BHF support, Professor Firth conducted a number of studies on myocardial (heart) capillaries. In one of these studies, he investigated the effects of cardiovascular disease (hypoxia and ischemia) on capillary permeability. Although the study was not explicitly geared at cleft structure research, it led to an important observation. Firth noticed some filament-like components in the wider ('non-junctional') cleft areas (eg Ward, Bauman and Firth, 1988). He thought the observation interesting and in line with a model of cleft structure that proposed the existence of a matrix of fibres with a substance filtering role (eg Curry and Michel, 1980, and Firth, Bauman and Sibley, 1983). He decided to conduct further research into the structure and composition of the observed filaments. This was facilitated by the 1989–1992 BHF grant we are investigating.

Figure 7-1 provides an overview of key research projects that Professor Firth was involved in around the time of this grant. Projects that had a direct influence on the idea to pursue the study of interest (ie the cleft structure project) are presented in white blocks. Projects that were directly influenced by the cleft structure project have arrows pointing to them and are elaborated on in Section 7.7.2. They include a BHF-funded study on paracellular cleft maturation and a Wellcome Trust-funded study on the formation of intracellular junctions in foetal capillaries. Appendix A provides a list of accompanying grants, specifying the funding body, title, duration and size. It reflects the core focus of Firth's group on capillary transport research. A list of equipment grants is presented in Appendix B.[4]

[4] The equipment grants the PI received were from various sources. The equipment available in the laboratory was utilised across projects.

Figure 7-1 An overview of the PI's grant landscape[5]



7.3.2 Scientific curiosity

In addition to Professor Firth's prior research and professional experiences[6], a general curiosity in the capillary transport field influenced his interest in paracellular cleft structure–function relationships.

Professor Firth's research had been curiosity driven all his life. He considered himself a basic scientist who valued academic autonomy in selecting research areas of personal interest. He never had more than three scientists assisting him at a time and never subscribed to the larger, programme-type commissioned work that supported the advancement of specific research agendas in a 'top-down' manner. He commented, 'I never got into the large plight-driven programmes' (Firth interview, 2008).

At the time of the cleft structure project, scientific communication between researchers was not very advanced. Professor Firth said, 'It was all about old pals and who knew whom' (Firth interview, 2008). As a new investigator with limited networks, Professor Firth kept

[5] For the BHF and Wellcome Trust grants that took place after the cleft structure project (ie those that have arrows pointing to them), Professor Firth was a co-applicant.

[6] For example, the MRC placental study (1979–1982), BHF-funded myocardial capillary work (1984–1989); and communications with cardiovascular experts.

informed about scientific advancements that interested him by reading the literature and through informal communications with a select few researchers he got to know.

The key scientific developments that influenced topic identification for the cleft structure project included a mix of research conducted by external groups and research in which Professor Firth was directly involved. They are summarised below:

- Advances in research on substance transport mechanisms:
  - One hypothesis was that the key substance transport mechanism is intracellular, ie that intracellular vesicles (small sacs) carry substances from blood to tissues by moving them through the inside of cells. Professor Firth said, 'Cells are full of vesicles, and they are the first thing that hits your eye when looking through a microscope' (Firth interview, 2008). This idea was introduced by cell-biologist Palade in the 1950s and developed by Palade and other researchers throughout the 1960s and 1970s (eg Burns and Palade, 1968).
  - The alternative hypothesis was that the key transport mechanism was paracellular (ie occurred through gaps between cells rather than through cells). Capillary physiologists (eg Pappenheimer, Renkin and Borrero, 1951 and 1953) proposed that pores (ie gaps and clefts) between endothelial capillary cells allow or restrict various compounds from passing.
  - Acceptance of the paracellular mechanism hypothesis was growing. Although there was a lot of debate regarding the two alternative hypotheses, by the mid 1980s it had become relatively widely accepted that the pore (cleft) system (rather than vesicles) was key in permitting the movement of water and solutes and in preventing some larger molecules from being transported to tissues (eg Crone, 1984, and Renkin, 1988).[7,8] The clefts were shown to have tight junctional areas towards the inside (luminal surface) of the capillary and wider areas towards the outer (abluminal) surface (eg Ward, Bauman and Firth, 1988). However, the identity of the cleft in terms of structural organisation and chemical composition remained elusive. According to Professor Firth, this was somewhat surprising given methodological advances in cell biology that could help further understanding.
- Advances in cleft structure research:
  - One hypothesis was that the structure of the cleft entailed a filtering 'matrix of fibres'. The known size limits to the movement of various compounds

[7] Water and some small solutes can pass relatively freely in and out of most capillaries, but proteins larger than the size of albumin (which is considered medium-sized) are generally restricted by the paracellular mechanism.

[8] The paracellular mechanism hypothesis did not exclude some role for vesicles in transport, but they are not considered to be the *primary* pathway for the exchange of water and solutes between blood and tissues. Vesicles are however involved in receptor-mediated endocytosis of specific *large* molecules. Endocytosis is the process by which cells absorb certain molecules from the outside by engulfing them with their cell membrane and then passing them on into transport vesicles. Many large molecules cannot pass through clefts. For example, receptor-mediated endocytosis is used for the specific uptake iron derivatives (eg ferritin and iron-oxide).

suggested that there is a filtering meshwork of molecular filaments within the clefts (eg Curry and Michel, 1980). This filter was assumed to be glycoprotein based but there was no solid empirical proof. In addition, researchers suggested (on theoretical grounds) that there may be linking bars of filaments as part of the matrix structure and structural stabilisation 'posts' in the wider ('non-junctional') cleft areas (eg Firth, Bauman and Sibley, 1983; Ward, Bauman and Firth, 1988; and Silberberg, 1988). If true, this could help explain both why clefts maintain their width under various pressures, such as osmotic[9] flows (Silberberg,1988), and how cleft structure helps regulate the movement of substances (Curry and Michel, 1980; Firth, Bauman and Sibley, 1983; and Renkin, 1988).

- Professor Firth and other researchers had observed filament-like structures in the cleft. Firth had already observed (during previous BHF-supported work) that clefts are not empty spaces but rather have some structures inside them (Ward, Bauman and Firth, 1988). Similar observations were made by Silberberg (1988).
- Methodological advances had been made that could facilitate more sophisticated ultrastructural studies of the paracellular clefts. This included advances in electron microscopy[10] and immunohistochemistry (IHC)[11] that had accumulated by the 1980s.

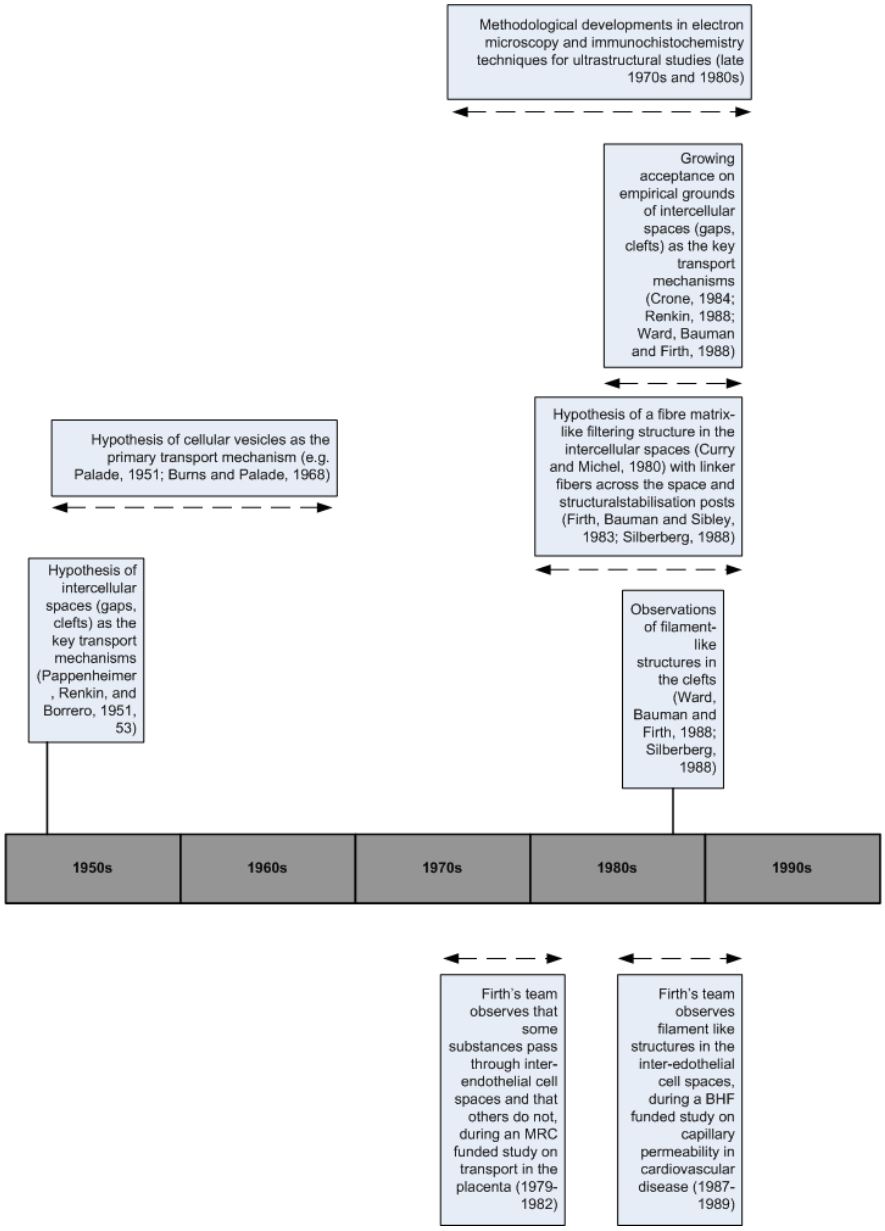
Figure 7-2 highlights key scientific advances that influenced the topic identification. These include the findings of other researchers and of Professor Firth's own research. General scientific advances are presented above the timeline. Observations specific to the work of Firth's team are presented below the timeline.

[9] Osmosis is 'the flow of a solvent by diffusion through a semipermeable membrane from a more concentrated solution to a less concentrated one, until the concentrations are equalized. It is a major factor in regulating the movement of water into and out of tissues in living organisms' (Encarta® World English Dictionary, 1999).

[10] An electron microscope uses electrons to illuminate a specimen and create an enlarged image. Electron microscopes have much greater resolving power than light microscopes. Light microscopes can achieve a maximum resolution of approximately 0.1 micrometres whereas transmission electron microscopes can achieve 0.1 nanometres (ie about a thousand times higher resolution).

[11] Immunohistochemistry refers to the process of localising proteins in cells of a tissue section exploiting the principle of antibodies binding specifically to antigens in biological tissues. It takes its name from the roots 'immuno', in reference to antibodies used in the procedure, and 'histo', meaning tissue (compare to immunocytochemistry). Immunohistochemical staining is widely used in the diagnosis of abnormal cells such as those found in cancerous tumours. Specific molecular markers are characteristic of particular cellular events such as proliferation or cell death (apoptosis). Immunohistochemistry is also widely used in basic research to understand the distribution and localisation of biomarkers and differentially expressed proteins in different parts of a biological tissue.

Figure 7-2 Timeline of some key scientific advances influencing topic identification



7.3.3 Identified gaps in scientific understanding

Professor Firth had a general frustration with the gaps in knowledge on capillary transport mechanisms, especially as he felt that methodological advances (eg in IHC and electron microscopy) could quite straightforwardly aid in clarifying the structure of capillary clefts and help provide further insights into their transport functions. This frustration, combined with knowledge gained through his own research engagements and familiarity with the work of others, provided a strong impetus to conduct the cleft structure project. Firth said, 'I was annoyed with the gaps in knowledge, and the research was timely'.

Lastly, it is interesting to note that Professor Firth felt that certain personal traits (ie the fact that he valued academic autonomy and that he was a bit of an introvert) influenced the research directions he took in his career – avoiding large, plight-driven, commissioned research and collaborative grants and staying committed to basic, curiosity-driven science.

7.4 Interface A – project specification and selection

Professor Firth had originated and formulated the idea to study capillary clefts, specified the project and drafted the grant proposal to the BHF largely by himself.

Informal, ie non-contractual, interactions with a colleague in the physiology department had an indirect influence on the specification of most projects the PI conducted at St Mary's Hospital, although not the BHF project specifically. There was an informal understanding that the Department of Anatomy (which Professor Firth headed) and the Department of Physiology would play to their individual strengths and attempt to build joint research capacity by applying for grants in complementary research areas and by sharing and exchanging knowledge. This implicitly influenced only the fact thatFirth's project specification in the BHF grant was to cover structural and histochemical research on capillary permeability and that complementary physiological research grants would be left to the physiology laboratory.

Prior to applying to the BHF, Professor Firth had applied for project support from the MRC. Despite an alpha rating, the MRC decided against funding the project; however, Firth was confident that he could get the cleft structure project funded. At the time, there was not much competition in the field of research on paracellular mechanisms of cardiovascular permeability, and he felt the proposal was unique and timely. Consequently, Firth approached the BHF (the contingency plan would have been the Wellcome Trust). He had attracted BHF support for previous research on myocardial capillaries, and they seemed well suited for funding the cleft structure project (since it included work on the heart and on blood vessels). In addition, Firth felt that the BHF would be open to funding a relatively small project-based basic-science study (unlike the MRC, which had tendency to support larger and more applied research programmes).

Lastly, and as far as Firth recollects, the peer review of the proposal suggested one or two minor modifications to the original application. However, no correspondences from the peer-review process were kept, which makes it difficult to understand in more detail the nature of modifications suggested.

7.5 Stage 1 – inputs to research

The key facilitators and inputs into the study were: funds; staff and their knowledge and expertise; equipment and scientific techniques; and informal collaborators.

7.5.1 Facilitators

The project was primarily facilitated by £102,720 of BHF funding. This allowed a research assistant to be hired through a process of open advertisement for the position and covered the purchase of equipment and consumables. The level of financial support for staff was

comparable to that provided by other grants Professor Firth received at the time. However, the grant was comparatively larger overall, due to nearly £40,000 support for the purchase of equipment.

7.5.2 Equipment

The nature of the proposed research required a then modern and important piece of equipment for IHC studies: the ultracryotome. This is a mechanical instrument used to cut frozen (structurally stable and preserved) biological specimens into transparent thin sections for electron microscope examination. Professor Firth was conscious that, if funded, this equipment would allow for interesting research not only in the topic supported by the grant but also for other projects going on in the department (at the time and in the future). The key techniques used in the project were IHC and electron microscopy.

7.5.3 Knowledge and expertise

At the time of the grant, Professor Firth was only beginning to become nationally recognised in academic circles. His expertise, the 'really good research idea and scientific foundation on which the project [was] built' (Firth interview, 2008) and the ability to recruit a 'very bright, diligent and energetic' research assistant 'with a colossal appetite for work' were key skill and knowledge-based inputs into the study.

7.5.4 Collaboration

Although there were no formal collaborations in the project specification, Professor Firth had an implicit agreement for informal collaboration and 'knowledge osmosis' with colleagues in the physiology department of St Mary's Hospital, on an as-needed basis.

7.6 Stage 2 – research process

The staff, equipment, methodological techniques and informal collaborators in the study were key in influencing how the research process evolved.

Professor Firth commented that the cleft structure project was one of the most productive and enjoyable projects he ever led. He commented: 'There really weren't any difficulties. It was a golden grant. It also interlocked well with other grants I had for placental research at the time from the Wellcome Trust. So there was a lot of cross-fertilisation of knowledge and experience, and this was both helpful for the study and personally rewarding'…[The combination of] a really interesting topic, existing evidence of the presence of some structure in the clefts we were to study, an excellent research assistant and my own expertise, appropriate techniques and little competition in the field by the research community…[were the key enablers of a] really good project experience' (Firth interview, 2008).

The research was also well received by other scientists at St Mary's Hospital (especially in the physiology laboratory), who adopted various insights that were developed during the course of the research to advance their own complementary projects. Their informal, ie non-contractual, interactions with Professor Firth were also helpful for progressing the cleft structure project.

A key informal external collaborator was a capillary specialist who worked on cell-adhesion molecules, using cell-culture techniques. She was based at the University of Milan (Mario Negri Institute) and met Firth at a conference in Denmark. This led to a brief but important collaboration, in that she provided Firth with antibodies that were central to characterisations of the glycoprotein structure of capillary clefts[15].

Interactions with the funder were limited to interim and final progress reports. Professor Firth commented that the BHF selected research projects carefully but did not micromanage.

The structural imaging method used in the cleft structure project combined electron microscopy and immunohistochemistry. Although they were fully appropriate for meeting the objectives of the proposed research, methodological advances (which were being developed for structural studies at the time) meant that building on the study's findings would need to entail new techniques (eg cell culture and confocal microscopy).

Lastly, Professor Firth (who had a strong professional commitment and personal dedication to education) saw the opportunity to train and empower the research assistant, who ultimately received her PhD via this grant, to be very important.

7.7 Stage 3 – primary outputs from research

The primary outputs from the project fall into two categories of the payback model: knowledge production (category 1) and benefits for future research and research use (category 2).

7.7.1 Knowledge production

This research produced more publications than any other grant Professor Firth held. It resulted in five articles in peer-reviewed journals and four meeting abstracts.

The publications contributed to knowledge about capillary cleft structure and chemical composition. First and foremost, they:

- exposed evidence of a regularly spaced array of molecular filaments (ie a matrix of linkers) spanning the clefts (eg Schulze and Firth, 1991; *Developmental Brain Research,* 1992; *Journal of Cell Science*, 1992; Schulze et al., 1992)
- identified constituent cell adhesion (transmembrane) proteins and showed that they are cadherin-5 glycoproteins, which ensure that cells are bound together and help localise and stabilise the paracellular cleft space (Leach et al, 1993, and Schulze and Firth, *Journal of Cell Science* , 1993 and *Cell Tissue Research,* 1993).

[15] The capillary specialist found out about the PI's work on capillary structures in the heart, brain and placenta and of the ultracryotome equipment in his laboratory. She provided Professor Firth with cadherin-5 cell adhesion antibodies, and he tested for and found the presence of the cadherin protein in placental paracellular clefts and myocardial capillaries.

The researchers also:

- found that the linker structures in the wider zones of clefts (ie non-junctional areas) were more widely distributed than previously thought (Schulze and Firth, 1991, *Developmental Brain Research,* 1992 and *Journal of Cell Science,* 1992)
- found differences in the 'tightness' of narrow zones of the clefts (ie tight junctions) in foetal and adult brain capillaries, suggesting that junctional structure changes during development (Schulze and Firth, *Journal of Cell Science*, 1992)
- found similarities in the structure and organisation of wider areas of clefts in brain and non-brain capillaries; this implied that differences in capillary permeability were most likely to result from differences in the structure of the tighter, junctional areas (Schulze and Firth, *Developmental Brain Research,* 1992 and *Journal of Cell Science,,* 1992)
- observed tighter and wider junction-like spaces between pericytes (cells on the external surface of heart capillaries) and the capillary wall (Schulze and Firth, *Cell Tissue Research,* 1993); this organisation suggested that pericytes might also have a role in capillary permeability[16].

These findings implied the need for further research on changes in paracellular clefts during development, the role of tight junctional areas in capillary permeability and the role of pericytes.

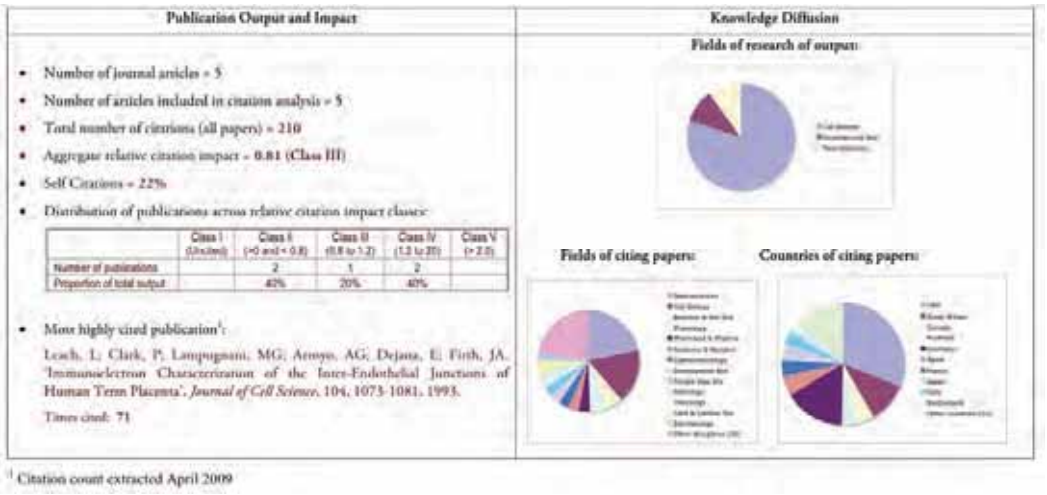
Although most of the research supported by the grant was conducted on rodent heart, brain and gut models, there were direct knowledge spillovers of findings and methods to a complementary Wellcome Trust-supported research project taking place in the laboratory at the same time (1989–1992). The core aim of the Wellcome Trust-funded study was to explore the adequacy of the (preserved) delivered human placenta as an experimental model for capillary permeability studies. This project was conducted by Professor Firth and another postgraduate student and contributed to findings on placental capillary junctions.

In Professor Firth's view, the methodological and knowledge-related contributions of the cleft structure project to the placental study were significant. The efforts of the cleft structure project to characterise the chemical composition of paracellular spaces of heart, brain and gut capillaries influenced the discovery of glycoprotein structures in the placenta as well. Firth felt that this validated co-attribution of a publication centred on the placenta and the capillary cleft structure within it (Leach et al., 1993) to both the Wellcome Trust-supported placental study and the BHF-supported research. However, the PI commented on the general difficulties of attribution in scientific research: 'It is often very difficult to really know what is one scientist's idea, and what is another's…It can be equally difficult to accredit discovery and research findings to the inputs of a single study. Researchers absorb each other's work, are influenced by it and share knowledge. Research is osmotic' (Firth interview, 2008).

[16] They are important in blood–brain barrier stability, as well as angiogenesis.

Figure 7-3 shows the results of bibliometric analysis for the case study grant.

Figure 7-3 Publication output and impact of directly related publications



7.7.2 Benefits to future research and research use

The key benefits of the cleft structure project for future research and research use (payback category 2) revolve around capacity building and career development, and targeting of future research.

Capacity building and career development

The key contributions of the grant to capacity building included: the training and career development of the research assistant; knowledge transfer to other scientists at the medical school; the introduction of ultracryotome equipment into the Department of Anatomy; and some spillovers on the ability to recruit researchers.

Training and career development of researchers

The research assistant received a PhD based on her work on the BHF grant. After Professor Firth decided to move away from research activities[17] (at the end of 1993), she went on to pursue a research career in other laboratories in London (at Eisai London Research Laboratories Ltd based at University College London (UCL) until 1997 and at the Institute of Ophthalmology, UCL). Firth believes that she left a research career in 2001 and returned to Germany with her husband. Efforts to trace her via previous laboratories and bibliometric analysis did not provide any leads.

The knowledge from this project was also transferred to other projects and scientists at St Mary's Hospital through informal communications. This included a researcher who was working on placental capillary research, and researchers in the physiology laboratory.

[17] This happened gradually, from 1993. The PI stayed involved in research as a secondary activity until 2001 but stopped leading and actively conducting research projects in the mid 1990s.

Equipment

A number of researchers at St Mary's were trained to use the ultracryotome and adopted it in their studies. In this context, the equipment was also key for facilitating other projects at the medical school and for enabling career development of postgraduate researchers.

Recruiting researchers

Lastly, Professor Firth believed that the project also helped attract students to the Department of Anatomy, because the associated publications helped build both his own and the department's reputation. He commented: 'A lot more people heard of me after this grant than before' (Firth interview, 2008). Nevertheless, the recruitment environment was challenging: the anatomy laboratory was a small group and the questions they were investigating were 'less popular' (Firth interview, 2008) at the time, than those being researched in some of the larger, more applied and programme-funded groups.

7.7.3 Targeting of future research

After the BHF grant completed, Professor Firth decided to 'gradually retire from a research career' (Firth interview, 2008) and move into educational activities and medical teaching. This was partially because of a personal commitment and interest in the educational aspects of medical science and partially because he felt that other researchers in the laboratory were better trained (in modern cell-culture and confocal microscope techniques) to build further on the body of knowledge he helped establish.

However, the cleft structure project helped attract follow-on funding for other research at St Mary's Hospital and also influenced studies in other research organisations.

Influence on future internal research

The project helped attract further funding from the BHF for a project investigating paracellular cleft maturation in capillaries of varying permeability (1993–1996) (see Figure 7-1).

Dr Peter Clark (a blood lecturer and specialist in cell adhesion) was PI in this study, and he also became the leader of Professor Firth's research group once Firth assumed educational management positions. Clark was key in advancing Firth's work on vascular permeability using more modern cell-culture and confocal techniques throughout the late 1990s. Clark's research established the presence of cadeherin 10 (another cell-adhesion protein) in the inter-endothelial junctions of the blood–brain barrier. Later yet, he moved into prostate pathology work, having found that cadherin 10 molecules have an important role in prostate disease. He was recently able to reengage in cardiovascular permeability studies by attracting BHF funding to explore the effects of ischaemia on the structure and function of cardiac microvessels (2005–2008).

The cleft structure project also helped attract further Wellcome Trust funding to investigate the formation of intercellular junctions in human foetal capillaries using in vitro models (1993–1996) (see Figure 7-1). One of Professor Firth's postdoctoral researchers, was a key researcher in the placental studies at St Mary's Hospital. She later became a professor of vascular biology at the University of Nottingham and continued to work in areas of areas of cell adhesion molecules, endothelial barrier (blood–brain and blood–retina) function and vascular dysfunction.

Influence on external studies

The cleft structure project also influenced research outside of St Mary's Hospital. This included studies on endothelial morphology changes in cardiovascular disease (eg Ward and Donelly, 1993) by another of Firth's former postdoctoral researcher in the early 1990s, who became a lecturer at the William Harvey Research Institute at St Bartholomew's Hospital.

A number of other researchers have frequently, in more recent times, cited the publications stemming from the BHF grant. The cleft structure project tends to be cited either in the context of background overviews to a study (eg introductions and literature reviews of accepted knowledge on paracellular cleft morphology) or in support of observations and new hypotheses made by more recent studies:

- Dr Hardwit Wolburg (University of Tubingen) references the study in a number of publications on tight junctions and the development, composition and regulation of blood–brain barrier structure in the 1996–2002 period (eg Wolburg and Lippoldt, 2002; Kniesel and Wolburg, 2000; Liebner, Gerhardt and Wolburg, 2000; Wolburg, Liebner, Reichenbach et al., 1999; and Kniesel, Risau and Wolburg, 1996).
- Drs Andrzej Vorbrodt and Danuta Dobrogowska (New York State Institute for Institute for Basic Research in Developmental Disabilities) cite the work in neuropathology studies and investigations of the blood–brain barrier in the 2001–2006 period (eg Vorbrodt et al., 2006; Vorbrodt and Dobrogowska, 2003 and 2004; and Vorbrodt, Dobrogowska and Tarnawski, 2001). A personal communication with Dr Vorbrodt revealed that the cleft structure project was very influential on his own research. He thought it advanced very important knowledge about endothelial cells and vascular permeability and impacted on the methods and research designs he adopted. Dr Vorbrodt commented: 'Most importantly, it provided a means for independently validating my own research findings on the molecular organisation of tight junctions and wider areas in paracellular clefts and provided support for my hypothesis about cleft structure formation processes during development' (Vorbrodt conversation, 2008).

Lastly, it is important to mention the inherent difficulty in assessing the range of potential impacts this study may have had on future research. The fact that Professor Firth left a research career in the mid 1990s meant that he was unfamiliar with developments in capillary studies for most of the last decade. Thus, we could only examine the work of a few researchers he knew of and led us to and complement this with a broad and general assessment of the context in which key publications from the study are cited in by authors who seem to reference the cleft structure project frequently. More detailed investigation was outside the scope of this retrosight project.

7.8 Interface B – dissemination

According to Professor Firth, there were no active efforts to disseminate research findings from the cleft structure project to external audiences. He commented, 'We were always very conservative about this, we never actively pursued conferences, but we would

sometimes go if invited' (Firth interview, 2008). Firth could not recollect the specific conferences at which he and his research assistant may have presented.

Professor Firth felt that the most useful way to keep up to date with developments in scientific knowledge was via reading publications. He said, 'Conferences are most useful for sourcing information about new research techniques and for identifying potential collaborators but less useful as inputs for new and groundbreaking research ideas. The really original ideas tend to be kept hidden at conferences…no one wants their ideas to be stolen' (Firth interview, 2008).

However, Professor Firth much earlier had had an instrumental role in the emergence of the European Placenta Group (EPG), a multidisciplinary society for placental researchers. The group founded and owns the journal *Placenta* and organises annual or biannual conferences. The idea for EPG arose at a conference in Hamburg (in the early 1980s) during which researchers from diverse disciplines exchanged knowledge on placental work. They decided to form a society to bring together anatomists, physiologists and other disciplines to share research experiences and discuss topical placenta research issues. Although Firth (who was one of the founding members) could not remember explicitly presenting the work of the BHF grant we are investigating at an EPG conference, he did informally share insights on the cleft structure project with other scientists in the EPG – it was a forum for knowledge exchange and communication.

Nevertheless, attributing and examining the impact of the BHF-funded study on the EPG is not possible given existing evidence. We followed up our research with another co-founder of the EPG. Although he also could not provide explicit evidence of the impact of the cleft structure project on the research of EPG members, he did suggest that some transfer of knowledge would have been very likely, because there is often overlap and exchange of insights from vascular studies using heart and placental models.

7.9 Stage 4 – secondary outputs

Although this basic research did inform further research, we did not identify any impact on policies or product development.

7.10 Stage 5 – adoption by practice and the public

Given that we could not identify any impact on policies or product development, there was no opportunity for identifying adoption of the findings in practice or by the public.

7.11 Stage 6 – final outcomes

There is no evidence of this basic research having made an impact in terms of health and broader economic gains.

7.12 Additional comments

According to Professor Firth, funding for research such as the cleft structure project would probably be more difficult to attain today than it was in the past. He commented, 'The coming of the research assessment exercise (RAE) has made it difficult to get money for anatomical and physiological, curiosity-driven basic research studies. The RAE has led researchers to focus on getting the 'right' funding, which generally needed to fit more clinical need, plight-driven donor agendas' (Firth interview, 2008).

7.13 Payback

The payback framework describes the process from research to several categories of impact. The categories allow us to compare across different case studies. Table 7-1 shows, in point form and by impact category, some of the impacts, described more fully above, that have emerged from this grant.

Table 7-1 Payback

Payback category	Impacts from case study
Knowledge production	• Five peer-reviewed articles • Four refereed abstracts
Research targeting and capacity building	Capacity building • HR capacity-building training and empowerment of the research assistant who received her PhD based on work on this grant • Physical capacity-building: introduction of ultracryotome equipment into St Mary's, which was used to advance other projects • Influence on recruiting researchers • Knowledge transfer to other investigators at St Mary's, and other research projects (especially Wellcome Trust funded placental studies). Benefits for future research and research use • Two follow-on funding grants from the BHF and Wellcome Trust • Influence on external studies: we identified influence on work of three external researchers (two groups)
Informing policy and product development	• Non e
Health and health sector benefits	• Non e
Broader social and economic benefits	• Non e

7.14 References

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APPENDIX

Appendix A: List of associated grants

Grant: Placental transport studies **Period:** 1979–1982 **Total:** £20,126 **Funding:** Medical Research Council

Grant: Gastric Acid Secretion **Period:** 1981–1984 **Total:** £23,094 **Funding:** Wellcome Trust

Grant: Project grant for studies of receptor mediated IgG and transfer in transport in human placenta **Period:** 1986–1989 **Total:** £44,676 **Funding:** Wellcome Trust

Grant: Studies on the perfused delivered term placenta as a human experimental model for microvascular permeability **Period:** 1989–1992 **Total:** £68,373 **Funding:** Wellcome Trust

Grant: Studies on the role of the endothelial cell glycocalyx in vascular physiology **Period:** 1990–1992 **Total:** £68,373 **Funding:** Wellcome Trust

Grant: Two-year extension for studies on the role of the endothelial cell glycocalyx in vascular physiology **Period:** 1992-1994 **Total:** £53,717 **Funding:** Wellcome Trust

Grant: Studies on formation of intercellular junctions between human microvascular endothelial cells using in-vitro models **Period:** 1993–1996 **Total:** £92,224 **Funding:** Wellcome Trust

Grant: One-year extension to studies on formation of intercellular junctions between human microvascular endothelial cells using in-vitro models **Period:** 1996–1997 **Total:** £35,456 **Funding:** Wellcome Trust

Grant: Project grant for myocardial capillary research **Period:** 1984–1987 **Total:** £56, 673 **Funding:** British Heart Foundation

Grant: Project grant for myocardial capillary research **Period:** 1987–1989 **Total:** £23,086 **Funding:** British Heart Foundation

Grant: Project grant for paracellular cleft research **Period:** 1989–1992 **Total:** £102,720 **Project title:** The Organisation and Nature of Formed Elements within the Paracellular Clefts of Capillary Endothelia **Funding:** British Heart Foundation

Grant: Project grant for research on paracellular cleft maturation in tight and leaky capillaries **Period:** 1993–1996 **Total:** £94,503 **Funding:** British Heart Foundation

Appendix B: List of equipment grants

Grant: Equipment Grant for the purchase of an ultramicrotome **Period:** 1977 **Total:** £8,686 **Funding:** Medical Research Council

Grant: Equipment Grant for the purchase of recording and perfusion equipment **Period:** 1979 **Total:** £3,837 **Funding:** Medical Research Council

Grant: Equipment Grant for the purchase of scanning integrative microdensitometer **Period:** 1981 **Total:** £23,217 **Funding:** Medical Research Council

Grant: Equipment Grant for confocal microscopy facilities **Period:** 1996 **Total:** £163,000 **Funding:** Medical Research Council

CHAPTER 8 Low-intensity warfarin and thrombosis

8.1 Overview of case study grant

The grant of interest to this case study, titled 'Low Intensity Warfarin and Thrombosis', was funded from 1993 to 1996 by the Medical Research Council (MRC) of Canada (now Canadian Institutes of Health Research (CIHR)). The work conducted through this grant investigated whether patients with antiphospholipid antibodies were resistant to the anticoagulant effects of low intensities of warfarin therapy. Antiphospholipid antibodies are a heterogeneous group of autoantibodies that are detected as lupus anticoagulants or anticardiolipin antibodies. In a randomised controlled trial, the team observed the effects of three low intensities of warfarin therapy on coagulation activation, as measured by a surrogate marker, in patients with antiphospholipid antibodies and systematic lupus erythematosus. The findings from this research formed the basis for subsequent larger studies, which ultimately concluded that lower intensities of warfarin are as effective as higher intensities and identified the best range for effective treatment with the lowest risk of bleeding.

8.2 Introduction to case study

Antiphospholipid antibodies (APLAs) are a heterogeneous group of autoantibodies that are detected as lupus anticoagulants (LAs) or anticardiolipin antibodies (ACAs). Antiphospholipid antibodies occur in 35 to 45 percent of patients with systemic lupus erythematosus (SLE) and are associated with an increased risk of a first and recurrent thromboembolism (TE) (Love and Santoro, 1996). Thromboembolism is a general term that describes both thrombosis, which is the formation of a blood clot inside a blood vessel that obstructs the flow of blood, and its main complication, which is embolisation. Embolisation occurs when an object (the embolus) migrates from one part of the body through the circulation and causes a blockage, also known as an occlusion, of a blood vessel in another part of the body. In patients with APLAs and previous TE, long-term oral anticoagulant therapy is recommended (Derkson et al., 1993).

At the time of this research, warfarin was the only oral anticoagulant drug available. It was, and still is, used to prevent and treat abnormal blood clotting by reducing the amount of blood clotting factors produced in the liver. This means that blood takes longer to clot and is therefore less likely to form harmful clots in blood vessels or the heart. Anticoagulants

can also stop existing blood clots from getting any larger. They are sometimes called 'blood thinners', although this name is misleading as anticoagulants do not 'thin the blood'. Warfarin has been used for more than 50 years and by millions of people. When used properly, warfarin is an extremely valuable drug. If warfarin is not used and monitored carefully, however, it can be one of the most dangerous drugs, as the anticoagulant response is unpredictable (Bartle et al., 2007).

The international normalisation ratio (INR) is a measure of warfarin's anticoagulant effect in humans. A healthy individual has an INR of 1. The optimal intensity of anticoagulation, the INR dose range that results in the lowest combined incidence of TE and bleeding episodes, was controversial at the time of this grant, although there was some speculation that lower intensities of warfarin might be just as beneficial in preventing clots but with a lower risk of bleeding.

A retrospective cohort study had found that patients with APLAs and previous TE who received oral anticoagulants (aspirin, heparin or warfarin) with a targeted INR of 2.0–3.0, were at risk of recurrent TE (Rosove and Brewer, 1992). However, in randomised controlled trials of different anticoagulant intensities involving patients with TE or a mechanical heart valve, oral anticoagulant therapy with a targeted INR of 2.0–3.0 was found to be as effective as a higher intensity regimen (INR 3.0–4.5) in preventing TE, with significantly fewer bleeding episodes (Saour et al., 1990; Turpie et al., 1988; Hull et al., 1982).

The case study grant titled 'Low Intensity Warfarin and Thrombosis', which was funded by the Medical Research Council, was a randomised controlled trial with two main objectives:

1. To investigate whether patients with APLAs and SLE are resistant to the anticoagulant effect of three low intensities of warfarin therapy
2. To determine the lowest intensity of warfarin therapy that suppresses thrombin generation.

This research was led by Dr Ginsberg, who received his medical training from the University of Ottawa from 1972 to 1978 and then became a Fellow of the Royal College of Physicians (Canada) (FRCP(C)) in Internal Medicine in 1985 and FRCP(C) in Haematology in 1986. In 1993, Dr Ginsberg was an associate professor in the Department of Medicine at McMaster University in Hamilton, Ontario. He has been director of the Thromboembolism Unit at Chedoke-McMaster Hospital since 1989 and a member of the Thrombosis Interest Group of Canada since the early 1980s.

8.2.1 The case study approach

Information for this case study was gathered via interviews with Dr Jeffrey Ginsberg (the principal investigator (PI)), Dr Mark Crowther (a current colleague who was a haematology resident at the time), and Dianne Donovan (one of the research nurses who also acted as the research coordinator). We have also reviewed the relevant scientific literature (publications and guidelines) and the PI's curriculum vitae and obtained bibliometric data on publications the PI identified as relevant to this grant. Unfortunately, the original grant application was not preserved.

8.3 Stage 0 – topic/issue identification

The idea for this project was a result of the PI's previous training, the environment at McMaster University and the PI's previous research. These three factors are elaborated on below.

8.3.1 Previous training

Dr Ginsberg began his career in thrombosis following a research fellowship at the Canadian Heart Foundation at McMaster University, where he was supervised by Dr Hirsh. Hirsh had an established a thrombosis programme at McMaster University that has been 'pre-eminent in thrombosis research for over three decades and has trained scores of scientists who now head up thrombosis units throughout the world' (Thrombosis Clinic, 2009).

Working with Dr Hirsh, Dr Ginsberg, with Roberts[1] and Weitz[2], studied impaired fibrinolysis (the process of breaking down a fibrin clot that is the product of coagulation) and the incidence of recurrent clotting in the veins. The same team also studied the prevention and treatment of post-phlebitic syndrome, which can occur following a blood clot in a vein in the leg and is marked by swelling in the leg. Through this work with Hirsh, Ginsberg recognised a need to optimise warfarin therapy using an inexpensive, creative model, and this became a goal.

8.3.2 Environment at McMaster University

The Henderson Research Centre (HRC) was established in 1988 as a joint initiative of the Hamilton Civic Hospitals (now part of the Hamilton Health Sciences) and McMaster University. The HRC had three main programmes, which interact closely: Experimental Thrombosis and Atherosclerosis (ETA), Clinical Trials Methodology (CTM) and Clinical Thromboembolism (CTE). Its mission is 'to conduct research into the pathogenesis, prevention, diagnosis and treatment of thrombosis and vascular disease and to provide a resource for key areas of research as outlined in the mission statement of the Hamilton Health Sciences' (Henderson Research Centre, 2010). To achieve its mission, the centre works 'to carry out basic, clinical, and epidemiologic research in thrombosis, atherosclerosis, and cardiovascular disease including venous thrombosis, pulmonary embolism, coronary heart disease, arrhythmias, congestive heart failure, cardiac thromboembolism, cerebrovascular disease, and peripheral vascular disease. The scope of the research is broad, extending from the basic laboratory to the bedside and beyond into the local, national and international communities'. At the Henderson Research Centre, research 'is driven by clinically relevant problems which, if solved, have the potential to be translated rapidly into more efficacious and cost-effective patient care or preventive strategies'.

The centre also acts 'as a resource and methods centre for the design, execution and analysis of local, national and international clinical trials and epidemiologic studies in

[1] Robin Roberts is a Professor Emeritus in the Department of Clinical Epidemiology & Biostatistics at McMaster University and a senior biostatistician and Chair at the Hamilton Civic Hospitals Research Centre.

[2] Dr Jeffrey Weitz currently holds a Canada Research Chair in Thrombosis at McMaster University.

cardiovascular disease, and for other clinical programmes which are part of the strategic plan of the Hamilton Health Sciences (Henderson Research Centre, 2010). As stated by one interviewee, thrombosis is 'an industry' at McMaster University (Crowther interview, 2008).

8.3.3 Previous research

The original research for this project was spawned by Susan Denburg, who is a neuropsychologist at McMaster University. Denburg was seeing patients with SLE who had 'brain rot': a neuro-degenerative disease connected to SLE and caused by clots in the brain. Previous research by Denburg and colleagues had shown in a clinical study that individuals with SLE who had APLAs had more clots than those who did not have these antibodies (Hanly et al., 1989). That finding led to a collaborative study with the Immunology Department within the hospital at McMaster University, as a result of which the team found that patients with SLE and APLAs had higher levels of the marker prothrombin fragment 1+2 than those with SLE but no such antibodies. That finding led Ginsberg and his team to investigate whether these markers would be a good surrogate or a sensitive biomarker of thrombin generation and coagulation activation. They also wanted to know whether one could reasonably treat patients with SLE with standard-intensity warfarin or lower intensity warfarin to prevent progression of their neuro-degenerative disease.

8.4 Interface A – project specification and selection

Specifically, the study involved a randomised controlled trial:

1. To investigate whether patients with APLAs and SLE are resistant to the anticoagulant effect of three low intensities of warfarin therapy
2. To determine the lowest intensity of warfarin therapy that suppresses thrombin generation.

Suppression of thrombin generation was defined as a statistically significant reduction in prothrombin fragment 1+2 (F1+2) levels. F1+2 is derived by factor Xa from prothrombin in the prothrombinase complex during thrombin generation and coagulation activation. Using F1+2 as a sensitive biochemical marker of thrombin generation and coagulation activities, it served as a surrogate marker of a prothrombotic state. Suppression of F1+2 indicates a reduced risk for TE.

The study population consisted of patients with SLE (as defined by the 1982 American Rheumatism Association revised criteria for SLE (Tan et al., 1982)) and APLAs (as defined by positive results to a test of the antibodies on two occasions at least three months apart (Douketis, et al., 1999)). Patients with any of the following conditions were excluded from the analysis: previous venous or arterial TE, contraindication to warfarin use, a need for long-term anticoagulant therapy, pregnancy and geographic inaccessibility for follow-up blood testing.

Eligible patients were randomly allocated to receive one of four treatment regimens for four months: placebo, warfarin with a targeted INR of 1.1–1.4, warfarin with a targeted INR of 1.5–1.9 and warfarin with a targeted INR of 2.0–2.5. For each patient, blood

samples for the measurement of INR and F1+2 were obtained at baseline; one, two, three and four months after the start of treatment; and one month after the treatment was stopped. During the five-month study period, patients were followed with clinic visits or telephone contact to document any episode of TE, bleeding or acute illness. The INR was measured regularly to guide dose adjustment of the study medication so as to maintain the INR within the targeted range. The dose of the study medication was adjusted by an independent observer, according to the INR, with the aid of a warfarin normogram. The patients and physicians involved in this study, and the laboratory personnel who performed the INR and F1+2 measurements, were unaware of the patients' treatment allocation.

The study population included 21 patients: 20 were women, as SLE predominantly affects women. The patients in the sample were aged between 18 and 49 years (mean age 34.5 years) and their disease was in the quiescent phase. Twelve patients were positive for ACAs, nine were positive for LAs and six were positive for both ACAs and LAs. In all patients, persistence of ACA or LA positivity had been documented to 14 months (mean 8.7 months) prior to study enrolment. Patients were randomly allocated to one of the four treatment groups.

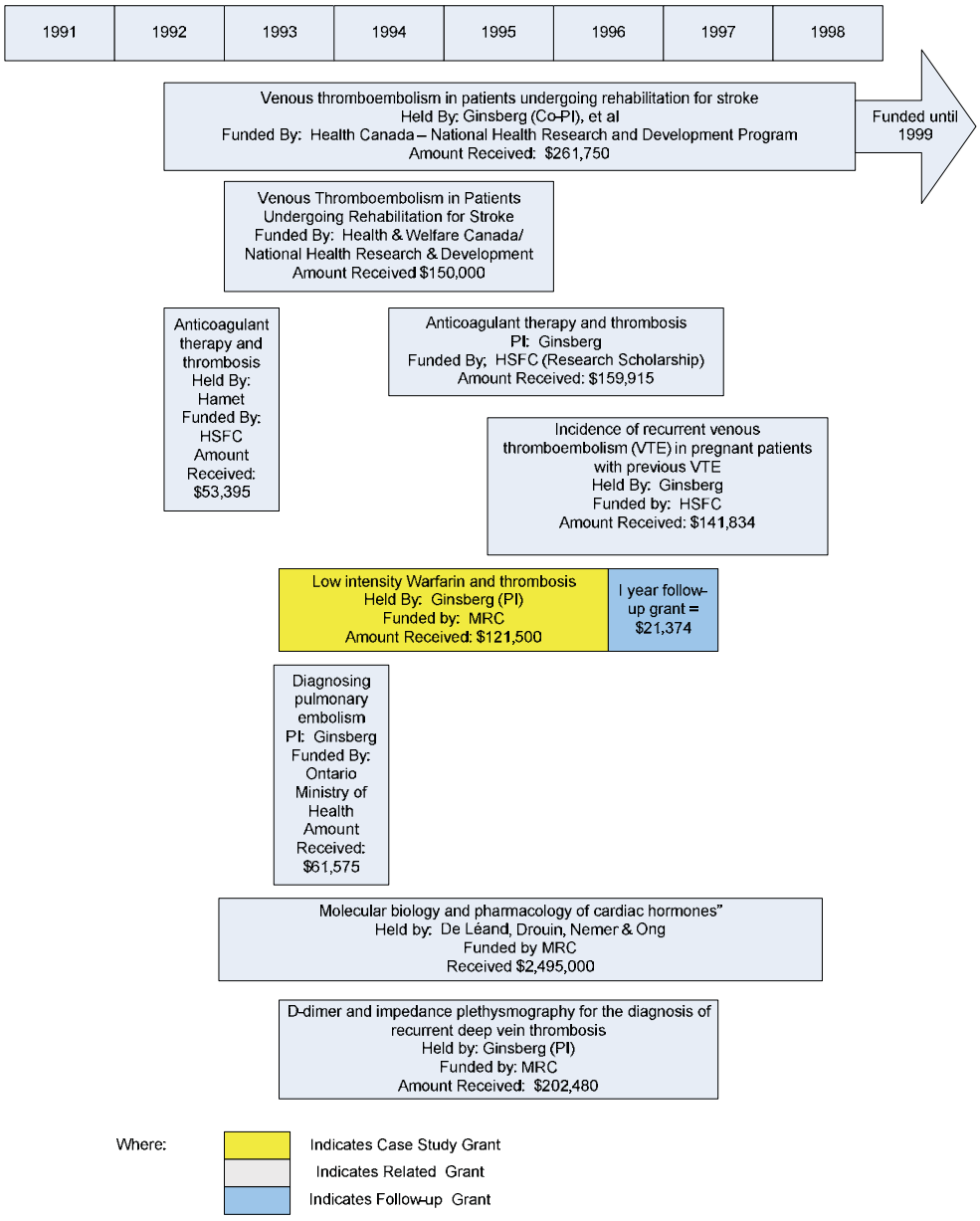
The main obstacle the team faced was getting patients to participate in their trial, because there was little personal gain expected for the patients other than more clinical oversight and making a contribution to science, so the team relied on their altruism and benevolence. Patients with SLE are often very sick, suffering miscarriages, painful and/or swollen joints, skin rashes, unexplained fever and fatigue. Although study participants were offered feedback, none requested it. Ginsberg thought that this was, in part, due to the time lag between collecting samples, creating the assays and drafting a manuscript.

Unfortunately, the original grant application was not preserved, so the author did not have access to comments made by the peer-review committee. The PI did not remember any substantive feedback or major criticisms and recalled that the proposal was well received.

The PI thought that the Heart and Stroke Foundation of Ontario (HSFO) might have funded this research if the MRC had not, although the PI mentioned that, at the time, the HSFO required more progress reports, which are time-intensive to produce.

This research fits well within the PI's ongoing research programme, as can be viewed in the Figure 8-1, which outlines all other grants held by the PI during a time period of two years prior to and two years after the funding of the grant that is the subject of this case study.

Figure 8-1 Peer-reviewed funding held by the PI from 1991 to 1998



8.5 Stage 1 – Inputs to research

8.5.1 Funding

Dr Ginsberg was a research fellow under Dr Hirsh at McMaster University from 1986 to 1988 and became an assistant professor in the Department of Medicine at McMaster University from 1989 to 1993 before becoming an associate professor in 1993. The three-year grant titled 'Low Intensity Warfarin and Thrombosis', which was funded from 1993 to 1996, thus occurred within the early stages of his career. The PI was quoted in a previous interview saying that his most trying professional moments were the 'frustration

[experienced] early in [his] career in getting funded by peer-reviewed funding agencies (Thomson Reuters, 2003).

The team requested Can$65,000 for each of the three years but received only Can$40,500 per year. Dr Ginsberg said that he did have enough to do the study and was granted funding in his first attempt (Ginsberg interview, 2008). This money did not directly pay for improvements to infrastructure, although Ginsberg says this funding did help improve communication between the clinicians and the research laboratory in terms of sending samples and receiving assays in a timely fashion.

The PI said the MRC was great and that the council had a 'good' set up in terms of trusting investigators and allowing researchers the freedom to make minor alterations to their research without having to write frequent progress reports to justify the changes. Other members interviewed indicated that they seemed to have adequate funding. McMaster University's clinical research is unique from that in most academic institutions in Canada, in that it is set up regionally, with some local money managed centrally. This money is used to ensure a high degree of stability among the research nurses and technicians by keeping their salaries consistent, thereby keeping experience within the group, which, as Dianne Donovan said 'is good for the science and the research' (Donovan interview, 2008).

Dr Ginsberg said during our interview that funding is always a challenge and thrombosis research is still begging for money. With limitations on the amount of public funding available, industry is now becoming a primary source of funding. That said, Figure 8-1 shows that the PI was awarded various grants to study thrombosis from various sources, including the MRC, Heart and Stroke Foundation of Canada and Ontario and the Ontario Ministry of Health. This figure was created using CIHR data sources, data from the Heart and Stroke Foundation of Canada and the PI's curriculum vitae.

8.5.2 Facilities

As stated above, McMaster University was very supportive, and researchers were well equipped to do thrombosis research at the Henderson Research Centre. Regionally, research was being undertaken at different sites in Hamilton, but the university coordinated and encouraged the various researchers to work together as a regional group.

The thrombosis community was very strong at McMaster University. Regional meetings were a place for Dr Ginsberg and his local colleagues, as well as those from neighbouring cities such as Toronto and London, to exchange ideas, support each other and participate in various studies. Many resources were shared within the regional group.

Space is frequently a concern, and clinical space for clinical studies is often hard to find. However, the population of interest for this study was a hyperspecialised group that was managed through an existing patient clinic. Thus the team did not require a lot of additional space or supplies. The facilities were perceived to be adequate.

Dr Crowther stated in his interview that a facilitator of the project was that the proximity of the clinics to each other (Crowther interview, 2008). Patients were largely recruited from Dr Denburg's Immunology Clinic at McMaster University. Minor contributions were made from St Joseph's Hospital in Hamilton and Mount Sinai Hospital in Toronto.

8.5.3 Techniques

Developments in laboratory testing were a great facilitator of this project, specifically with regard to the availability of new and improved assays, which were very sensitive. Using these assays, the team could look more closely both at the propensity of patients with APLAs to form blood clots, which is the root cause of TEs, and their risk of developing brain lesions. For this study the team studied D-dimer, a breakdown product of pre-forming clots. It absence implies that there is no increased clotting tendency in the body; its presence can be due to clotting.

8.5.4 Research team

This research benefitted from a group of researchers who had different interests and who formed a 'true multi-disciplinary organisation' (Crowther interview, 2008). The research team consisted of James Douketis, Mark Crowther, James Julian, Katheryne Stewart, Dianne Donovan, Elzbieta Kaminska, Carl Laskin and Jeffrey Ginsberg. The team shared patients and had common observations, so they had a common research agenda.

James Douketis did his Clinical Fellowship in Thromboembolic Disease at McMaster University from 1993 to 1996, with Dr Ginsberg as his supervisor. His prior training included a doctor of medicine degree (MD) from the University of Toronto from 1984 to 1988 and an FRCP(C) in Internal Medicine, also at the University of Toronto, from 1988 to 1993.

Mark Crowther, at the time, was a haematology resident who was interested in being trained in thrombosis – a specialised area that existed at only a few centres in Canada. Dr Ginsberg was a world leader in this area and attracted fellows. Crowther had come to McMaster University to train in haematology, because it was known as the best place to do so, and his interest in thrombosis developed from there.

James Julian was pursuing a masters degree in epidemiology and biostatistics in the Department of Clinical Epidemiology and Biostatistics at McMaster University and was responsible for analysing study outputs. Carl Laskin, MD, was in the Department of Medicine at Toronto General Hospital, University of Toronto. His involvement was through referrals of his patients to the study. This collaboration emerged through a collegial friendship and through a knowledge and appreciation of what each other was doing. Elzbieta Kaminska, a rheumatologist within the Department of Medicine at McMaster University, also referred patients to the study. Laskin and Kaminska had minimal involvement in the research project.

Katheryne Stewart, a research assistant from the Department of Medicine at McMaster University, was responsible for identifying and enrolling patients into the study. Dr Ginsberg described her as a 'very useful, helpful research assistant'. Dianne Donovan was an experienced research nurse. She coordinated this research project and helped with patient enrolment, data collection and monitoring of patients.

8.6 Stage 3 – Primary outputs from research

It is difficult to explicitly say which outputs were produced by the specific grant that is the focus of this case study. The reasons for this include the existing collaborations that were

ongoing at the time of this research, the fact that this grant was only part of a research programme at McMaster University and the continued research that occurred after the MRC grant ceased. This is evident from the fact that the paper identified by the PI as most relevant to the MRC grant funded from 1993 to 1996 was published three years later, in 1999.

8.6.1 Knowledge production

The PI identified three publications that were directly related to the MRC grant, with the first being the most relevant to the case study grant.

1. Douketis, J.D., M.A. Crowther, J.A. Julian, K. Stewart, D. Donovan, E.A. Kaminska, C.A. Laskin and J.S. Ginsberg, 'The Effects of Low-Intensity Warfarin on Coagulation Activation in Patients with Antiphospholipid Antibodies and Systemic Lupus Erythematosus', *Thrombosis and Haemostasis*, Vol. 82, 1999, pp. 1028–1032.
2. Crowther, M.A., J.S. Ginsberg, J. Julian, J. Denburg, J. Hirsh, J. Douketis, C. Laskin, P. Fortin, D. Anderson, C. Kearon, A. Clarke, W. Geerts, M. Forgie, D. Green, L. Costantini, W. Yacura, S. Wilson, M. Gent and M.J. Kovacs, 'A Comparison of Two Intensities of Warfarin for the Prevention of Recurrent Thromboembolic Events in Patients with Antiphospholipid Antibodies: Results of a Randomized Clinical Trial', *New England Journal of Medicine*, Vol. 349, 2003, pp. 1133–1138.
3. Kearon, C., J.S. Ginsberg, M.J. Kovacs, D.R. Anderson, P. Wells, J.A. Julian, B. MacKinnon, J.I. Weitz, M.A. Crowther, S. Dolan, A.G. Turpie, W. Geerts, S. Solymoss, P. van Nguyen, C. Demers, S.R. Kahn, J. Kassis, M. Rodger, J. Hambleton and M. Gent, 'Comparison of Low-Intensity Warfarin Therapy with Conventional-Intensity Warfarin Therapy for Long-Term Prevention of Recurrent Venous Thromboembolism', New England Journal of Medicine, Vol. 349, No. 7, 2003, pp. 631–639.

The first article explains the results of the team's findings related to the effect of lower intensity warfarin therapies on patients with SLE (Douketis et al., 1999). This study, which was supported by the case study grant, provided biochemical evidence that patients with SLE and APLAs are not resistant to the anticoagulant effect of lower intensity warfarin therapy (INR 2.0–2.5), as this intensity of anticoagulation is effective in suppressing activation of coagulation. The team found a significant and persistent reduction in F1+2 levels in patients receiving warfarin with a targeted INR of 2.0–2.5 but not with lower intensities of anticoagulation. In patients receiving warfarin with a targeted INR of 1.5–1.9, there was a downward trend in F1+2 levels compared with the placebo group, but the difference was significant only at the three-month time interval. The authors thus concluded that, in patients with SLE and APLAs, warfarin therapy with a target INR of 2.0–2.5 is effective in suppressing coagulation activation and so might be effective in preventing thromboembolic events. This conclusion was based on the assumptions that:

- elevated F1+2 levels are a reliable surrogate marker of coagulation activation and a prothombotic state
- suppression of F1+2 levels is a reliable predictor of a reduced risk for TE.

The results of the MRC-supported project led to a much larger randomised double-blind trial involving 114 patients with APLAs and previous thrombosis to test the effects of high-intensity warfarin on thrombosis prevention as described in the second publication listed above (Crowther et al., 2003). This study, also known as the Patients with Antiphospholipid antibodies: Prevent Recurrent Events (PAPRE) trial, concluded that high-intensity warfarin was not superior to moderate-intensity warfarin in preventing recurrent thrombosis in patients with APLAs. The team observed a low rate of recurrent thrombosis among patients in whom the INR target was 2.0–3.0, which suggests that moderate-intensity warfarin is appropriate for patients with APLAs.

The preliminary work of this pilot study was the impetus for subsequent studies and a much larger clinical trial – known as the Extended Low-Intensity Anticoagulation for Thrombo-Embolism (ELATE) study – that was funded by CIHR and has changed the way many clinicians use warfarin in practice and in further research. Listed above as the third reference (Kearon et al., 2003), this paper reported the results of a randomised double-blind study, in which 738 patients who had completed three or more months of warfarin therapy for unprovoked venous thromboembolism were randomly assigned to continue warfarin therapy with a target INR of 2.0–3.0 (conventional intensity) or 1.5–1.9 (low intensity). Patients were followed for an average of 2.4 years. The team found that 16 of 369 patients assigned to low-intensity therapy had recurrent venous thromboembolism (1.9 per 100 person-years) compared with six of 369 assigned to conventional-intensity therapy (0.7 per 100 person-years) (hazard ratio 2.8 (95 percent confidence interval 1.1 to 7.0)). A major bleeding episode occurred in nine patients assigned to low-intensity therapy (1.1 events per 100 person-years) and eight patients assigned to conventional-intensity therapy (0.9 event per 100 person-years) (hazard ratio 1.2 (95 percent confidence interval 0.4 to 3.0)). There was no significant difference in the frequency of overall bleeding between the two groups (hazard ratio 1.3 (95 percent confidence interval 0.8 to 2.1)). The team concluded that conventional-intensity warfarin therapy (INR 2.0–3.0) is more effective than low-intensity warfarin therapy (INR 1.5–1.9) for the long-term prevention of recurrent venous thromboembolism in patients who have had unprovoked venous thromboembolism, reducing their risk by about two thirds. Therefore, the low-intensity warfarin regimen does not reduce the risk of clinically important bleeding. These findings were consistent among various subgroups.

Bibliometric analysis was conducted on the three publications identified by the PI as directly related to the case study grant (Table 8-1).

Table 8-1 Publication output and impact

Number of journal articles:	3
Number of articles included in citation analysis:	3
Total number of citations (all papers):	339
Aggregate relative citation impact:	5.87 (Class V)
Self-citations:	10%

Distribution of publications across relative citation impact classes:

	Class I (uncited)	Class II (>0 and <0.8 citations)	Class III (0.8 to 1.2 citations)	Class IV (1.2 to 2.0 citations)	Class V (>2.0 citations)
Number of publications		1			2
Proportion of total output		33%			66%

Most highly cited publication[3]:	Kearon, C., J.S. Ginsberg, M.J. Kovacs, D.R. Anderson, P. Wells, J.A. Julian, B. MacKinnon, J.I. Weitz, M.A. Crowther, S. Dolan, A.G. Turpie, W. Geerts, S. Solymoss, P. van Nguyen, C. Demers, S.R. Kahn, J. Kassis, M. Rodger, J. Hambleton and M. Gent, 'Comparison of Low-Intensity Warfarin Therapy with Conventional-Intensity Warfarin Therapy for Long-Term Prevention of Recurrent Venous Thromboembolism', *New England Journal of Medicine*, Vol. 349, No. 7, 2003, pp. 631–639
Times cited:	221

8.6.2 Dissemination

The team at McMaster University is a very prominent and productive group in the area of thromboembolism research. Dr Ginsberg claimed that the thromboembolism group at McMaster University is 'the best in the world' (Ginsberg interview, 2008). This reputation assists in their dissemination activities, their research findings are widely known and they tend to chair meetings of the international societies and write review articles. This recognition started with Jack Hirsh, who is an 'internationally recognized clinician and scientist in anti-coagulant therapy and thrombosis' (McMaster University, 2009). He came to McMaster University in the early 1970s and established a centre of excellence. The university is known to have high standards and expects people who work there to be high achievers. These standards and expectations have helped the university maintain a high profile worldwide.

The main method of disseminating the findings from the case study grant was via publications. Dr Ginsberg said that the paper published in 1999 in the journal *Thrombosis and Haemostasis* is read by patients (clotters and bleeders) and haematologists who are interested in blood-thinning therapy (Douketis et al., 1999). Ginsberg claimed there was an international reach, which, he says, typically happened with his thrombosis trials. He claims to be well known in Holland, Europe, the US and other countries.

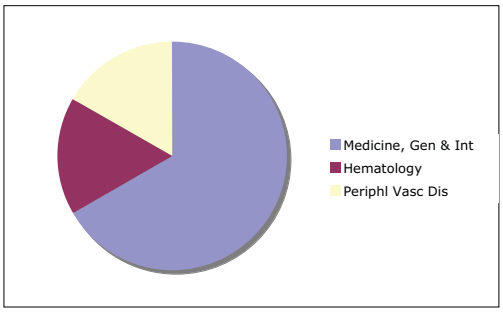
[3] Citation count extracted April 2009.

With regard to this specific study, Dr Ginsberg and/or members of his team did present their papers and conduct poster presentations nationally and internationally, although none was a keynote or invited speaker at a conference. This research has not been covered by the media, and the team did not present their information in a forum directed to health policymakers. Ginsberg told us that there were informal interactions and discussions with potential users of this research, such as clinicians, practitioners and other academics within McMaster University, and through networking at various meetings and conferences.

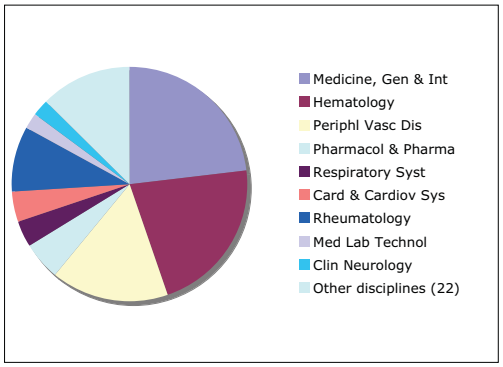
The bibliometric analysis also investigated knowledge diffusion. As anticipated, it was found that Dr Ginsberg and his team most commonly publish in the area of general and internal medicine. Their work is most commonly cited by those also working in the same area of medicine in the US. The work has been cited by researchers working in 21 different countries.

Figure 8-2 Knowledge diffusion: fields of research of output (a), fields of citing papers (b) and countries of citing papers (c)

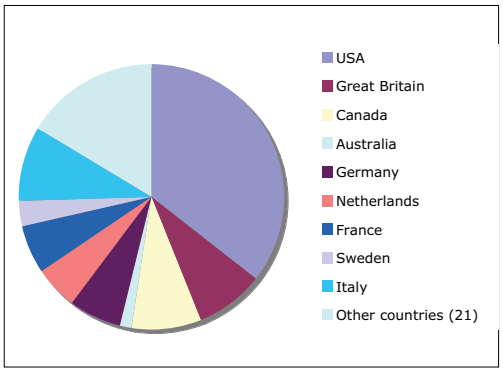
(a)



(b)



(c)



8.6.3 Training and capacity building

Overall, Dr Ginsberg told us that this project, in addition to the other ongoing projects and the reputation of McMaster University, did help his ability to recruit and expand his research group. He did not believe that this specific grant helped influence the reputation of the research group, although this project was comparable with other ongoing projects. He also explained that having support from the MRC always adds a level of prestige to their research and helps in attaining subsequent funding.

Although this pilot study intended to explore the utility of different levels of warfarin, the team found that traditional levels of warfarin were best. This confirmation of ongoing practices prevented the team and other researchers from wasting additional time and money exploring lower intensity doses.

Dr Ginsberg is currently a professor in the Department of Medicine at McMaster University in Hamilton, Ontario. He has been the director of the Thromboembolism Unit at Chedoke-McMaster Hospitals since 1989. In 1998, Ginsberg received the Stephen Garnett Distinction Award for Outstanding Physician, which was awarded by the medical staff at Hamilton Health Sciences. He also serves as a career investigator for the Heart and Stroke Foundation of Canada.

Dr Ginsberg's research interests are still focused on the clinical complications of APLAs, although he is no longer using surrogate markers. Ginsberg claimed that the use of markers has fallen out of favour because clinical outcomes are thought to be more reliable and are preferred. His current research interests also include the clinical development of novel antithrombotic agents (some of this research involves industry support), the diagnosis and management of thrombosis during pregnancy, the prevention and treatment of post-phlebitic syndrome and the diagnosis of venous thrombosis and PE.

Dr Crowther says that this project was really important to him. The results of the work supported by the MRC grant were mirrored through a larger follow-up study (described below), which was funded by the CIHR. Crowther took over the project as the PI in 1999 and the project went on to be published in the *New England Journal of Medicine* in 2003, ten years after the initial research commenced (Crowther et al., 2003). Crowther stated that publication in this journal has had a giant impact on his career and claims he is now one of the world experts in the area.

Dr Crowther is still looking for optimum therapies for patients. In 2007, his team published some of their findings in *Blood*, the leading subspeciality journal, in a paper called 'How We Diagnose and Treat Thrombotic Manifestations of The Antiphospholipid Syndrome: a Case-Based Review' (Garcia et al., 2007). It is a recommendation to the haematologists of the world about how patients should be treated, which, Crowther says, springs directly from the grant investigated by this case study. Crowther has written many of the recommendations for care of patients with APLAs over the last ten years (for example: Crowther, 2004, and Schunemann et al., 2004).[4]

8.6.4 Benefits to future research and research use

Dr Ginsberg said that although the MRC grant funded a small study, it changed the way many researchers used warfarin in clinical trials. Two such clinical trials were the PAPRE study and the ELATE study, which were discussed previously. The findings from both of these studies were consistent with those of the Prevention of Recurrent Venous Thromboembolism (PREVENT) study, a placebo-controlled trial in some of Ginsberg's

[4] Dr Crowther continues to publish prolifically in the area of antiphospoholipid syndrome. Although outside the temporal scope of this case study, it should be noted that publications directly related to the case study research continue to flourish, most notably a current review article: Ruiz-Irastorza G, M. Crowther, W. Branch, and M.A. Khamashta. "Antiphospholipid Syndrome." *Lancet* (2010).

patients, which evaluated warfarin therapy with a target INR of 1.5–2.0 for extended treatment of patients who had had unprovoked venous thromboembolism. Among patients assigned to low-intensity warfarin therapy in the PREVENT study, the rate of major bleeding was 0.9 per 100 person-years and the rate of recurrent venous thromboembolism was 2.6 per 100 person-years; these rates are similar to those in the low-intensity–therapy group (1.1 and 1.9 events per 100 person-years, respectively) (Ridker et al., 2003).

Collectively, the results of the PREVENT, ELATE and PAPRE studies, which all investigated different intensities of warfarin, suggested that low-intensity anticoagulant therapy reduces the risk of recurrent thrombosis by about 75 percent, whereas conventional-intensity therapy reduces this risk by more than 90 percent. These studies, which all investigate optimum dose setting, relate back to the MRC-supported project, in which Dr Ginsberg and his team had previously shown in a smaller pilot study that low-intensity warfarin was better than placebo but not optimal.

8.7 Stage 4 – Secondary outputs

The above findings are included in the guidelines for thrombotic practice in North America from the American College of Chest Physicians (ACCP) Consensus Conference on Anti-thrombotic Therapy (Schunemann et al., 2004). Dr Crowther is one of the authors on the chapter about warfarin anticoagulants. Recommendation 2.7.1 is drawn from the PAPRE study, which is based on the MRC grant.

In addition, the journal *Chest* recently published a paper from a Consensus Conference on Anti-Thrombotic Therapy that included broad recommendations for anti-thrombotic therapy across several different patient populations (Hirsh, Dalen and Guyatt, 2001). These recommendations are widely accepted as being useful around the world, and the recommendations for warfarin, although indirectly related to the case study grant, were influenced by the findings of the case study grant.

Dr Ginsberg also explained that the research community has made a lot of effort to educate patients and practitioners. The team has been involved in writing pamphlets pertaining to dose setting and misconceptions held by practitioners about the fundamentals of warfarin (such as targeting an INR of 2.0–3.0) and monitoring of INR through blood test results.

8.8 Stage 5 – Adoption by practice and the public

In the 1980s and early 1990s, doctors had a one-size-fits-all approach to treating thrombosis: the first episode of clotting would be treated for three months, the second for a year and the third for life. Based on the results from basic research and clinical studies, in part resulting from the case study grant, practitioners now know that patients can be categorised according to the risk of recurrence. Clots are being treated better today and doctors now know which INR to target; a lower INR is less effective and there is no evidence of a lower bleeding rate with a lower INR. An INR range of 2–2.5 yields the best range for effective treatment and a reduced risk of bleeding.

This work has also had a huge impact on the care of patients with APLAs. The guidelines mentioned above recommend a certain treatment path for these patients, which is based on the original research conducted within the grant first funded by the MRC in 1993. It has affected patient care around the world (Ginsberg interview, 2008).

8.9 Stage 6 – Broad health and economic outcomes

Standard therapy to prevent recurrent venous thromboembolism includes 3–12 months of treatment with full-dose warfarin with a target INR of 2.0–3.0. However, for long-term management, no therapeutic agent has shown an acceptable benefit-to-risk ratio (Ridker, 2003).[5]

8.10 Payback

The payback framework describes the process from research to several categories of impact. The categories allow us to compare across different case studies. Table 8-2 shows, in point form and by impact category, some of the impacts, described more fully above, that have emerged from this grant.

Table 8-2 Payback

Payback category	Impacts from case study
Knowledge production	• Three related peer-reviewed articles • Papers presented and poster presentations at various meetings
Research targeting and capacity building	• Informal methods of knowledge transfer within the laboratory, organisation and colleagues at meetings • Douketis obtained his MSc in Biostatistics
Informing policy and product development	• Refinement of assays
Health and health sector benefits	• Work is referenced in guidelines for warfarin therapy • Clots are being treated better today and doctors now know which INR to target • The treatment path for patients with APLAs is much improved
Broader social and economic benefits	• Refer to Section 1.7

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[5] In follow-up correspondence with Dr. Crowther, who now leads the research programme, he stated that: "There is no doubt that this work has modified and improved the treatment of patients around the world with APS". Although beyond the scope of this case study, ongoing publications in the field clearly support this statement.

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CHAPTER 9

Analysis of the glucose transporter compliment and function in metabolically important tissues of the Milan hypertensive rat

9.1 Introduction to the case study

9.1.1 Overview

At the time of this grant, it was known that there were links between diabetes and heart disease, but these links were not well understood at the molecular level. One link was the hormone insulin, which is chiefly responsible for regulating blood sugar levels but also has effects on lowering blood pressure by dilating blood vessels. Insulin decreases blood sugar levels by activating the glucose transporter protein GLUT4, which then transports sugar out of the bloodstream and into body tissues for storage. This grant aimed to investigate whether GLUT4 was also important in how insulin affected blood pressure, using the Milan rat as an animal model of high blood pressure. The team was particularly interested in whether defects in glucose transport protein expression exist in hypertension and whether these defects may, in fact, have a role in causing the condition. Although the study resulted in some publications, the findings of the research were inconclusive. However, an important contribution of the study is that it provided the methodological underpinning for additional research and helped the researchers involved develop their careers.

9.1.2 Background

This case study examines the evolution and impacts of a British Heart Foundation (BHF)-funded basic research grant between 1994 and 1996. The principal investigator (PI), Professor Gwyn Gould, was then a senior lecturer and reader at the Lister Institute for Preventative Medicine (Department of Biochemistry and Molecular Biology, University of Glasgow). He started his career as a cell biologist with specific expertise in protein biochemistry and has continued to pursue interests in this field until the present day. After completing undergraduate and doctor of philosophy (PhD) qualifications at the University of Southampton (Department of Biochemistry) and a postdoctoral degree at Dartmouth Medical School (in the United States), he joined the University of Glasgow in 1989. Since 2005, he has been a professor of biochemistry and molecular biology at the university.

This grant supported research into the cellular and biochemical basis of hypertension. The research team wanted to explore whether there was a link between hypertension and defects in carbohydrate metabolism (ie the processes by which various sugars are formed, broken down and interconverted in living organisms).

Glucose transport proteins play an important role in the metabolism of carbohydrate. When levels of sugar in blood are high, they transport glucose out of the blood stream and into the tissues (eg muscle and fat cells) for conversion into glycogen (a source of stored energy). When blood sugar levels are low, they do the reverse, transporting glucose from the tissues, where it had been stored as glycogen, back into the bloodstream for use as an energy source by the body.

There are different types of glucose transport proteins; one of them (GLUT4) responds to insulin's signals. Insulin is a hormone that plays an important role in controlling and regulating carbohydrate metabolism. In a healthy state, insulin 'instructs' GLUT4 to bind glucose and take it to fat and muscle for conversion into glycogen. When there is insulin resistance in muscle and fat tissues, GLUT4 does not respond properly to insulin's signals. As a consequence, levels of glucose in the bloodstream remain abnormal. This is associated with conditions such as diabetes.

In a healthy body state, insulin also acts as a vasodilator (ie it widens blood vessels), but when insulin resistance is present, the vasodilatory properties are overridden. As a result, insulin resistance is also associated with hypertension (ie high blood pressure).

By the time of the grant, it was known that:

- individuals with mild forms of insulin resistance are at increased risk of heart disease[1]
- individuals with insulin resistance-related diabetes often also develop heart disease (eg coronary heart disease and artherosclerosis)
- there are defects in glucose transport protein expression in patients with diabetes.

Professor Gould suggested that the relationship between diabetes and cardiovascular disease was a 'hot research topic' at the time of the grant (Gould interview, 2008). He was keen to explore whether defects in the expression of insulin-responsive glucose transport proteins (eg GLUT4) might be implicated in hypertension, as they were in diabetes. Gould's laboratory had built up expertise in glucose transport and diabetes research but had not carried our work in the cardiovascular field specifically. Gould wanted to apply his knowledge and experience to this new challenge in cardiovascular research. To avoid

[1] The metabolic syndrome (a combination of medical disorders including diabetes and cardiovascular disease) was discovered and gained interest as a topic for research. In 1988, Gerald Reaven (a endocrinologist in the United States) proposed insulin resistance to be a key underlying factor of the increased risk of type 2 diabetes, heart disease and stroke. He called this group of associated conditions: Syndrome X. Today, the terms 'metabolic syndrome', 'insulin resistance syndrome' and 'syndrome X' are often used interchangeably. Most of the patients with metabolic syndrome were older, obese and had a degree of insulin resistance. Although the causes of the syndrome were unknown (and remain so today), it was known to be associated with ageing, genetic factors and lifestyle conditions (eg high calorie diet and little exercise). For an overview of the metabolic syndrome, see Alberti (2005).

confusion with other projects Gould conducted, we refer to this study as the 'hypertension project'.

The case study approach

The key sources of information for this case study were interviews with the PI and a PhD student who was a key researcher on the grant; Professor Gould's curriculum vitae; relevant scientific literature (publications)l and bibliometric data. Unfortunately, all grant-related documents (applications and interim and final reports to the BHF) were destroyed in 2002, so we could not review the information from archival reports.

9.2 Stage 0 – topic/issue identification

There were three key interrelated influences on Professor Gould's identification of the research topic and decision to pursue the study. These were:

1. opportunistic motivations: the ability to conduct research in a 'hot topic' field
2. scientific curiosity
3. PI's prior research.

We elaborate on each below.

9.2.1 Opportunistic motivations

As mentioned previously, research into the links between diabetes and hypertension was topical in the early 1990s. Professor Gould said, 'Without a doubt, there was a significant opportunistic element with this grant. All the cards stacked…the money was there, we had the skills, it was a very hot topic to research…it was the right project, at the right time' (Gould interview, 2008).

There were a number of enabling conditions and strategic considerations in choosing to pursue the project:

- availability of experimental resources (ie access to animal models) – a colleague at the Cardiovascular Research Centre at Glasgow University had established a colony of hypertensive rats, which also ended up showing insulin resistance (ie the hypertensive Milan rat)[3]; she was willing to provide Gould access to the animals, which were a suitable model for the experimental work he intended to conduct
- funding environment – the proposed research was topical and according to Gould of interest to biomedical research funders globally; in addition, the BHF was known to be funding research (in general) at high levels at the time and with high success rates for applicants

[3] The Professor had purchased the rat strain from the United States and established an animal facility in her laboratory. Professor Gould had first come across the strain a couple of years earlier, when he was asked to (informally) assist a cardiovascular medicine researcher at the cardiovascular research centre with biochemistry aspects of a study she was then conducting on the Milan rat.

- opportunity for high impact – Gould felt that the research could make an important contribution to the field of hypertension and be highly cited (if they could show a link between defects in glucose transport protein expression and hypertension)
- institutional environment – at the time of this grant, there was a drive by Glasgow University to increase cross-faculty interactions; access to the colony of rat models at the medical school would provide an opportunity for interactions between the Department of Biochemistry and Molecular Biology and the Cardiovascular Research Centre at the University, which housed the animal laboratory, and the medical school would assist with genetic analyses of the animal model.

9.2.2 Scientific curiosity

Professor Gould's core research interest was in cellular signalling[4] in health and disease. Having worked on the role of cell signalling and glucose transport in diabetes for a number of years, he found the opportunity to explore glucose transport in a new area such as cardiovascular disease intellectually attractive. He familiarised himself with research advances in the cardiovascular disease field by reading relevant literature.

In addition to prior research in Gould's laboratory (see Section 9.2.3), a number of scientific advances that had accumulated by the time of the grant provided the knowledge foundation for the hypertension project (see Figure 9-). Most notably, these included:

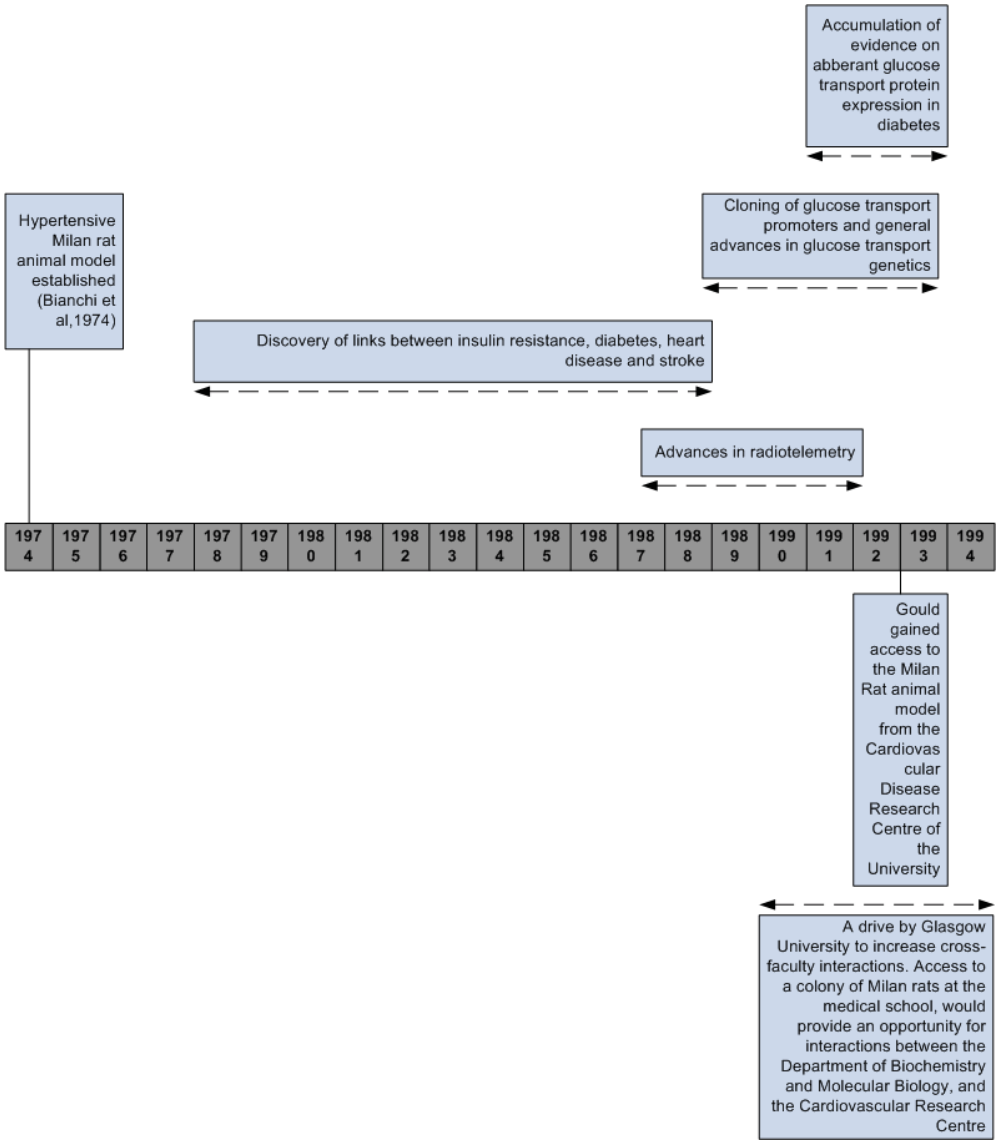
- the discovery of links between insulin resistance, heart disease and stroke (eg Haller, 1977; Phillips, 1978; and Reaven, 1988) and an accumulation of clinical studies in human populations on these links (by a large number of research groups predominantly in the United States)
- advances in the genetics of glucose transport, including the cloning of glucose transport protein promoters in the 1990s (eg Fukumoto et al., 1988; Kaestner et al., 1990[5]), which allowed for more sophisticated molecular and cell biology studies
- an expanding body of evidence describing the aberrant expression of glucose transporters in diabetes associated with insulin resistance, which included studies carried out by Gould's group and other groups (eg Chen et al., 1990; Gould et al., 1992; and Gould and Holman, 1993)
- the availability of new research tools that enabled more sophisticated studies in animal models (eg radiotelemetry – a technique that allowed the blood pressure of a rat to be remotely monitored and reported over 24 hours)
- the development of more sophisticated animal models for hypertension research.

[4] Cell signalling is part of the communication system that controls cell activities and coordinates their actions. Cells are told what to do, when to do it and how to do it through various signals.

[5] The research was published at the same time by a large number of different groups in the United States, which makes attribution difficult. The references provided are two frequently cited examples.

Figure 9-1 illustrates a timeline of key developments that influenced idea generation for the hypertension project. General scientific developments are presented above the timeline, whereas influences specific to Gould's home institution and engagements are presented below the timeline.

Figure 9-1 A timeline of key developments that influenced idea generation for the hypertension project



9.2.3 PI's prior research

Prior to the grant we investigated, Professor Gould had received a one-year grant from the BHF to examine potential defects in glucose transport proteins in the Milan rat (1993–1994). Although the ultimate purpose of that grant was strikingly similar to the study we are investigating, the predecessor grant essentially served to confirm that the Milan rat was a suitable experimental model for studies of carbohydrate metabolism defects in

hypertension. The research showed that the techniques and equipment with which Gould's laboratory had experience could be used to conduct research with this animal model.

Although contractually distinct, the case study grant and its predecessor grant were perceived as one by the researchers involved. The research team on the predecessor grant was the same as that in the hypertension project.

In addition to the BHF predecessor grant, two other projects (on which Gould was the PI) influenced the hypertension project. Both of these grants involved a postdoctoral researcher in Gould's laboratory, and although they did not feed directly into the hypertension grant, they provided the laboratory with additional expertise.

In the first grant, which started in 1992, the post-doc became involved with a project on the role of the GLUT5 glucose transport protein in diabetes.[6] This project was funded by the Scottish Hospitals Endowment Research Trust (SHERT) between 1992 and 1994. The post-doc was a clinician by training who (at the time) had left medical practice because of a desire to pursue research activity. During the course of the SHERT project, he developed a strong interest in glucose transport across conditions and tissues and was keen to look at transport mechanisms not only in diabetes but also in hypertension. Although the findings from the SHERT grant did not directly feed into the idea for the BHF hypertension project, the experience and skills that he developed during the SHERT study significantly influenced how the hypertension project evolved.

In the second grant, which started in 1993, the post-doc assumed a key role on another study funded by the MRC (1993–1996). Together with Professor Gould, he investigated the structure of various transport proteins in fat cells and oocytes (ie egg cells) to identify functional motifs (ie shared structures between various proteins). As per the SHERT grant, the skills and experience he gained through the MRC study were transferred and applied to the BHF hypertension project.

When the opportunity arose to apply for the grant for the BHF hypertension project (1994–1996), then, as he had done with the predecessor BHF grant (1993–1994), the post-doc agreed to contribute to the study. Although his involvement was informal (ie non-contractual[7]), he regularly mentored and helped the more junior researcher who was pursuing his PhD through the hypertension research.

Figure 1-2 shows research grants Professor Gould held at the time of the hypertension project. The grants that directly influenced the idea to pursue the BHF hypertension project are represented in white blocks. The future research that was directly influenced by the hypertension project has an arrow pointing to it. This is an MRC-funded study on the genetic and molecular basis for insulin resistance and hypertension (elaborated on in Section 0).

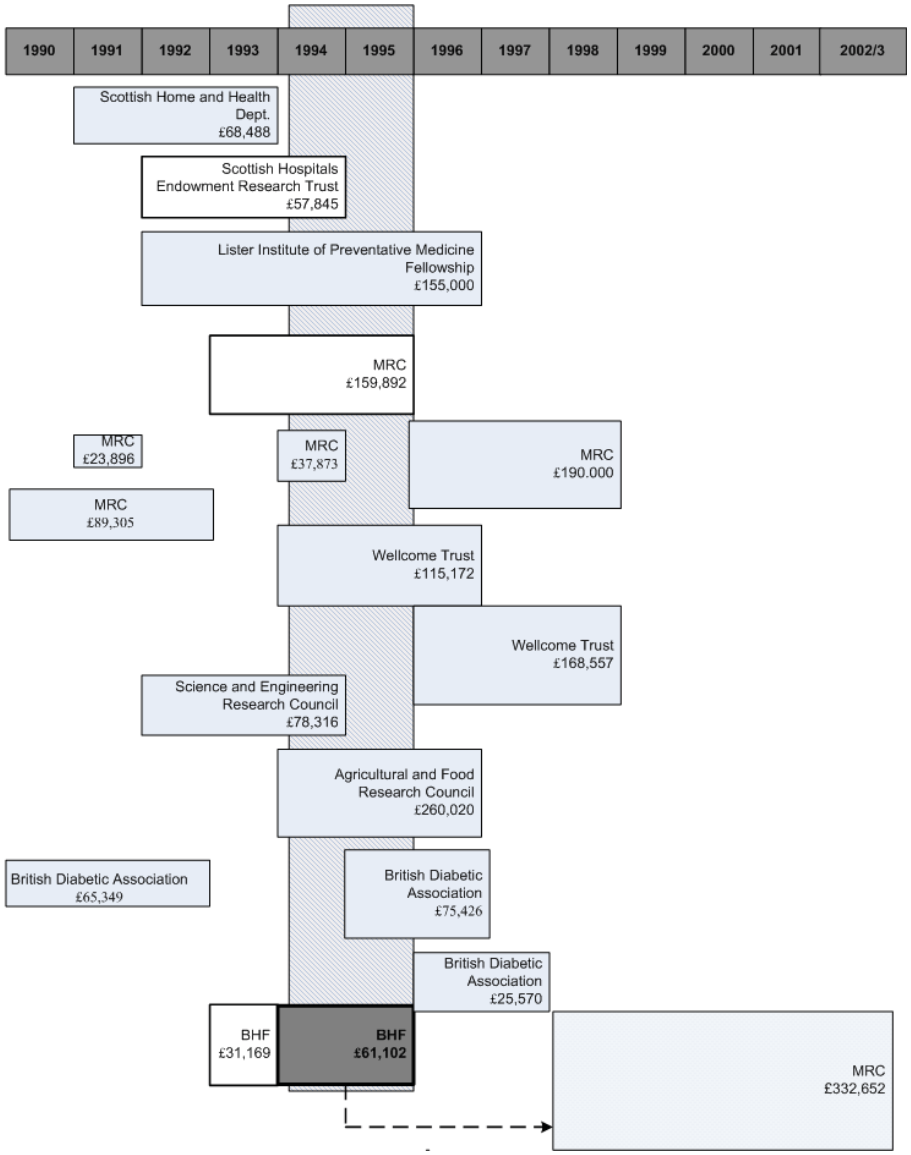
An accompanying list of the project grant titles is provided in Appendix A. It reflects the core focus of Professor Gould's group on cell-signalling research and especially glucose

[6] GLUT5 is found in intestinal cells and it is not stimulated by insulin.

[7] Dr Livingstone was not named on the grant.

transport in healthy and insulin-resistant states. A list of equipment grants[2] is presented in Appendix B of the case study.

Figure 9-2 Overview of the PI's grant landscape in the 1990–1999 time window



9.3 Interface A – project specification and selection

Professor Gould had the original idea for the hypertension project. Using the Milan rat as an animal model of high blood pressure, he wanted to explore whether there was a link

[2] The equipment grants were not from the BHF, but the equipment available in the laboratory was utilised across projects.

between hypertension and defects in carbohydrate metabolism and – more specifically – to investigate whether GLUT4 was important in how insulin affected blood pressure.

Professor Gould drafted the project specification and the proposal to the BHF, with some input and feedback from researchers involved in the predecessor BHF grant The project collaborator at the Cardiovascular Research Centre also provided input; she was to assist with any potential genetic studies on the animal model, which were to complement the largely cell biology and biochemical investigations.

Professor Gould did not recall the BHF requesting any modifications to the original proposal. The PhD student commented, 'In essence, this was an extension to our previous BHF grant. Although we did have to put in a formal proposal, it was all quite straightforward' (Interview, 2008).

The informants could not comment on whether anyone else would have funded the project had the BHF refused support. Although the topic of research was 'popular' (Gould interview, 2008), cardiovascular research was not a core focus in Gould's laboratory (ie diabetes was): '[Cardiovascular research] was a side-stream'. Professor Gould believes they could have 'bent the application' to receive grants from a diabetes research funder (eg Diabetes UK), but they did not have any intention of doing so.

According to both Gould and the PhD student, the peer review process was fair.

9.4 Stage 1 – inputs to research

The key facilitators and inputs into the study were grant funding; staff knowledge and expertise; time for conducting the study; techniques, consumables, collaborators; and reputation.

9.4.1 Funding

The project was primarily facilitated by £61,202 of BHF funding. This allowed the PhD student to pursue his PhD with the grant support and covered the costs of reagents and consumables. The post-doc's involvement was informal (ie non-contractual and not covered by the BHF grant). The level of financial support for staff was comparable to that provided by other grants Professor Gould held at the time[9].

9.4.2 Knowledge/expertise

According to Professor Gould, all staff where highly committed, dedicated, knowledgeable and productive.

9.4.3 Time

In terms of duration, this grant differed from most grants Professor Gould held at the time, which were for three years (eg see Figure 1-2), whereas the hypertension project grant was a two-year grant. The investigators had to first validate the feasibility of the study with

[9] Dr Livingstone's postdoctoral work included research on number of projects in Professor Gould's laboratory, and he was supported by other grant sources.

a predecessor one-year grant (also from the BHF) and then apply for the two years of BHF support to conduct the hypertension research project.

9.4.4 Techniques

The key cell biology and biochemistry methods used in the project included immunohistochemistry (IHC) techniques and confocal microscopy, cell fractionation and protein quantification. These were relatively basic and standard techniques in cell biology at the time. Most of the equipment used in the project was already available in the laboratory and not sourced via the BHF grant. According to the post-doc, some minor pieces of equipment (eg an ultracentrifuge) had to be purchased through internal resources.

9.4.5 Collaborators

There was one formal collaboration on the grant between the Department of Biochemistry and Molecular Biology and the Cardiovascular Research Centre at the university. The contribution of the latter to the project was limited to the provision of the Milan rat animal model and some assistance with genetic analysis of the animals.

9.4.6 Consumables

Some reagents were provided by external laboratories in the United Kingdom (eg from a colleague of Professor Gould's at Bath University) and abroad (University of Queensland, Australia). However, these organisations were not formal (ie contractual) collaborators in the study (despite researchers from the Australian group being listed as co-authors on one of the resulting publications).

9.4.7 Reputation

Professor Gould could not comment on whether his own reputation helped in securing the grant but thought that the reputation of the Professor (who was then Chair of the Cardiovascular Research Centre) may well have been helpful. Gould was becoming established at the time but was rather young (32 years old). He commented that this 'could have been an advantage in terms of donors wanting to give a young researcher a chance' (Gould interview, 2008) (and he believed this was the case with his earlier grants) but could not do more than speculate on the issue.

9.5 Stage 2 – research process

According to Professor Gould, the research process was straightforward and productive. Interactions with the funder (BHF) were limited to progress reports (unfortunately the documents have been destroyed so we could not assess how the research was received by the BHF). The commitment and enthusiasm of the investigators maintained a positive team dynamics and 'good momentum' throughout (Gould interview, 2008). He commented: 'I was very lucky to get such committed and driven people to work on this. Attracting good people is often down to luck and not easy. On the one hand it is a self-fulfilling prophecy. If you get good people, you have a higher chance of doing good work, which in turn helps you get more good people. Unfortunately, sometimes the outputs have little to do with the quality of the researchers. You can have really good researchers, but an

unlucky project that leads to negative findings and no major discoveries' (Gould interview, 2008).

9.6 Stage 3 – primary outputs from research

The primary outputs from the project fall into two categories of the payback model: knowledge production (category 1) and benefits for future research and research use (category 2).

9.6.1 Knowledge production

At the time of the interview, Professor Gould felt that the outputs were modest, 'because the study did not reveal anything groundbreaking and is unlikely to ever be highly cited because of the negative findings' (Gould interview, 2008). He referred to the outputs as a 'meat and two vegetable grant, not terrible but not brilliant, sort of solid'.

The key findings from the BHF hypertension project were inconclusive:

- Although reduced levels of the GLUT4 transporter were observed on the surface of muscle cells in hypertensive Milan rats, there seemed to be no significant reduction in the fat cells (Campbell et al., 1995, and Rice et al., 1996).
- Levels of some other glucose transport proteins (including those which are not affected by insulin) were not altered in fat and liver cells, but some transport protein levels were profoundly altered in brain tissues (Campbell et al., 1995; Livingstone et al., 1995; and Livingstone et al., *Proceedings of the Nutrition Society*, 1996).

The team had 'ideally hoped to find that defects in glucose transport protein expression exist in hypertension and to see whether these defects may in fact be involved as a cause' of the condition (Gould interview, 2008). However, the findings failed to establish whether changes in GLUT4 levels were linked to, or affected, hypertension.

In addition, a later MRC-supported study (elaborated on in 0) showed that the observation of reduced levels of GLUT4 on the surface of muscle cells (Campbell et al., 1995, and Rice et al., 1996) did not have to do with defects in protein expression bur rather with a defect in the mechanism of transporting the GLUT4 to the cell membrane (Collison et al., 2005).

Some other findings that emerged from the experimental observations made during the project were also reported in peer-reviewed journals. For example:

- The researchers found that the intracellular pool of GLUT4 that is available to respond to insulin is lower in human than in rat adipocyte cells (Kozka et al., 1995) and suggested that this is the reason why human adipose cells are much less responsive to insulin stimulation of glucose transport activity than rat cells.
- The researchers also tried to elucidate the pathway by which GLUT4 reaches the cell surface upon insulin stimulation, by examining the co-localisation of GLUT4 and other proteins (membrane proteins and receptors). They found only limited

co-localisation (Livingstone et al., *Biochemical Journal*, 1996, and Martin et al., 1996).

In total, the BHF project produced eight journal articles[10], one meeting abstract and two editorial material publications.

One of the papers, although classified as an article, was, according to Professor Gould, actually a review of current knowledge on mammalian glucose transporters, intracellular signalling and transporter translocation, which included insights pertaining to GLUT4 implications in hypertension (Arbuckle et al., 1994).

One of the articles was, in fact, from the later MRC (1998–2003) grant but was also attributed to the BHF study because of significant knowledge and methodological inputs (Collison et al., 2005).

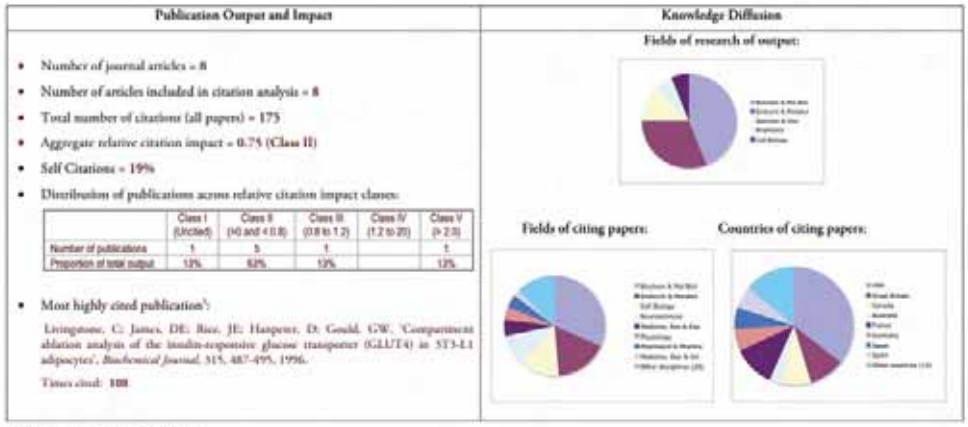
Interestingly, only one of the journal articles attributed to the BHF study is authored exclusively by the core hypertension project research team (Campbell et al., 1995). The other articles are predominantly authored by the hypertension project researchers and other members of Professor Gould's group and/or researchers at the Cardiovascular Research Centre at the university (eg Livingstone et al., 1996; Arbuckle et al., 1994; Rice et al., 1996; and Livingstone, Lyall and Gould, 1995). This indicates a degree of knowledge cross-fertilisation between projects and departments.

One publication includes co-authors from the University of Bath (Kozka et al., 1995). Their involvement was limited to providing reagents. In addition, two of the publications attributed to the BHF study also involved international co-authors from the University of Queensland in Australia (eg Martin et al., 1995, and Campbell et al., 1994). Like the researchers from the University of Bath, the Australian research team also helped the BHF study by providing needed reagents.

Figure 9-1 shows the results of bibliometric analysis for the case study grant.

[10] Although classified as articles in the Web of Science database, Professor Gould felt that two of the eight articles were actually reviews.

Figure 9-1 Publication output and impact of directly related publications



[1] Citation count extracted April 2009

9.6.2 Benefits to future research and research use

The key benefits of the hypertension project for future research and research use (payback category 2) revolve around 1) capacity building and career development and 2) targeting of future research.

Capacity building and career development

Training and career development of researchers

Unfortunately, the hypertension project did not lead to any major discoveries (as discussed in Section 9.6.1). Although Professor Gould felt that the links between glucose transport protein levels and hypertension merited further research, he decided to withdraw from cardiovascular studies following this project. There were a number of reasons for his decision:

- Although the cell biology and biochemistry methods used in the hypertension project were appropriate for the study, building or revisiting the findings would have called for molecular genetic approaches. Professor Gould was not trained in such techniques and was not very interested in developing the new expertise that would have been required.
- Professor Gould's core interests lay in the diabetes rather than the cardiovascular research field, and he decided to focus his efforts on the diabetes area rather than deflecting his attention and resources to other fields.
- Lastly, Professor Gould felt that competitor laboratories were at an advantage in terms of advancing glucose transport studies in hypertension. A study conducted by a group in Oxford at the time of the BHF grant arrived at similar conclusions at around the same time as Gould's group. However, the Oxford group was well trained in genetic approaches and equipped with genetic research instrumentation, which 'placed them in a better position' to take the research forward .

Interestingly, Professor Gould also highlighted the dangers of pursuing research outside a core field of interest and expertise. He said, 'With hindsight, I probably would not have engaged in this project, and especially not if I had considered the need for molecular

genetic approaches to address the research questions over the longer term' (Gould interview, 2008).

Despite the scientific outcomes, Professor Gould felt that providing the PhD student with the opportunity to study for a PhD was an important output from the grant. The PhD student himself felt that the project and qualification he received were very important for his subsequent career development. After the completion of the grant, he went on to pursue a brief postdoctorate career in the same laboratory but in the group of a researcher who had experience in molecular genetic (rather than cell biology) methods. Following the postdoctorate, he left academia to pursue scientific research and business development in the instrumentation and medical devices commercial sector. This choice was personal preference for a career in the private sector based partially on an opinion that there was less bureaucracy in industry than in academic settings. However, Gould mentioned that the PhD student 'would have struggled to find a good position in academia had he wanted to stay due to the negative findings of the project (ie 'bad luck') and through no fault and no reflection on his research quality' (Gould interview, 2008).

Despite leaving academia, the PhD student felt that his academic experience was crucial for attaining jobs in industry and that he applied the skills he had developed to challenges in the medical device sector. He is currently the Chief Executive Officer of a medical device start-up company exploiting diagnostic micro-fluid chip technology[11].

The other key postdoctoral researcher who assisted with the hypertension project stayed in research for a few more years (looking at glucose transport but outside of the cardiovascular field). He then returned to clinical practice, the field in which he was originally trained.

There was no evidence of the impact of the BHF grant on further recruitment in Professor Gould's laboratory. Gould commented that attributing recruitment to a specific grant is often challenging because projects supported by various donors are frequently interlinked.

Targeting of future research

The study formed the foundation for one other MRC-funded project in Professor Gould's laboratory (1998–2003). This was a cooperative grant between the Cardiovascular Research Centre and Gould's 'home department' of Biochemistry and Molecular Biology. At the time, the MRC had established a scheme looking to support cross-disciplinary research. The availability of funding through this scheme, coupled with complementary expertise between the two University of Glasgow departments, was a major impetus behind the MRC grant application.

The study looked at cell signalling and molecular genetics in metabolic and cardiovascular syndromes using the stroke-prone hypertensive rats as an experimental model. The Cardiovascular Research Centre took the lead on the project using genetic techniques, and Gould's group contributed with cell biology and biochemistry expertise in cell-signalling research. The findings from the BHF-funded hypertension project input significantly into

[11] Prior to this he was the business development director of an acoustic biosensor instrumentation spin-out from the University of Cambridge. His prior commercial experience was at other medical device and instrumentation companies.

the knowledge base for the MRC research, and the BHF study also contributed in terms of methods.

According to Professor Gould, the MRC project was of higher impact than the BHF grant. The study found that the reduced levels of GLUT4 on the surface of the muscle cells of hypertensive rats (reported in the BHF study findings), were essentially a research artefact. The levels of intracellular GLUT4 were not reduced; instead, the availability of GLUT4 to bind glucose at the cellular membranes was reduced due to a defect in the mechanism through which GLUT4 was brought to the membranes. Hence, a defect in the mechanism of transporting the GLUT4 to the cell membrane, rather than in protein expression, was shown to be responsible for reduced glucose binding.

Apart from the MRC study, Professor Gould did not build on the hypertension project findings to inform his subsequent research. The same is true of the further engagements of Dr Campbell.

Because both Professor Gould and the PhD student left the cardiovascular field, it was not possible to gain insights (based on interviews) into whether external groups may have built on the study findings. We attempted to follow up by analysing the citation profile of the key article stemming from the hypertension project (as identified by Gould and the PhD student[12]). The article was cited nine times. Six of the citing articles were available for full text analysis; for three, only abstracts could be accessed.

All of the citations of the key paper were in the context of:

- background information on glucose transport protein localisation in peripheral tissues (ie in introduction and literature review sections as part of reviews of accepted knowledge on glucose transport proteins)
- support for the observations and related hypotheses of other studies (eg in discussion sections)
- validating the feasibility of the hypertensive rat as a suitable experimental model (ie referencing the hypertension project and other studies that successfully conducted research using the Milan rats).

Overall, the citation analysis did not reveal any particular external group building significantly on the project findings.[13]

[12] This article was titled 'Analysis of the glucose transporter compliment of metabolically important tissues of the Milan hypertensive rat' (Campbell et al., 1995). It is important to emphasise that a key article is not necessarily the most highly cited article attributed to the project. It can also be (as is the case here) the article that most closely corresponds to the outputs of the specific project/grant under investigation (as opposed to an article that is the output of the project under investigation as well as complementary projects).

[13] Most of the citing articles are by distinct research groups.

9.7 Interface B – dissemination

The key dissemination vehicles for the study's findings were publications and conferences. The PhD student recalled presenting at four national conferences in hypertension and diabetes research fields but did not have records of the details of these conferences.

The findings from the study, together with those from other studies Professor Gould conducted, were also disseminated to patient groups through talks by Gould. Although he could not remember ever presenting the findings of the BHF study specifically, he was relatively confident that they were disseminated as part of overall advancements in glucose transport research in his laboratory. To the best of Gould's knowledge, the BHF never explicitly asked for public engagement activities (at least at the time), unlike some other funders (eg Diabetes UK). However, Gould was always active in talking to patient groups and believed this to be important both as an academic and moral responsibility, as well as beneficial in terms of mobilising the interest of youth in science education.

9.8 Stage 4 – secondary outputs

We did not find any evidence of impacts on policies or product development from this basic research project.

9.9 Stage 5 – adoption by practice and public

Given there was no evidence of impact on policies or product development, it is unlikely that there was opportunity for adoption of the findings in practice or by the public. We did not find any evidence of such adoption.

9.10 Stage 6 – final outcomes

This basic research study did not make an impact in terms of health and broader economic gains.

9.11 Summary of case study impacts

The payback framework describes the process from research to several categories of impact. The categories allow us to compare across different case studies. Table 9-1 shows, in point form and by impact category, some of the impacts, described more fully above, that have emerged from this grant.

Table 9-1 Payback

Payback category	Impacts from case study
Knowledge production	• Eight peer-reviewed journal articles • One meeting abstract • Two editorial material contributions
Research targeting and capacity building	Capacity building: • One researcher received his PhD and the grant also helped his career development Benefits for future research and research use: • Knowledge and method inputs into follow-on collaborative MRC grant
Informing policy and product development	• Non e
Health and health sector benefits	• Non e
Broader social and economic benefits	• Non e

9.12 Additional comments

Professor Gould suggested that the opportunity to respond to peer-reviewer comments, particularly when a grant is rejected, would be a valuable aspect of a peer-review processes. To his knowledge, the BHF does not practice this – or at least did not at the time[14].

9.13 References

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Chen, I., T. Alam, J.H. Johnson, S. Hughes, C.B. Newgard and R.H. Unger, 'Regulation of Beta-Cell Glucose Transporter Gene Expression', *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 87, No. 11, 1990, pp. 4088–4092.

[14] The Biotechnology and Biological Sciences Research Council (BBSRC) always has and the MRC has in more recent times.

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APPENDIX

Appendix A: List of associated grants

Grant: Mechanistic studies of insulin-stimulated glucose transport **Period:** 1991–1994 **Total:** £68,488 **Funding:** Scottish Home and Health Department

Grant: Evaluation of the role of GLUT 5 in insulin-stimulated glucose transport in normal and NIDDM tissues **Period:** 1992–1994 **Total:** £57,845 **Funding:** Scottish Hospitals Endowment Research Trust

Grant: Targeting of glucose transporters to intracellular compartments: implications for insulin-stimulated glucose transport **Period:** 1992–1997 **Total:** £155,000 **Funding:** Lister Institute of Preventive Medicine Fellowship

Grant: Co-operative Grant. "Genetic and molecular basis for insulin resistance and hypertension in the SHRSP **Period:** 2000–2003 **Total:** £332,652 **Funding:** Medical Research Council, London

Grant: The use of Xenopus oocytes as a model system for the study of insulin-stimulated glucose transport **Period:** 1990–1993 **Total:** £89,305 **Funding:** Medical Research Council, London

Grant: Isolation of a cDNA encoding the prostaglandin F2-α receptor **Period:** 1991–1992 **Total:** £23,896 **Funding:** Medical Research Council, London

Grant: Targeting of glucose transporters in adipocytes and oocytes: implications for insulin-stimulated glucose transport **Period:** 1993–1996 **Total:** £159,892 **Funding:** Medical Research Council, London

Grant: Synthesis of phosphono analogues of phosphotyrosine-containing peptides for use as tools for signal transduction research **Period:** 1994–1995 **Total:** £37,873 **Funding:** Medical Research Council, London

Grant: Identification and characterisation of a unique intracellular compartment in adipocytes: targeting of GLUT4 to this pool and implications for insulin-stimulated glucose transport **Period:** 1996–1999 **Total:** £190,688 **Funding:** Medical Research Council

Grant: Molecular mechanism of mitogen-stimulated glucose transport **Period:** 1994–1997 **Total:** £115,172 **Funding:** Wellcome Trust

Grant: The use of green fluorescent protein tagged GLUT4 to study insulin-dependent transporter translocation in real time **Period:** 1996–1999 **Total:** £168,577 **Funding:** Wellcome Trust

Grant: Structure-function relationships in the human glucose transporter family **Period:** 1992–1995 **Total:** £78,316 **Funding:** Science and Engineering Research Council

Grant: Glucose transport across the enterocyte: mechanisms, structure and function of GLUTs 3 and 5, and the Na+-dependent carrier, SLGT1 **Period:** 1994–1997 **Total:** £260,020 **Funding:** Agricultural and Food Research Council

Grant: Insulin-stimulated glucose transport: the behaviour of "'insulin-responsive"' glucose transporters in foreign cells **Period:** 1990–1993 **Total:** £65,349 **Funding:** British Diabetic Association

Grant: Analysis of potential lesions in glucose transport proteins in the Milan hypertensive rat **Period:** 1993–1994 **Total:** £31,169 **Funding:** British Heart Foundation

Grant: Glucose transport in hypertensive rats **Period:** 1994–1996 **Total:** £61,202 **Funding:** British Heart Foundation

Grant: Insulin signalling pathways in permeabilised adipocytes **Period:** 1995–1997 **Total:** £75,426 **Funding:** British Diabetic Association

Grant: Analysis of the targeting of GLUT4 to an insulin-responsive compartment **Period:** 1996–1998 **Total:** £25,570 **Funding:** British Diabetic Association

Appendix B: List of equipment grants

Grant: Equipment Grant towards the purchase of an automated DNA sequencing unit. **Period:** 1992 **Total:** £35,000 **Funding:** Scottish Home and Health Department **Collaborative grant**

Grant: Equipment Grant towards the purchase of an automated DNA sequencing unit. 1992 **Period:** 1992 **Total:** £21,200 **Funding:** Scottish Hospital Endowments Research Trust **Collaborative grant**

Grant: Equipment Grant towards the purchase of a computer and software for the laboratory **Period:** 1992 **Total:** £5,000 **Funding:** Lister Institute of Preventive Medicine

Grant: Equipment Grant towards the purchase of a Phosphorimager system **Period:** 1993 **Total:** £97,380 **Funding:** The Wellcome Trust **Collaborative grant**

Grant: Purchase of ultracentrifuge for the subcellular analysis of glucose transporters **Period:** 1994 **Total:** £29,910 **Funding:** Medical Research Council

Grant: Equipment Grant towards the purchase of a confocal microscope and computer workstations **Period:** 1995 **Total:** £187,210 **Funding:** The Wellcome Trust **Collaborative grant**

Grant: Improvement of DNA sequencing and genotyping facilities for Wellcome-funded research groups **Period:** 1996 **Total:** £134,242 **Funding:** The Wellcome Trust **Collaborative grant**

Grant: Equipment Grant towards the purchase, support and staff to run a BIAcore facility **Period:** 1996–1999 **Total:** £229,573 **Funding:** The Wellcome Trust **Collaborative grant**

Grant: Co-operative Group Component Grant. "Genetic and molecular basis for insulin resistance and hypertension in the SHRSP." **Period:** 2000–2003 **Total:** £332,652 **Funding:** Medical Research Council **Collaborative grant**

CHAPTER 10 The role of coagulation and fibrinolysis in the pathogenesis of recurrent stroke

10.1 Introduction to the research project

10.1.1 Overview

The research undertaken on the grant 'The Role of Coagulation and Fibrinolysis in the Pathogenesis of Recurrent Stroke' looked at whether clear links could be established between the genetic profiles of individuals and their risk of having a stroke. It did so by focusing on improving what was then quite limited understanding of the role of genetics in blood clot formation and breakdown. Was it possible to show how genetic variations between individuals affected their likelihood of suffering a stroke or other acute ischaemic[1] events? Could particular genes or proteins be singled out as especially significant?

The demonstration of links between particular genetic profiles and the risk of stroke would, it was hoped, ultimately open potentially significant therapeutic avenues. However, as this was a relatively new field at the time the research was undertaken, the investigators saw therapeutic applications as a fairly distant prospect (in common with many of their colleagues). The project was based at the University of Leeds Teaching Hospital and was conducted between 1994 and 1997.

10.1.2 Understanding the broader research field

By the late 1980s and early 1990s, a substantial body of research had been carried out to investigate a range of risk factors for stroke – some environmental, others behavioural and still others describing the familial and personal histories of patients involved. Much of the supporting evidence had been derived from landmark studies conducted using very large cohorts of patients and controls, including – among others – the Framingham and Prospective Cardiovascular Münster (PROCAM) studies in the United States and Europe respectively.[2] Some of this work had helped to generate risk profiles for stroke to help

[1] Ischaemia describes a situation of restriction in blood supply – with resultant damage to tissues and organs.

[2] The Framingham Heart Study is an ongoing cardiovascular research project based in Framingham, Massachusetts in the United States. Launched in 1948, it began by tracking cardiovascular events in 5,209 adults and is now looking at patterns in its third generation of participants. The PROCAM, or Prospective Cardiovascular Münster Heart Study is one of the largest prospective, epidemiological studies of coronary heart disease risk factors in Europe.

predict stroke events before they occurred (see, for example, Wolf et al., 1991).

At the same time, though, there was growing interest in the potential contributions of various genetic factors to the risk of stroke. Firstly, the evidence from epidemiological research of the importance of genetic factors, especially twin studies, was increasingly clear (Brass et al., 1992; Khaw et al., 1986; and Brass and Shaker 1991). Secondly, research showing the apparently central importance of blood-clotting proteins such as fibrinogen in cardiovascular events seemed to suggest that genetic factors might have a role to play. Findings from the Framingham Heart Study published in 1987 (Kannel et al., 1987) demonstrated that elevated levels of fibrinogen were a reliable predictor of cardiovascular disease (CVD). Finally, a small number of disorders caused by single genes, such as sickle-cell anaemia and lupus, had been identified that were thought to strongly predispose individuals to strokes in later life (for example, Carreras and Vermylen, 1982).

Although the impact of single-gene disorders was discussed in a number of review articles during this period (for example, Hart and Kanter, 1990), research efforts showed that their overall impact was small. Various studies published in the mid to late 1980s suggested a prevalence of stroke attributable to single-gene haematological disorders ranging between just 1% and 7% (Bogousslavsky et al., 1988, and Klein and Seland, 1984).

Attention therefore switched increasingly to the potential role of complex genetic factors – in other words, those simultaneously controlled by a number of genes. It was hoped that, in alliance with further exploration of other risk factors, including environmental factors, this would explain the high levels of stroke-related mortality and morbidity in the United Kingdom (UK): in 1990, figures published in a background document supporting the *Health of the Nation* white paper suggested that 9% of all deaths in men and 15% of all deaths in women in the UK were primarily caused by stroke (Department of Health, 1994). From a clinical perspective, then, the impetus for research in this area was clear.

Methodologically, the growing focus on complex genetic traits presented much greater challenges than the single-gene studies that had gone before (Table 10-1 summarises some of the key confounding factors). However, important improvements in molecular genetic methods during the late 1980s made research work in this area much more feasible than it had been previously. The research supported by this grant was very much part of the first wave of findings to emerge from this shift; a review of the genetics of ischaemic stroke published in 2000 identified the first major paper on the role of complex genetic factors in the disease as having been published in 1994 – the year that the grant began (Hassan and Markus, 2000).

Table 10-1 Some well-known confounding factors facing investigators when researching the impact of complex genetic disorders on disease symptoms (adapted from Alberts, 1990)

Area	Confounding factor	Examples
Background factors	Environmental factors	Extrinsic influences including diet, smoking and stress may contribute to the modified expression of certain genetic traits
	Risk factors	Some acknowledged risk factors for stroke (eg hypertension and diabetes) may in fact reflect the composite effects of a range of environmental and genetic risk factors
Genetic factors	Polygenic traits	Abnormalities in a number of genes may come together to cause a given trait
	'Phenocopy'	Sometimes, environmental factors can bring about effects identical to those that are caused by defective genes

10.1.3 The case study approach

The case study based on this research grant involved a combination of: face-to-face interviews with senior investigators on the project; a review of the curriculum vitae of the principal investigator (PI); and documentary analysis of key citing papers, publications and conference abstracts arising from it. In this case, we were also fortunate enough to be able to obtain supporting grant documents from the Stroke Association, which provided funding for the grant research.

10.2 Stage 0 – topic/issue identification

One of the unusual features of this grant is its surprisingly open title. Coagulation and fibrinolysis describe complex processes of blood clot formation and breakdown that are controlled or mediated by a large number of proteins and – by implication – a large number of genes. The open remit of the grant application hints at how open this field was at the time during which the research was undertaken. It also reflected a desire on the part of the researchers to leave open the possibility of developing a range of ideas and workstreams. Indeed one of the co-investigators suggested at interview that some of the research conducted on the grant had been quite 'opportunistic'. The researchers were keen to take an exploratory approach to what was a new and emergent research area.

The key unifying feature of the range of research topics considered as part of the grant was a concern with the theme of risk factors. The researchers sought to understand which of a number of agents contributed most significantly to the risk of stroke – and particularly recurrent stroke. Indeed, the funding application for this grant made reference to the impact of past completed stroke events on the risk of further CVD events, stating a recurrence rate of 13% by the end of the first year after stroke and up to 30% after five years.

Key factors determining the topic identification for this particular grant included:

- the clinical focus and career orientation of the PI, who was (and is) also a practising clinician

- the presence of a key partnership with an established stroke researcher
- timing, in light of the career stage of the PI and the particular development of his research skills
- the status of the wider research field at the time.

The ideas for the project arose from discussions bridging the diverse – but closely related – research interests of the PI and the main co-applicant on the grant application. Both were working in hospitals at Leeds at the time. In this section, we examine how the research topic was identified and show that four factors were crucial.

10.2.1 Clinical focus of the PI

The PI is a clinician, and it was clear during our interview that a key driving force throughout his career has been the pursuit of research with potential clinical applications. His early research work (towards a medical doctorate (MD) that he completed at Bristol University in 1987) had been on hormonal aspects of the control of blood coagulation. During a year abroad in Switzerland at the end of the 1980s, however, he had worked extensively on molecular aspects of thrombosis[3] and blood coagulation. He returned to the UK in 1989 with a strong interest in looking at how molecular genetics tools might be applied to problems of blood coagulation.

10.2.2 The presence of a key partnership with an established researcher

In April 1993 the PI had secured a large research grant from the Stroke Association to investigate the link between a particular gene cluster and stroke incidence by partnering with a researcher with an established reputation in this field. This meant that he already had a track record of winning funding from the Stroke Association, but at first glance, the PI's chances of securing funding for this grant do not seem to have been strong. He had a limited track record in research overall at the time at which he made his grant application, and much of it had focused on relationships between diabetes and CVD. Furthermore, he did not yet have any substantive results from the first grant, which he suggested was usually a prerequisite for winning follow-on funding.

In this context, the partnership with the esteemed researcher was, in the view of the PI, crucial to the shape of the research on this grant. This researcher's reputation was an important factor in winning funding at the outset. His involvement also played a key role in defining the direction of the PI's research at this time, as he consciously moved away from the diabetes research that had previously been his main focus when putting together the application. At interview, the PI expressed some regret that, despite the efforts of his research group and others in this area, links between diabetes and stroke research remain poorly defined.

10.2.3 Timing

This grant was complementary to a substantial one he had won previously from the Stroke Association in April 1993. He had used the funding from the earlier grant to help set up a

[3] The term 'thrombosis' refers to the process of blood-clot formation. Coagulation is an important precursor, as it is concerned with the way in which red blood cells are bound together to stem blood flow.

patient cohort for further genetic studies. Without further funding, however, fuller characterisation of this cohort was impossible. The timing of the second research grant was therefore crucial, as the funding it provided enabled the PI to purchase the laboratory material for his first major strand of research using this patient cohort. Without the grant, the PI felt that it probably would not have been possible to proceed with the research at this time.

10.2.4 The state of knowledge in the wider cardiovascular research field at the time

This area of research was a new and relatively open one at the beginning of the 1990s. In this sense, it represented a great opportunity for an appropriately trained PI looking to establish his reputation as a researcher. By moving away from his direct research interests, and allying with an established stroke researcher, the PI was able to exploit this research 'niche'. It also provided him with an ideal opportunity to ask fundamental questions about the genetics of stroke, using the patient cohort that he had assembled. Do certain blood clot-forming proteins differ between patients with stroke and healthy controls? And do these differences then predict outcomes later in life – particularly the risk of a stroke?

10.3 Interface A – project specification and selection

The project specification was jointly agreed by the PI and two researchers. The first of these (the afore mentioned esteemed researcher) played an important role in managing clinical aspects of the research and was a key member of the research team – not least by bringing extensive clinical experience of stroke management in practice. As it involved working closely with a predefined patient cohort, preserving relations with the patients themselves, and their managing general practitioners (GPs), was vital to the long-term prospects of the research group that the PI was seeking to set up, and both he and the esteemed researcher had important roles in this regard. The final grant application was submitted with the second leading researcher as an additional co-signatory; he was to conduct much of the benchside research on the grant.

The application was submitted in open competition and won funding at the first time of asking from the Stroke Association. Interaction with the Stroke Association throughout the process – as for many other researchers during this period – was limited. Indeed, the PI suggested that there was considerable doubt as to whether funding would be secured until he finally received an acceptance letter. By applying so soon after the start of his cohort-forming grant from the Stroke Association, the PI was attempting to win further funding even without any preliminary data from the first grant yet being available. In practice, few researchers expected to receive support from funding bodies under these circumstances.

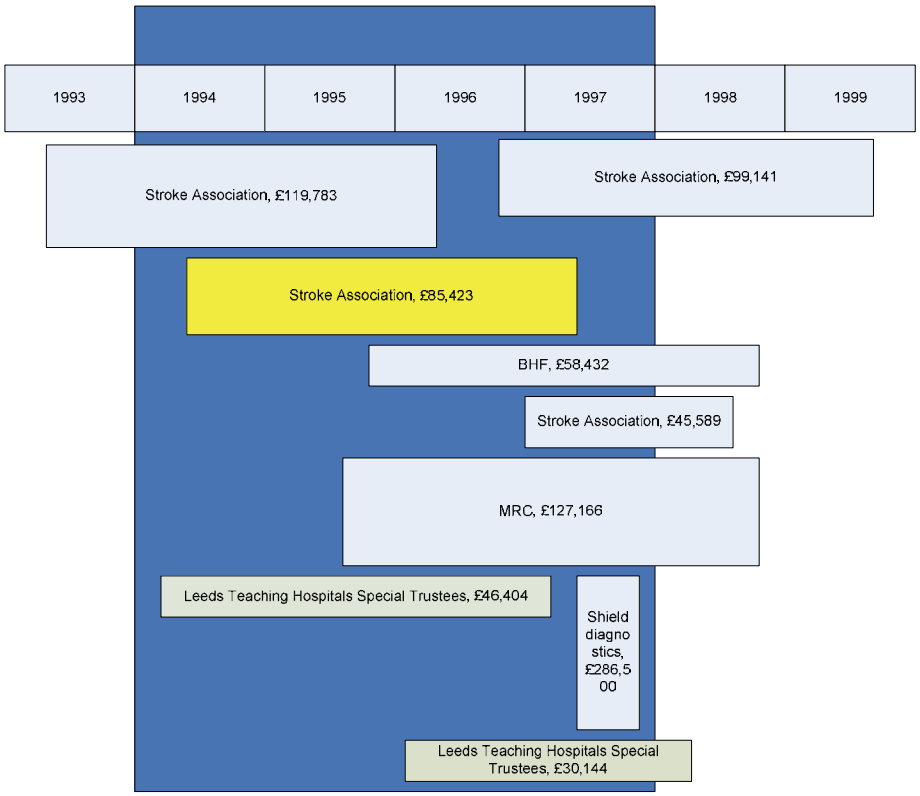
10.4 Stage 1 – inputs to research

In this section, we outline how a number of key inputs contributed to the research on this grant.

10.4.1 Facilitators

The key facilitator for the research was £85,423 in direct funding from the Stroke Association, over half of which was allocated to paying medical staff salaries.[4] However, as the grant map in Figure 10-1 shows, the funding picture is in fact more complex. The PI had received an earlier grant from the Stroke Association in 1993, which provided the foundations for the research conducted on this one, as it supported the recruitment of both patient and control cohorts. Indeed, in several of the grant documents that we were able to gather from the Stroke Association, the PI recommends that reports on the two grants be viewed in parallel. The PI was also the recipient of large sums of funding from a variety of sources over the later years of the grant. In particular, he won substantial support funding from industry in 1995 (£65,000 from Sandoz Pharmaceuticals).

Figure 10-1 Funding map for the PI's research group; this shows major inputs to the PI's laboratory, relating to stroke research, over the period 1993–1999



In reputational terms, the presence of the esteemed researcher as a co-applicant on the grant was a key facilitator. With an established track record in the field of stroke research, the PI felt that his involvement brought the reputational capital required to help win funding from the Stroke Association to help recruit study subjects in and around Leeds.

[4] Of £85,423 originally allocated, some £44,000 was allocated in medical staff salaries (according to the grant application; details from the end of grant report were not available).

This was important because the PI himself was still a relatively junior researcher, with around 30–40 publications to his name at the time the grant was awarded.

10.4.2 Knowledge/expertise

Core knowledge and expertise inputs to the grant came from the PI and the esteemed researcher, who brought a wealth of experience in stroke research and clinical practice (see, for example, Bamford et al., 1989, and Bamford et al., 1991). This was key given that the PI's core area of research focus up until the start of the grant had been diabetes.

Although the core research team was small (only seven or eight individuals), two doctor of philosophy (PhD) students in the PI's laboratory made major contributions to the work on this grant. , One of the co-applicants performed many of the assays and was, in fact, the only clinician directly involved in the research on a day-to-day basis. Another researcher, who received her PhD in 1998, was also extensively involved in the laboratory work as a trained phlebotomist; indeed she collected and analysed many of the samples from haematology outpatients herself.

10.4.3 Techniques

The research on the grant involved relatively simple techniques. Indeed, many of the assays could readily be performed by one researcher. Other kinds of test, however, depended on a team of researchers working together – plasma phenotyping, for example.

No new methods were developed, although some minor modifications were made to ensure higher throughput given the very large number of samples involved. What *was* new was the systematic way in which these methods were applied (for further details, please refer to 10.5 below).

10.4.4 Samples/study recruits

The presence of a defined patient cohort was a crucial underpinning factor for this grant. By the time funding was received, a good deal of the study recruitment had occurred (using funding from the Stroke Association grant in 1993). The research under this grant built on this platform by initiating the first round of data collection, but it also continued recruitment, particularly of the control cohort. By the final year of the grant, 680 cases and 652 controls had been recruited.

10.4.5 Consumables

A significant proportion of the funding provided by the Stroke Association for this grant was spent on consumables (£6,500). Although the first grant the PI received was largely used to offset the costs of recruiting the patient cohort, he now needed inputs to cover coagulation assays, plastic-ware and so forth.

10.4.6 Space

Space was a key constraint on the PI's activity as he looked to establish a new research group at Leeds University. For most of the period covered by the first two grants from the Stroke Association, the PI worked from an under-resourced laboratory. He suggested that his group might well not have been awarded funding had the Stroke Association visited the research group's premises.

10.5 Stage 2 – research process

In terms of the research process, this grant needs to be seen alongside the earlier Stroke Association funding won by the PI in 1993. The crossover in both time and method between these two grants was substantial. Broadly speaking the research involved four streams:

- recruiting study subjects and controls
- gathering records and other supporting information
- sample collection and analysis
- follow-up procedures.

10.5.1 Recruiting study subjects and controls

The first major hurdle facing the project team was recruiting a suitable patient cohort. Much of the work on this aspect was completed using funding from the earlier Stroke Association grant, although it continued under the grant considered here. All patients were recruited in hospitals in the area, identified mainly through admissions records and on the basis of the results of computed tomography (CT) scans. According to a co-investigator on the grant, Leeds General Hospital was unusual in having a register of CT scan results even at this time, and the key criterion for selection of patients became that individuals had had both a CT scan and a diagnosis. All CT scan results were reviewed by the esteemed researcher and his fellow co-applicant and then classified using the Oxford Community Stroke Project classification[5] of stroke events to ensure that appropriate patients were recruited.

Personal contact with potential study subjects was regarded as essential in order to secure their consent in the study and thereby satisfy ethics committee requirements. The project team often met with patients directly to explain the context and aims of the study and how individuals would be involved if they agreed to participate. One of the co-investigators noted that this approach paid dividends; patients were often quite happy to be involved in the study.

Recruitment of the control group proved more challenging. It was time and resource intensive: this section of the study involved at least three research nurses, a PhD student and a number of technical staff at various stages. It also proved difficult to recruit appropriate controls. Firstly, the required demographic profile of the control group was mainly elderly (to ensure match up with the subject group), and this meant that the project team required approval to visit daycare centres and distribute information about the study to potential participants. The project team also went out to local businesses to recruit younger controls for the small number of young patients with stroke – and found that businesses readily cooperated with the study by sending out information by post to their employees.

[5] The Oxford Community Stroke Project was a research initiative led by the esteemed researcher and others; a key paper arising from this project (Bamford et al., 1991) identified a series of hallmark symptoms and diagnostic test results that could be used to classify stroke patients on a scale and ensure that they received the most appropriate forms of treatment then available.

10.5.2 Gathering records and other supporting information

Following recruitment of subjects and controls, the researchers turned to gathering medical records in support of the research. Hospital medical records were an important source for validating clinical events reported in the main body of patients. For the control cohort, gathering supporting evidence depended on a trawl through primary care records.

10.5.3 Sample collection and analysis

The laboratory-based element of the research involved taking blood samples from acute stroke patients within ten days of their stroke event. These were analysed for a range of blood proteins and clotting factors. Genomic DNA was also extracted, although significant results from this would not emerge until later research grants. Results from these tests were compared with similar analyses for the control cohort.

10.5.4 Follow-up procedures

All cases were followed up three months after initial samples were taken. Details of completed patient events were gathered in 486 subjects of the original 680 case subjects originally recruited. Deaths were reported in 257 cases to the middle of 1997, the point at which the final grant report was submitted. Recurrent stroke was identified in 86 cases and ischaemic cardiac events in 106. Where follow-up in person was possible, this was conducted.

The research on this grant was conducted largely independently of other researchers in this field, with one important exception. Analysis of the clotting factor, Factor VII, was undertaken jointly with an epidemiologist specialising in this area of research and then director of the MRC Epidemiology and Medical Care Unit. Although the PI would subsequently develop quite strong links – for example, with industry – little of this was in place in the early 1990s.

10.6 Primary outputs from the research

10.6.1 Knowledge

The research on this grant made an important contribution to mapping links between various agents in blood clotting and clot breakdown pathways and cerebrovascular stroke. Interestingly, the focus in the grant application on recurrent stroke was not one that was ultimately reflected in the body of research conducted using this block of funding. The PI claimed up to 18 papers in internationally recognised journals arising from this grant, although only five of these could be directly attributed to the grant. The grant clearly contributed data to some of the indirectly attributable papers.

The five publications that emerged directly from the grant explore a variety of more or less complex links between genetic factors and stroke. Some describe associations with genetic variations in specific agents or proteins; others are concerned with wider issues, such as gender-related linkages. We provide details of a sample below, to illustrate the range of ground covered, rather than presenting a comprehensive picture of all of the publications.

Carter et al. (1997) looked at variations in the levels of circulating fibrinogen – another agent involved in the blood clotting pathway and a known independent risk factor for

stroke. By focusing on a particular genetic variation (polymorphism *Bβ 448*[6]), the investigators hoped to understand how variations in the levels of circulating fibrinogen come about. They found a clear discrepancy in degrees of association between this particular genetic variation and fibrinogen levels in male and female subjects. In males, increasing fibrinogen levels seemed to be influenced by environmental factors; in females, the investigators suggested that a functional difference in the fibrinogen molecules themselves might result in greater risk of stroke – rather than absolute levels in circulation or the genetic variation per se.

Catto et al. (1997) evaluated the links between two agents in the blood-clotting process – von Willebrand factor and Factor VIII:C – and cerebrovascular stroke. The role of von Willebrand factor had not previously been examined. Based on analysis of blood samples taken from the cases and control cohort, the paper found an association between elevated levels of both of these agents and the risk of stroke. No claim was made that these links were predictive, however, and the paper suggested that further investigation was needed to establish how firm the links were.

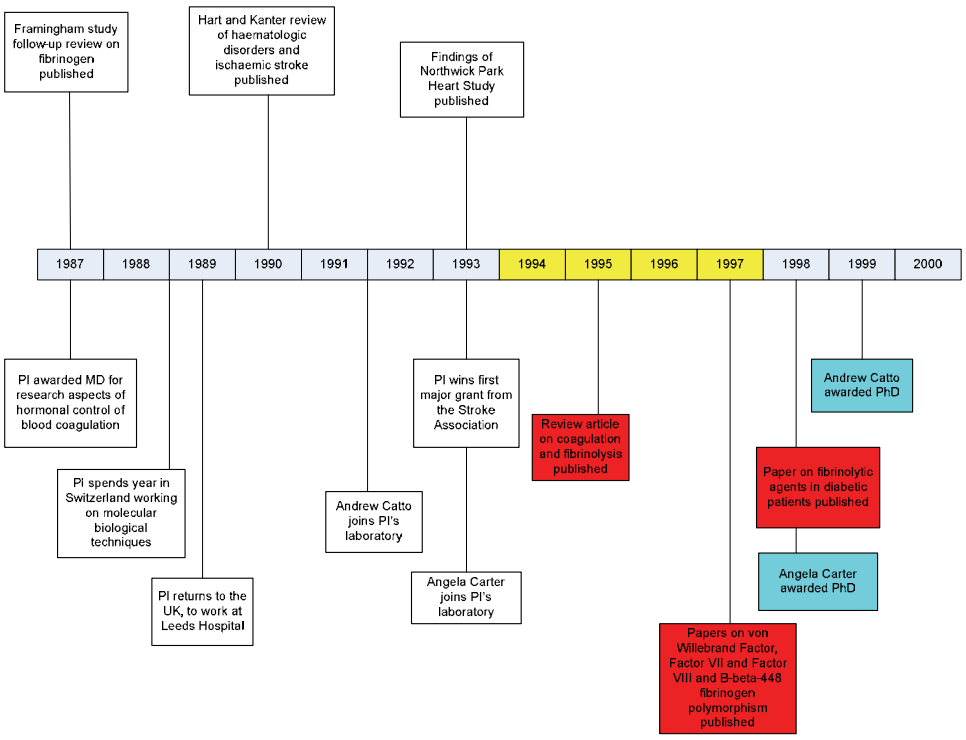
Heywood et al. (1997) focused more closely on genetic aspects. It examined a number of variant forms of the gene for Factor VII (also a clotting agent) and the relationship between these forms and circulating levels of Factor VII:C. The results suggested that neither variant forms of the gene nor the presence of Factor VII:C in circulation were associated with cerebrovascular disease.

Mansfield et al. (1998) sought to bridge work on risk factors for stroke with the PI's core research interest, diabetes, by investigating disturbances in the fibrinolytic pathway in patients with type 2 diabetes who also had acute ischaemic stroke. The results suggested that no such disturbances occurred – in marked contrast to the apparently clear links between impaired fibrinolysis and coronary artery disease in patients with type 2 diabetes and those without diabetes.

Figure 10-2 shows a timeline of key events associated with the grant; important developments relating to the wider research field are shown above the line, while grant-specific events are shown below it.

[6] The term 'polymorphism' describes the observation that a specific gene may exist in a number (ie more than two) variant forms within a given population.

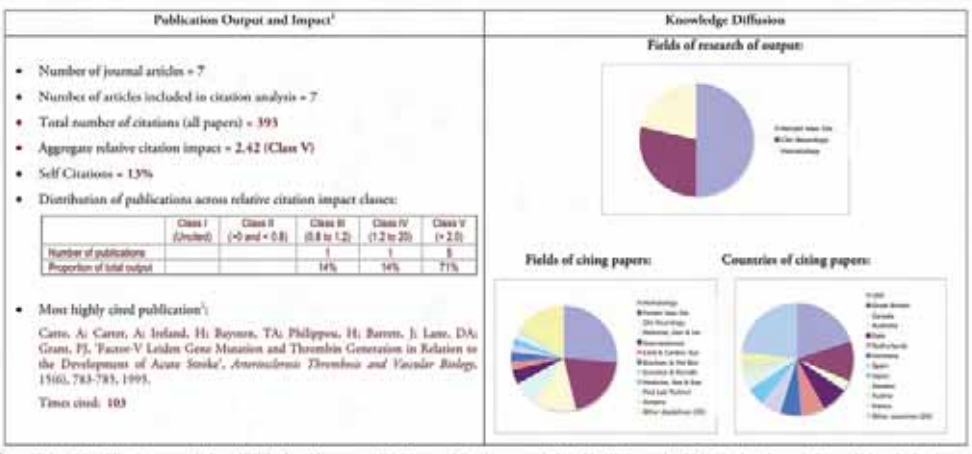
Figure 10-2 Timeline showing key events associated with the grant



The group has continued to publish papers using data gathered during the grant in question. A 2007 paper published in the journal *Stroke* (Carter et al., 2007) was based almost entirely on data gathered during this grant but only recently analysed. Indeed, one of the co-investigators felt that a key constraint on maximising the impact of the grant had been finding time to take forward some of the insights gained at this time.

Figure 10-3 shows the results of bibliometric analysis for the case study grant.

Figure 10-3 Publication output and impact of directly related publications



[1] In addition, 12 publications were indirectly linked to this grant. All of these publications were indexed in WoS, received 299 citations in total, for a relative citation impact of 1.23. They were spread across the five relative citation impact classes, with one in I, four in II, one in III, four in IV, and two in V. Their self citation rate was 12%.
[2] Citation count extracted April 2009

10.6.2 Benefits to future research and research use

Capacity building and career development

This grant played a significant role in launching the PI's research career. Although it is difficult to distinguish clearly between the impacts of this grant and the first one received from the Stroke Association, the two grants provided the basis for recruiting a viable patient cohort for long-term study and vital preliminary data without which it would have been very difficult to secure follow-on funding. Moreover – as the funding map in Figure 10-1 illustrates – the PI's success rate in securing large-scale funding to support his research increased markedly after these two grants were awarded. This included a large grant from the MRC for continued research in this area in 1995.

The grant was also important in helping the PI to build his research group. A co-applicant on the original application wrote his PhD theses using data obtained from the research; one of the researchers involved was subsequently inspired to complete a PhD part-time, and the grant provided 50% of the data for her research. The research also made a major contribution to the thesis towards a medical doctorate of at least one student in the PI's laboratory at the time. Several of these individuals remained in the laboratory for some time after completing their qualifications, and the researcher who went on to complete a PhD part-time continues to work in the same group today – 11 years after the end of the grant. However, for the practising medics who were part of the original study team, the long-term impact has been less certain. Most have left research to pursue clinical practice full-time; one of the co-applicants, for example, is now a hospital Director of Geriatrics.

More broadly, however, there is some suggestion that the grant may have had an important role in building capacity in this research area within Leeds University as a whole. The PI is founding director of a recently established interdisciplinary research unit – the Leeds Institute of Genetics, Health and Therapeutics (LIGHT) – that brings together researchers looking at a range of complex chronic disorders, including CVD and diabetes. The aim of the centre is to 'perform internationally competitive translational research…and to improve the delivery of patient care' (LIGHT, 2008). Although there is no suggestion of a direct link between the research on this grant and the establishment of LIGHT, the researchers felt that its findings and impact had helped to build a case for a multidisciplinary unit focusing more closely on bridging the gap to clinical practice.

In a similar vein, one of the aspects highlighted by both the PI and the co-investigator we spoke to was the opportunities offered by the grant to work across the research–clinical practice boundary. The PI practised throughout the research period and continues to do so today. The researcher who went on to complete a PhD part time was clear that one of the defining features of the research from her perspective had been the opportunities it offered to work closely with families and individuals affected by stroke – and that this had given her unusual skills compared with others who had pursued more conventional laboratory-based career paths. She also felt it had strengthened capacity by boosting mechanisms for cross-cutting work with National Health Service (NHS) staff. The most important

implication of this has been an improved ability to move from clinical observations to a clear delineation of the most appropriate scientific approach for investigating them.[7]

In a wider sense, however, the PI and supporting interviewees were clear that the reputational impact of the research on the grant had been substantial. It helped to establish the PI's research group and raise the profile of the group and the department as a whole.

Targeting future research

Perhaps most importantly, the grant played a vital role in establishing a very fruitful research stream in which the PI continues to work today. In combination with the 1993 Stroke Association grant, the investigators were able to assemble a large patient group and control cohort as the basis for a long-term and comprehensive mapping exercise, during which they have examined a range of linkages between genotypes, intermediate phenotypes and the risk of stroke. The systematic methods adopted by the PI and his group – in terms of medical record analysis alongside data collection from samples – mean that the patient cohort and control group are now well characterised, and this has attracted involvement from other researchers looking to conduct particular tests. Indeed, a research group based in Germany focusing on the possible role of nitric oxide in endothelial cell dysfunction has recently made use of the extensive bank of samples the PI's research group had collected from stroke and CVD patients to test for possible links using an assay that they have developed.

The research unveiled a host of linkages and research lines that the PI and his team have been looking at throughout the intervening period, including Factor XIII (now with therapeutic potential). In recent years, the group's work has moved towards trying to understand in greater depth how protein structure and function affects blood-clot formation – using a range of new technologies. This ongoing stream of research continues to produce publications, including a paper in *Stroke* in 2007 that brought together many of the findings from the preceding 15 years in single discussion of predictive variables for mortality (Carter et al., 2007).

The research also helped bring about changes in methodological approach; specifically, the PI's group has gradually moved away from retrospective studies. A co-investigator suggested that within 10 days of a stroke, there are often so many potential confounding factors that could be influencing results that there were always questions about what might be being missed. She highlighted the possible impact of transient ischaemic attacks (TIAs) as a possible example.[8] In general, she suggested that prospective studies were now viewed much more favourably.

For the department as a whole, however, the implications of the grant in terms of research targeting (as opposed to capacity building) have been less clear. LIGHT has since moved

[7] The co-investigator we spoke to largely credited the PI with this, suggesting that it was his particular vision for research that transcended the basic–applied boundary that really motivated laboratory staff to pursue it.

[8] The term TIA describes a situation in which the blood supply to the brain is interrupted for a very brief period of time. Often referred to as a 'mini-stroke', the symptoms of a TIA are similar to a full stroke and include visual impairment or slurring of speech. Typically, though, symptoms last for only a few minutes to a couple of hours and may disappear altogether within 24 hours.

more towards basic research, with a growing number of chemists and biochemists recruited to work on defined systems. Although clinicians continue to work as part of the unit, the co-investigator felt that the historical focus on large-scale clinical studies had arguably been lost since the completion of the grant.

10.7 Interface B – dissemination

Findings from the grant were disseminated quite widely. The PI was regularly invited to national and international events both during and after the grant and presented data at eight seminars, conferences and symposia – almost all of which addressed the cardiology or haematology research communities (through the British Society for Haemostasis and Thrombosis or the International Society for Thrombosis and Haemostasis).

However, the investigators we spoke to admitted that dissemination attempts had not been as successful as they had hoped. Despite the large number of publications arising from the grant, the PI had never been invited to speak at an event addressing stroke specialists. He suggested that a possible explanation for this might have been the difficulty the research group had in communicating its findings to a community to which he was not directly linked (since his own specialisation was diabetes research). One of the co-applicants was able to present some of the findings of the research in stroke-specific events, but the impact of this campaign overall was uncertain, partly because research addressed risk factors and its clinical implications were still uncertain. The co-investigator we spoke to also highlighted difficulties the group had had in achieving recognition in the United States.

The PI does not seem to have engaged in concerted public engagement work stemming from the research findings.

10.8 Stage 4 – secondary outputs

The research conducted on this grant has not contributed to the development of any drugs. The PI suggested that the grant arguably came too early in the process of research to have contributed directly in this way. In fact, the focus had been on establishing a viable cohort and conducting basic mapping experiments to explore potential linkages and identify biomarkers for stroke.

Recent discussions with a pharmaceutical company based in Cambridge suggest that the research stream as a whole may contribute to therapeutic developments in the future. Indeed, the co-investigator highlighted a grant recently won by the PI to investigate the development of Factor XIII-based therapeutic agents – with much of the evidence for the move in this direction having come from the original grant in question. Specifically, evidence gathered on the grant pointed to a genetic variant of Factor XIII as being protective against the risk of stroke. This observation had spurred a stream of in-vitro research, which now seemed likely to yield therapeutic applications. The PI is currently working on this in collaboration with chemists based at Leeds University. He is also in contact with a drug development company based in Cambridge with a view to potential clinical applications – but emphasised again that this represented the culmination of a stream of research rather than an impact of this grant in particular.

In terms of clinical applications, the story seems to have been similar, with some important observations made during the study but little evidence thus far of translation into clinical practice. For example, a co-investigator highlighted the researchers' surprise at how few people had been seen by their GPs when they were conducting three-month follow-up. She found that beyond provision of aspirin for some patients, follow-up seemed to have been negligible.

10.9 Stage 5 – adoption by practice and public

The results of the research do not yet seem to have been taken up by the health service, doctors, public health officials and so forth. The PI made a case that the work conducted on the grant, and indeed much of the stream of research he and his group have been involved with since, may well contribute to policy in the future, but that it had been largely preclinical in focus. Much of his work has focused on characterising risk factors in a field that was new and untested when he began his research career.

10.10 Stage 6 – final outcomes

The findings of this grant do not yet seem to have had any demonstrable effect on society more broadly – eg through improved health in the population, spin-off companies employing people, sale of products by the pharmaceutical industry and so forth.

10.11 Summary of case study impacts

The payback framework describes the process from research to several categories of impact. The categories allow us to compare across different case studies. Table 10-2 shows, in point form and by impact category, some of the impacts, described more fully above, that have emerged from this grant.

Table 10-2 Payback

Payback category	Impacts from case study
Knowledge production	• 18 full, peer-reviewed academic papers and letters – five of which are directly attributable to research conducted on the grant • 12 abstracts
Research targeting and capacity building	Research targeting and development: • Helped develop patient cohort and control group that has formed the basis for an ongoing stream of research in this area over the past 15 years, looking at an ever-increasing number of genetic linkages with risk factors for stroke • Established a range of genetic linkages that the PI and his group have since gone on to examine in greater depth Capacity building: • Two PhD students based their theses wholly or substantially on research conducted on this grant • One MD student completed their thesis using data gathered wholly or substantially from research on this grant • Grant helped PI to establish his research group – and many of the researchers involved have either remained within it or collaborate with its work today
Informing policy and product development	• Non e
Health and health sector benefits	• Non e
Broader social and economic benefits	• Non e

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CHAPTER 11 Nimodipine binding in cerebral ischaemia

11.1 Overview of case study grant

This case study examines the grant titled 'Nimodipine Binding in Cerebral Ischemia', which was funded by the Heart and Stroke Foundation of Canada (HSFC) for a two-year period from July 1990 to June 1993. Led by Dr Antoine Hakim and conducted at the Montreal Neurological Institute (MNI), the team measured the in-vivo binding of [3H]-nimodipine[1] to brain cells in a rat model of reversible cerebral ischaemia. The team found that infarcts were observed only in regions showing persistent elevation of nimodipine binding, as determined by histology performed in a separate group of rats (n=8) after 24 hours of reperfusion. The team concluded in separate studies that increased nimodipine binding to ischaemic tissue, which is an index of tissue vulnerability to ischaemic cell death, is initially reversible. The conclusion, therefore, was that nimodipine binding was a sensitive indicator of early and reversible ischaemia-induced cerebral dysfunction.

The results of this study, together with findings from many others at the time, led to recognition that the negative effects of ischaemic stroke could be attenuated if the brain is given back its blood supply rapidly. The possibility that brain function may be salvaged after infarction if the brain is reperfused rapidly was subsequently proven in human studies with tissue plasminogen activator. Through the creation of various networks, campaigns designed to educate the public and healthcare practitioners about the way strokes are treated and managed have led to improved lives for many patients and millions of dollars saved for the healthcare system.

11.2 Introduction to case study

Scientists used to think that when a patient had a stroke, the affected part of the brain would die within minutes. It was then discovered that when the brain is deprived of its

[1] Nimodipine is a dihydropyridine calcium channel blocker originally developed for the treatment of high blood pressure.

blood supply, the brain cells allow an intracellular influx of ionic calcium through calcium channels that act like 'open gates', which leads to necrosis of neurons exposed to anoxic conditions (Choi, 1985, and Picone et al., 1989).

At the same time, the calcium channel was known to be regulated by the N-methyl-D-aspartate (NMDA) excitatory amino acid receptor (Choi et al., 1988). Scientists then found that administration of non-competitive NMDA antagonists that were able to penetrate the blood–brain barrier could markedly reduce ischaemic brain damage (Buchan, 1990; Gill et al., 1987; and Germano et al., 1987). On the other hand, competitive antagonists of the NMDA receptor were found to penetrate the blood–brain barrier poorly, yet after systemic administration they were also found to be effective in reducing ischaemic brain damage (Swan and Meldrum, 1990). CGS-19755, a prototype of this class of drugs, was found to reduce hippocampal brain damage resulting from transient forebrain ischaemia in gerbils, even when administered four hours after ischaemia (Boast et al., 1988). Thus, although stroke was recognised as a frequently devastating disease, these findings meant that when stroke does occur, a region of the brain adjacent to the most damaged area remains viable (alive) even if it does not function for a while. This meant that at least part of the deficit experienced by a patient after stroke may be reversible if doctors could identify these viable regions and were able to maintain and prolong the viable state of the tissue.

The main objective of the research conducted via Dr Hakim's grant titled 'Nimodipine Binding in Cerebral Ischemia', which was funded by the HSFC from 1990 to 1993, was to study global reversible cerebral ischaemia by further understanding the fundamental problem of blood–brain oxygen transfer in post-ischaemic and normal brains. By measuring the activity of the calcium channels that allow the passage of harmful calcium ion into cells, the research team believed that they could identify the regions of the brain affected by the stroke that were still salvageable.

The study proposed to explore the potential use of a previously developed nimodipine model to distinguish among the ischaemic regions of rat brains those regions that will recover and those that will die due to a lack of oxygen. This was achieved by exposing rat brain to short periods of ischaemia followed by reperfusion (restoration of blood flow). This model allowed the research team to test the overarching hypothesis that measurement of the binding capacity of the calcium channels would indicate the severity and duration of the ischaemia and predict whether the region had been reversibly or irreversibly injured.

In previous studies in their rat model, the team had found that when they restored previously restricted blood flow to the area around the middle cerebral artery, it recovered more quickly. Nimodipine binding was high in areas with restricted blood supply and declined when the blood flow was restored. Nimodipine binding persisted in areas where tissue death due to lack of oxygen had occurred, and so nimodipine was thought to be a sensitive indicator of early and reversible brain dysfunction caused by restricted blood flow (Berger and Hakim, 1989, and Vogel and Hakim, 1988).

The primary investigator (PI) on the case study grant was Antoine Hakim. Hakim had completed a postdoctoral fellowship in cerebral metabolism at the Montreal Neurological Institute and Hospital in 1980. In 1984, Hakim became a certified fellow of the American Board of Psychiatry and Neurology. When he prepared the application for the case study

grant, Hakim was an associate professor in the Department of Neurology and Neurosurgery at McGill University.

11.2.1 The case study approach

The findings presented in this case study are based on a combination of two face-to-face interviews with the PI (Dr Hakim) and a collaborator (Dr Diksic); a telephone interview with Dr Gjedde, who worked with Hakim as a methodologist on similar projects involving human studies using positron emission tomography (PET) during the early 1990s; a review of the original grant application and supporting documentation; a review of the PI's curriculum vitae; and documentary analysis of the scientific literature and bibliometric analysis.

11.3 Stage 0 – topic/issue identification

The idea for the project arose from the PI's training and clinical interest in stroke and a desire to improve patient outcomes and add to the state of knowledge in this area of research. The grant application was submitted to and funded by the HSFC through an open operating grants competition. In this section, we further examine how the research topic was identified based on the two critical factors:

1. training and clinical interest
2. a desire to improve patient outcomes and fill the gaps in knowledge by building on previous findings.

11.3.1 The PI's training and clinical interest

Dr Hakim's laboratory-based research activities were frequently guided by his clinical exposure to patients with neurological problems. During his neurology residency at the Montreal Neurological Institute and Hospital, he was intrigued by patients with Wernicke-Korsakoff syndrome, who exhibit severe memory deficits in association with alcoholism and thiamine deficiency but show only very limited brain lesions. Hakim then began to study how systemic conditions result in focal brain damage, which led to an interest in stroke because of the selective nature of the damage caused by ischaemic stroke.

The idea for this research initially arose from clinical and scientific curiosity and dissatisfaction with current practice. The PI stated that, at the time, clinical practice after a stroke tended to be focused on palliation; there was no urgency to reperfuse the affected area, as it was believed that once a stroke had occurred, the damage was done and only time would tell regarding the patient's functional levels and outcomes. As previously discussed, research was beginning to indicate that some of the cerebral deficit following an ischaemic stroke could be prevented or reversed if the viable regions could be identified and if it was understood how to maintain and prolong the viable state of the tissue.

Research on this issue was being undertaken internationally, but the crucial step was being able to 'create a stroke' in the laboratory. The PI had shown, in previous work, a viable model to simulate stroke in a rat model in the laboratory.

11.3.2 Improve patient outcomes and building on previous findings

This proposal was to build on the data obtained from a permanent focal ischaemia model that was already in use. The PI referred to his 1989 paper, published in the *Journal of Cerebral Blood Flow and Metabolism*, as one of his most important influential papers leading up to the case study grant (Hakim et al., 1989). In this paper Hakim et al. described temporarily occluding the middle cerebral artery in rats and showed how nimodipine bound to brain tissue, thereby indicating the areas of the brain that were facing potential death via open calcium channels. This paper also indicated that brain tissue facing potential death could recover if blood flow resumed to the affected areas. Hakim believes that these results helped focus people's minds on the fact that there is time to treat patients after they have a stroke. Up until this time, the general belief was that once an individual had a stroke there would be no return of function.

Additional previous work conducted by the research team suggested that:

1. the more profound the ischaemia to which a region is subjected, the more rapid the activation of its calcium channels
2. persistent binding to a dihydropyridine ligand in a region may indicate that this area is not inevitably committed to infarction.

At the time of this grant proposal, when using the permanent occlusion model, it was found that all the brain regions exposed to ischaemia eventually infarct but at different rates. Thus it had not then been possible to test the ability of the nimodipine binding model to distinguish the ischaemic regions that could be salvaged and those that could not.

Cell death occurs when there is an unregulated and excessive passage of calcium ions into cells. By measuring the activity of the calcium channels, it was believed that the regions of the brain affected by the stroke that are still salvageable could be detected. Previous studies had shown that by administering non-competitive NMDA antagonists, which are lipophilic and therefore able to penetrate the blood–brain barrier following systematic administration, clearly reduced ischaemic brain damage (Buchan, 1990; Gill et al., 1987; and Germano et al., 1987). Thus it was believed that damaged cells could be saved with timely re-supply of oxygen and glucose to the ischaemic areas through restitution of blood flow. Hakim called it the 'window of opportunity for treatment'. Animal research indicated that the window of opportunity for treatment was 4–6 hours (Boast et al., 1988). The window of opportunity was thought to be patient specific, depending on the state of the vessels – and whether there are deposits within them – and the extent of occlusion. Much is still unknown in this area of stroke research.

When writing this grant proposal, the PI was Director of the Cerebral Ischaemia Laboratory at the MNI and had extensive experience in the field.

11.4 Interface A – project specification and selection

The PI developed the idea and drafted the grant application on his own, although he stated that he discussed his proposal with colleagues and thus was influenced by others who were not involved in the project.

The animal model used was the one developed by Pulsinelli (Pulsinelli et al., 1988). This model involves occluding the middle cerebral artery, thereby creating damage similar to that seen in patients with stroke. Hakim described it as a well-accepted model of global ischaemia that allows for reperfusion. He also indicated that it correlated well with the varying degrees of neuronal injury. Using this model, the team could insert radioactive nimodipine through calcium channels into neuronal cells. The model presented several advantages, which included ease of preparation, a high rate of predictable ischaemic neuronal damage, a low incidence of seizures and the absence of prolonged anaesthesia (Pulsinelli et al., 1988). Four experimental programmes were set up to:

1. investigate the most appropriate duration of ischaemia for evaluation of dihydopyridine binding
2. investigate the effect of reperfusion on dihyropyridine binding
3. evaluate non-specific binding
4. evaluate the regional maximum number of binding sites and an index of binding affinity following two ischaemic intervals.

For a number of years, Dr Hakim's laboratory had extensively used dihydropyridines[2] to label voltage-sensitive calcium channels. *In-vitro* binding with this ligand has proved to be extremely insensitive to pathological change, whereas in-vivo binding had shown marked sensitivity to ischaemic insult. Specifically, the team had shown that in-vivo binding of [3H]-nimodipine (a radiolabelled dihydropyridine) was initially increased in areas of severe ischaemia but this was followed by a complete absence of binding capability (Hakim and Hogan, 1991). In areas of more moderate ischaemia, a less pronounced and delayed increase in nimodipine binding occurs. It was thought that the appearance and disappearance of dihydropyridine binding was a reflection of the severity and duration of cerebral ischaemia and thus a potential marker for cell death (Hogan and Hakim, 1992).

Administering radiolabelled nimodipine molecules after stroke would allow radiographic imaging to be used to determine which brain cells had been affected. Knowing which part or parts of the brain are infarcted could allow doctors to identify and target these areas for treatment, thus preventing further cell death.

The study also intended to test the following hypotheses:

- Oxygen consumption of cortical regions of the brain *in vivo* fails to increase during activation because the functional density of capillaries in the brain tissue is close to the maximum achievable density of perfused capillaries.
- In patients recovering from cerebral ischaemic stroke, the oxygen consumption of post-ischaemic brain tissue will remain low until the functional density of capillaries has returned to the normal average.

[2] Dihydropyridine is a molecule based on pyridine and is the parent of a class of molecules well known in pharmacology as L-type calcium channel blockers.

The methods were descried as relatively simple. The team injected nimodipine into the bloodstream of a rat and then measured its concentrations in the bloodstream and brain tissue. By comparing the concentration of the molecule in the bloodstream with the concentration in the brain tissue, the team estimated how many more molecules would be found in brain tissue compared with what would be expected if uptake was completely passive. That is, did the brain seem to hold on to more of this drug than would be predicted with passive distribution between the bloodstream and the brain tissue? This difference, this excess of the molecule in the brain at given times, is known as the binding potential.

The experimental outline was as follows:

1. Using autoradiography, [3H]-nimodipine binding was measured regionally in the brains of rats exposed to 10, 20 or 30 minutes of carotid clamping. The interval that would maximise binding to [3H]-nimodipine in the regions not committed to histologic infarction was to be chosen for subsequent experiments.
2. The effect of varying the duration of reperfusion following a set interval of carotid clamping on nimodipine binding and subsequent histologic outcome was determined.
3. Experiments were planned to determine the degree of non-specific binding to nimodipine in normal and ischaemic rats.
4. The regional maximum number of binding sites and an index of binding affinity following two ischaemic intervals were measured.

Interpretation of the results was dependent on outcome studies on the role of the blood–brain barrier in the model and the influence metabolites of nimodipine may have had on the autoradiographic measurements.

The feedback from the review committee was predominantly positive and the proposal received a strong score. The PI was known to the committee as an 'outstanding investigator who had made important contributions to the field of ischaemic injury' (Scientific Review Committee Report, 1990).

Some concern was voiced by the committee with respect to the terminology used in the proposal (e.g. necrosis/infarction versus selective neuronal damage). This was important, because although nimodipine binds to infarcted regions in focal models of rodent ischaemia, this may not occur in the restricted and less severe damage of a global ischaemic model. One reviewer expressed concern that the binding may not be restricted to voltage-sensitive calcium channels or strictly active ones. However, this did not deter reviewers from recognising and acknowledging the potential of the anticipated findings. Others wanted to know about the dynamics of the receptor binding, how it opens and closes, and how the calcium influx could be measured.

Additional concern was expressed regarding overlap between Dr Hakim's multiple grants (the details of these grants are discussed later on in the section called 'Funding') and the uniqueness of this proposal. It was decided, however, that this grant was sufficiently different from his other funded grants and represented a new research direction.

The overall decision of the reviewers was that this application was excellent and had the potential to produce novel and important information for the field of cerebral ischaemia. One reviewer stated that 'the ability to image cerebral ischaemia at the early, critical time points is essential if evaluation of new therapies is to be performed' (Scientific Review Committee Report, 1990). Ultimately, the reviewers and the funding organisation were excited by this research proposal.

11.5 Stage 1 – inputs to research

In the sections that follow, we outline how the inputs contributed to the research undertaken on this grant.

11.6 Funding

Dr Hakim applied for a three-year grant from the HSFC for a total of Can$145,309. During an interview with the PI, he claimed that it was very difficult, at the time, to get funding for stroke research because the common belief was that not much could be done to assist patients after a stroke and that damage and cell death were not preventable or reversible (Hakim interview, 2008). An annual breakdown of the amounts requested is shown in Table 11-1. The funding requested was intended to pay for a technician, a summer student, experimental animals (rats), radioactive supplies and other consumables. The methods for this work were already established in the laboratory, so there was no request for additional equipment.

Table 11-1 Annual breakdown of funding requested

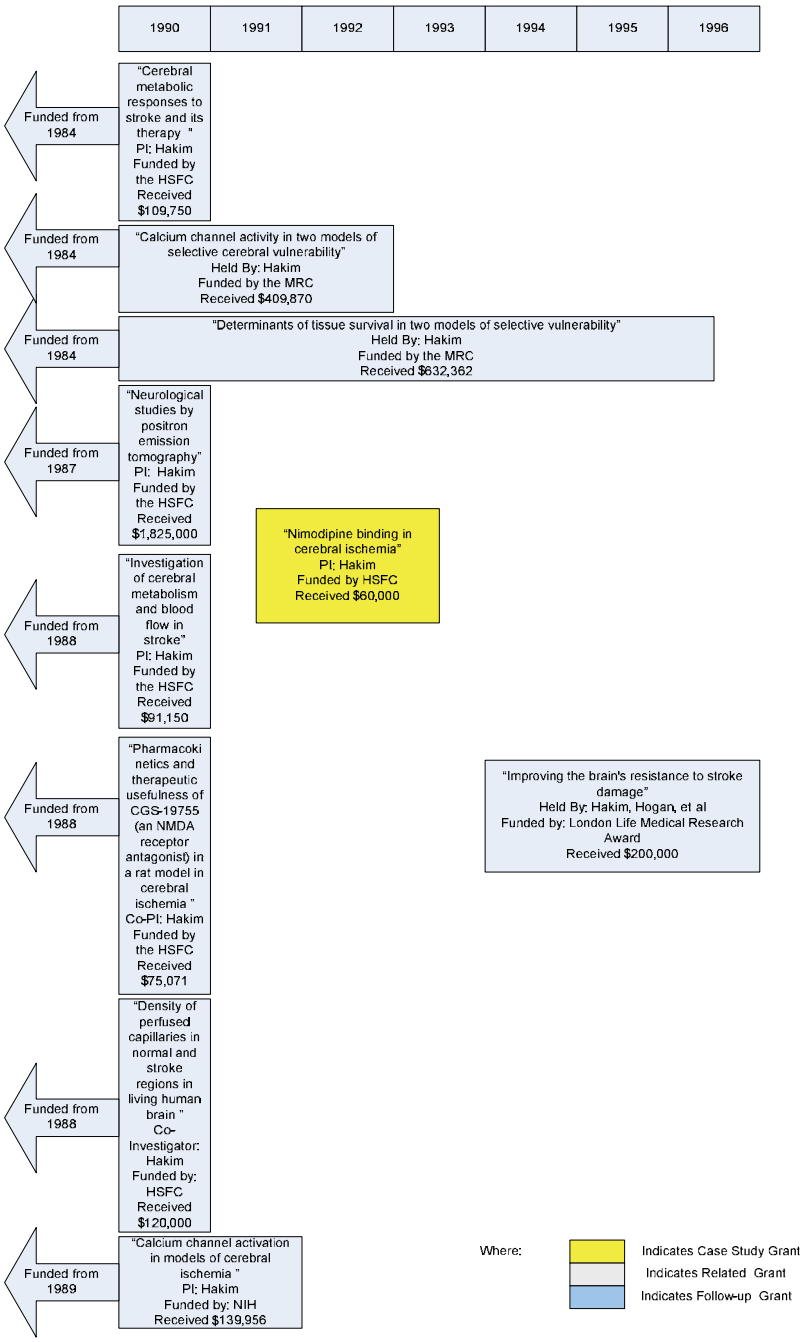
Year	Amount (Can$)
1991–1992	45,394
1992–1993	48,397
1993–1994	51,518
Total funding requested	**145,309**

The PI received two years of funding at Can$30,000 per year, which the PI did not consider adequate.[3] This grant was complemented by several other grants, including two grants from the Medical Research Council (MRC): one to study focal ischaemia and another to study thiamine deficiency. Dr Hakim also held a grant from the National Institute of Health in the United States to study calcium channel activity in focal ischaemia. Another two grants – one from the MRC and another from Ciba-Geigy – were to examine the competitive antagonist CGS19755 in focal ischaemia. The PI also collaborated with Dr Gjedde on a grant from the HSFC that involved both rat autoradiography and human and primate PET. As the PI stated, 'no research is an island in and of itself', by which he meant that research work and findings are cumulative and

[3] The PI recalled not receiving the amounts for which he applied, and records from the MRC show that he received Can$60,000 over two years from the HSFC for this grant. However, data obtained from the HSFC showed that he received $30,000 in the first year and Can$45,387 in his second year. Thus, the amount of Can$60,000 may be a conservative figure and could be as high as Can$75,387.

inter-related. It is important to recognise that the PI was working on various aspects of focal ischaemia and that the various grants collectively contributed to greater knowledge and may have had some overlap. Figure 11-1 shows the related funding from peer-reviewed grants the PI had access to over the same period as the case study grant plus and minus two years.

Figure 11-1 Related peer-reviewed funding held by the PI from 1990 to 1996



11.6.1 Collaborations

The research was greatly facilitated by collaboration with Dr Diksic and his team, who made the isotopes required for the studies. Doing so required the work of 12 individuals and thus was a very expensive process.

11.6.2 Facilities

The research was conducted at the MNI, where all interviewees said equipment was a huge problem. When the team initially began to conduct studies using nimodipine, they found they suddenly needed different kinds of equipment, as systems had to be developed to detect nimodipine in the brain and analyse the results. For instance, the team needed access to video equipment, cassettes and a dark room. The challenges of obtaining this new equipment were faced prior to this grant. Laboratory space was thought to be sufficient by the interviewees.

Dr Diksic also claimed that there was good support from the institute, who funded about 50 percent of the team's operating costs. The remaining funds would have come from private donations or peer-review grants.

11.6.3 Research team

As indicated on the grant application, the research team officially consisted of the PI (Dr Hakim), a research student (Matthew Hogan) and a technician (Jocelyn Barthe).

At the time of this grant, the PI, who holds a doctor of philosophy (PhD) degree in biochemical engineering, as well as a medical degree, was an associate professor in the Department of Neurology and Neurosurgery. He was also the coordinator of the Brain Imaging Center at the MNI. The PI was known in the field of focal ischaemia as an accomplished researcher with an extensive publication record and a successful record of obtaining peer-review funding. He and his group at the MNI have been recognised internationally for their research work.

Dr Hogan was a fellow at the time and now specialises in neurology and stroke research at the Ottawa Hospital Research Institute. He was first author on many of the publications.

Dr Hakim described the laboratory technician, Jocelyn Barthe, as someone who understood what the research team was trying to accomplish and helped them greatly. Barthe completed the first occlusion in a rat in Hakim's laboratory.

The team also included visiting postdoctoral students from Japan: Hiroto Kuwabara and Shunya Takizawa. Gjedde explained that Hakim involved a mixture of basic and clinical researchers with a mixed skill set and expertise in his projects and by doing so strengthened his studies.

11.7 Stage 3 – primary outputs from research

Dr Hakim explained that the most important finding resulting from this and other work he was doing at the time was that it indicated that there is a 'window of opportunity' in which brain tissue can be restored to normal function following ischaemia.

With the funds obtained through the HSFC grant titled 'Nimodipine Binding in Cerebral Ischemia', the team measured the in-vivo binding of [3H]-nimodipine to brain cells in a rat model of reversible cerebral ischaemia. By exposing living rat brain cells to short periods of ischaemia followed by restoration of blood flow (reperfusion), the team intended to investigate the ability of nimodipine to cross the blood–brain barrier and to determine whether it would interact with specific receptors. They found that it was possible to determine that binding had occurred, but it was not clear that the nimodipine molecules had the expected effects. In general, infarct was observed only in regions that showed persistent elevation of nimodipine binding following reperfusion, as determined by histology performed in a separate group of rats (n=8) after 24 hours of reperfusion. The team concluded that increased nimodipine binding to ischaemic tissue, which is an index of tissue vulnerability to ischaemic cell death, is initially reversible with prompt reestablishment of cerebral blood flow and is a sensitive indicator of early and reversible ischaemia-induced cerebral dysfunction. Thus, nimodipine was found to be effective and helpful as a marker to identify open calcium channels.[4]

This grant also allowed the team to further refine their methods and to define and clarify the concept of the binding potentials that would subsequently be used in humans to show the binding potentials of other experimental drugs. This grant resulted in the onset of a whole new approach to monitoring indirectly the appearance and effects of the brain's own molecules. It was a very productive period from a technical point of view and paved the way for a whole new era in brain studies in humans using PET (Hakim interview, 2008).

11.7.1 Knowledge production

The PI identified 23 publications, including peer-reviewed, invited and non-refereed articles and manuscripts, as directly related to the case study grant. A selection of these articles is described below in an effort to outline the main findings created by the research team. It should be noted that this grant was part of a much larger programme of research, so attribution to the case study grant may not always be completely clear.

1. Hogan, M. and A.M. Hakim, 'Pathophysiology of Stroke: Laboratory and Clinical Insights', *Current Opinion in Neurology and Neurosurgery*, Vol. 3, 1990, pp. 46–49.
2. Hogan, M., A. Gjedde and A.M. Hakim, 'Nimodipine Binding in Focal Cerebral Ischemia', *Stroke*, Vol. 21, Suppl. IV, 1990, pp. IV78–IV80.
3. Hakim, A.M., M.J. Hogan and S. Carpenter, 'Time Course of Cerebral Blood Flow and Histological Outcome after Focal Cerebral Ischemia in Rats', *Stroke*, Vol. 23, 1992, pp. 1138–1144.
4. Takizawa, S., M.J. Hogan, A.M. Buchan and A.M. Hakim, '*In Vivo* Binding of [3H] Nimodipine in Rat Brain after Transient Forebrain Ischemia', *Journal of Cerebral Blood Flow and Metabolism*, Vol. 14, 1994, pp. 397–405.

[4] Hakim was working simultaneously with Gjedde and Evans in human studies, with the support of Eli Lilly, to test nimodipine as a neuroprotective drug for patients with stroke. Nimodipine as a drug was found to be ineffective in cardiac studies and in changing cardiac function in humans.

5. Hogan, M.J., S. Takizawa and A.M. Hakim, 'In vitro binding of [3H] nimodipine and [3H] CGS-19755 to rat brain in focal cerebral ischemia', *Experimental Neurology*, Vol. 134, 1995, pp. 56–63.

The first article listed above, which was published in 1990, represents a review in which the authors, Hakim and Hogan, highlighted recent evidence showing that the loss of neurological function within the penumbra that results from ischaemia is reversible although time dependent (Hogan and Hakim, 1990). They explained that the initial changes observed in ischaemic tissue include a rapid loss of high-energy metabolites and a fall in intracellular pH. Shortly thereafter, the extracellular potassium concentration increases, while extracellular concentrations of calcium and sodium decrease and the extracellular fluid space shrinks. The ionic and fluid shifts were attributed to changes in cell membrane permeabilities associated with cell-membrane depolarisation. A large body of evidence indicated that the shift of calcium ions into the cell led to cell death. In addition, there was evidence that calcium channel and NMDA receptor antagonists may be beneficial in cerebral ischaemia, which suggested that an improved understanding of ischaemic events could lead to better therapy of stroke.

In the second article, the team reported research investigating the binding properties of the voltage-sensitive calcium channel antagonist nimodipine in a rat model of focal cerebral ischaemia (Hogan, Gjedde and Hakim, 1990). Male Sprague-Dawley rats weighing 250g underwent occlusion of both the proximal middle cerebral artery and the ipsilateral common carotid artery. [3H]-nimodipine (130Ci/mmol) was infused intravenously and circulated for 30 minutes. The rats were killed at various time points; five minutes or 4, 24 or 48 hours after occlusion. The brains were removed and examined by autoradiography. The team observed a focal increase of nimodipine binding in severely ischaemic regions five minutes after occlusion, which also appeared in regions with presumed penumbral blood flow four hours after occlusion. These results led the team to hypothesise that nimodipine binds to activated calcium channels in ischaemic tissue. However, this increased binding depended on the duration and severity of cerebral ischaemia. The team concluded that sequential measurement of nimodipine binding might allow identification of regions with potentially reversible effects of ischaemia and monitoring of their response to therapy.

The third article listed above describes the relation between time-dependent changes in cerebral blood flow and the appearance of infarction after focal cerebral ischaemia (Hakim, Hogan and Carpenter, 1992). The study aimed to measure perfusion after simultaneous occlusions of the left middle cerebral artery and ipsilateral common carotid artery in rats and to correlate the measures with the timing and distribution of histological changes. The team studied histological and cerebral blood flow changes five minutes or 4, 24 or 48 hours after the onset of focal ischaemia. Blood flow was determined autoradiographically. A coronal template subdivided into regions of interest was applied to the autoradiographs and the histological data. The team observed that cerebral blood flow five minutes after occlusion fell below 50 percent of normal in some regions of the non-occluded hemisphere. Many ischaemic structures showed stable blood flow for 48 hours after occlusion, confirming that reperfusion was minimal in this model. Infarction eventually occurred in all areas in which blood flow at five minutes fell below ten percent of that in control rats, but infarction appeared earlier in regions in which blood flow at five

minutes was below five percent of that in control rats. When blood flow at five minutes rose above 12 percent of that in control rats, the occurrence of infarction became unpredictable. These results led the team to conclude that, despite the general dependence of infarction on perfusion levels, blood flow alone was not a reliable indicator of those regions committed to infarction and that duration of ischaemia also had to be taken into account.

The fourth article reports on the regional variation in relative in-vivo binding of the L-type voltage sensitive calcium channel (VSCC) antagonist [3H]-nimodipine to brain cells following transient forebrain ischaemia in the rat (Takizawa et al., 1994). At 30 minutes of reperfusion, after 20 minutes of forebrain ischaemia, [3H]-nimodipine binding was significantly increased in two areas of the brain – the striatum and dentate – which suggests that VSCCs were responding to ischaemic depolarisation. Two hours after ischaemia and reperfusion, binding in all brain structures returned to normal levels, which indicated repolarisation of cell membranes. After 24 hours of recirculation, increased [3H]-nimodipine binding was again observed in the striatum and dentate. Binding remained elevated in the striatum and dentate, and increased binding became evident in the CA1 region of the hippocampus after 48 hours of reperfusion. With the exception of the dentate gyrus, the second increase in [3H]-nimodipine binding anticipated or coincided with the observed regional ischaemic cell changes. These observations in global cerebral ischaemia support previous work that indicated that in-vivo binding of [3H]-nimodipine to the L-type VSCC may be an early and sensitive indicator of impending ischaemic injury. Such measurements were felt to be of use in identifying vulnerable brain regions and defining a therapeutic window of opportunity in models of cerebral ischaemia.

As focal cerebral ischaemia resulted in increased in-vivo binding of calcium channel antagonists to both the L-type VSCC and the NMDA receptor-linked calcium channel, the team then investigated the effect of focal cerebral ischaemia on the in-vitro binding of calcium channel antagonists to rat brain (Hogan, Takizawa and Hakim, 1995). Quantitative autoradiography was used to measure regional in-vitro binding of the VSCC antagonist [3H]-nimodipine and the competitive NMDA receptor antagonist [3H]-CGS-19755 to rat brain following four hours of irreversible focal cerebral ischaemia. [3H]-Nimodipine binding to the non-ischaemic hemisphere was characterised by one binding site, with regional binding affinity (KD) estimates ranging from 221pM to 482pM and maximal binding site densities (BMAX) ranging from 13.2pmol/g (9.6–17.5pmol/g) tissue (estimate and 95 percent confidence interval) in the CA1 region to 32.5pmol/g (26.5–39.9pmol/g) tissue in dentate. [3H]-CGS-19755 binding to the non-ischaemic hemisphere was characterised by KD estimates ranging from 59nM to 97nM and BMAX values ranging from 143pmol/g (108–192pmol/g) tissue in the cortex to 569pmol/g (515–641pmol/g) tissue in the CA1 region. For [3H]-CGS-19755, a model of two binding sites was applicable in several brain regions. No difference in binding site densities or binding affinities was observed between ischaemic and paired non-ischaemic structures (cortex and striatum) with either ligand. The team concluded that in-vitro binding of [3H]-nimodipine and [3H]-CGS-19755 to ischaemic brain failed to identify ischaemic-induced changes in calcium channel function previously reported by in-vivo binding methods.

Bibliometric analysis was conducted on 13 peer-reviewed manuscripts of the 23 publications identified by the PI as directly related to the case study grant. The analysis

excluded reviews, notes and position papers. Eight of 13 articles were included in the citation analysis, as five articles included in the broader bibliometric analysis were not indexed in the Web of Science and thus could not be included in the citation analysis.

Table 11-2 Publication output and impact

Number of journal articles:	13
Number of articles included in citation analysis:	8
Total number of citations (all papers):	114
Aggregate relative citation impact:	0.31 (Class II)
Self-citations:	42%

Distribution of publications across relative citation impact classes:

	Class I (uncited)	Class II (>0 and <0.8 citations)	Class III (0.8 to 1.2 citations)	Class IV (1.2 to 20 citations)	Class V (>2.0 citations)
Number of publications		7	1		
Proportion of total output		88%	13%		

Most highly cited publication[5]:	Hakim, A.M. and M.J. Hogan, 'In Vivo Binding of Nimodipine in the Brain: I. The Effect of Focal Cerebral Ischemia', *Journal of Cerebral Blood Flow and Metabolism*, Vol. 11, 1991, pp. 762–770
Times cited:	38

11.7.2 Dissemination

The methods of disseminating the findings obtained through the case study grant included publishing papers, as described above, and attending national and international meetings and conferences at which a series of abstracts, lectures and poster presentations were communicated to various audiences. Dr Hakim was not a keynote speaker at a conference in relation to the specific results from this case study grant; however, he was invited to organise and chair a lecture titled 'Reversibility in Cerebral Ischemia' at a symposium for the 17th International Joint Conference on Stroke and Cerebral Circulation via the American Heart Association in Phoenix, Arizona, in January 1992. He was also an invited lecturer in Istanbul, Turkey, in October 1990, giving a presentation entitled 'Cerebral Ischemia: From Pathophysiology to Treatment'.

Communication was also open between the team and the pharmaceutical company Eli Lilly regarding the effectiveness of nimodipine as a marker. Eli Lilly was interested in the potential of nimodipine as a drug. Hakim added that the team was doing human studies at the same time to test the effectiveness of nimodipine on cardiac function. Eli Lilly provided the team with the required compounds and allowed them to manufacture nimodipine in the laboratory so they could do their human studies using radiolabelled nimodipine.

[5] Citation count extracted April 2009.

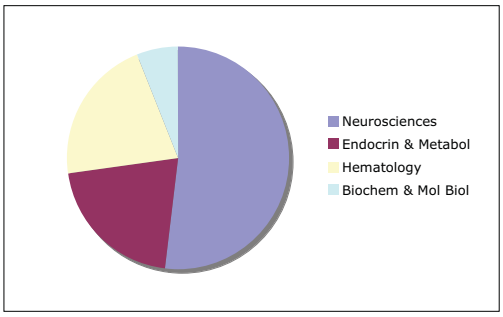
Dr Hakim, who was an associate professor at McGill University at the time, said that this work did influence his teaching, as he would incorporate research findings into his lecture slides.

The PI stressed that another enabler for the success of this and other research was the networking and collaborating that took place nationally and internationally. The PI acknowledges that these activities have been central to him achieving his desired research outcomes. Through formal and informal networks, the PI has disseminated his findings to academics, patient groups, practitioners, industry and health-system decisionmakers.

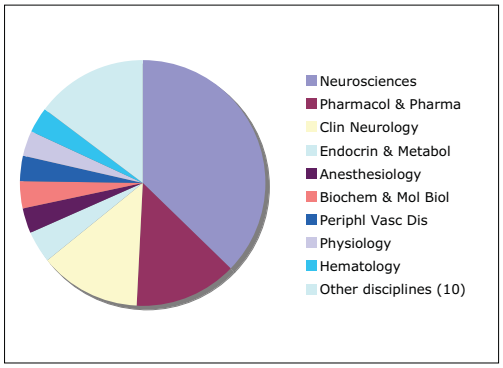
The bibliometric analysis also investigated knowledge diffusion, which showed that Dr Hakim and his team most commonly publish in the area of neuroscience. Their work is most commonly cited by those working in neurosciences and pharmaceuticals systems in the United States.

Figure 11-2 Knowledge diffusion: fields of research of output (a), fields of citing papers (b) and countries of citing papers (c)

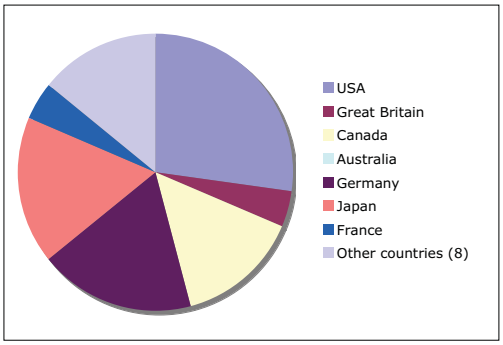
(a)



(b)



(c)



The PI, who is currently the Scientific Director of the Center of Excellence for the Heart and Stroke Foundation's Centre for Stroke Recovery, continues to be aware of the need to communicate stroke research, as well as the social consequences of stroke. He currently involves social scientists and economists in his grant applications and research in an effort to make the research more multidimensional and inclusive of the social sciences aspects.

The PI was and still is a large advocate of stroke therapies. He has communicated the need for drugs to the public and the pharmaceutical industry and has informed them of the huge social and economic potential. Years after this specific grant, dissemination of his

work has reached policymakers via his role as Chief Executive Officer (CEO) of the Canadian Stroke Network (CSN).

11.7.3 Training and capacity building

This grant was a smaller contribution to a much wider research programme that was ongoing within the PI's laboratory at the time. Thus, unless otherwise stated, the impacts presented here are a result of the entire programme of research and cannot be attributed solely to the case study grant.

Overall, Dr Hakim felt that this grant and research programme had assisted in his ability to recruit students and research staff to his laboratory. He said that young recruits want to go to a 'happening place' and that any notoriety from a research perspective will influence those recruits. Due to this grant, and others held within his laboratory at the time, Hakim was able to expand his group and improve the physical infrastructure of his laboratory.

Dr Hakim has had an extremely successful research career focused on stroke research. In 2000, Hakim became, and remains at the time of writing, CEO and Scientific Director of the CSN. Hakim is also Director of the Neuroscience Research Program at the Ottawa Hospital Research Institute, a position he has held since 2001, and he has maintained a position in the Department of Cellular and Molecular Medicine, as well being Professor and Chair of Neurology, at the University of Ottawa, since 1992. In addition, Hakim became Scientific Director of the Heart and Stroke Foundation's Centre for Stroke Recovery in 2005 and accepted the position of Chair of the Guideline Committee for the World Stroke Organization a few years ago.

While participating on this grant Dr Matthew Hogan completed a fellowship in stroke research. Hogan currently holds two positions: associate professor in the Department of Medicine and Department of Cellular and Molecular Medicine at the University of Ottawa and neurologist within the Division of Neurology at the Ottawa Hospital General Campus. He also maintains a laboratory at the Neuroscience Research Institute in Ottawa, where his research is focused on the study of physiologic events that occur during acute cerebral ischaemia. These investigations include both laboratory-based basic science projects and clinical imaging studies in humans to study how the human brain responds to both normal and pathological challenges. Hogan's work with nimodipine was just part of the spectrum of work he did with Dr Hakim. Hakim believes the skills Hogan gained through the fellowship have been influential for his career and current research interests. Hogan's current work relies on metabolic and functional imaging techniques. The implementation and development of these computer-based imaging methods forms a secondary area of study within Hakim's laboratory.

Albert Gjedde, who collaborated with Dr Hakim on the research project and on Hakim's broader stroke research programme, is now a professor and Chair of the Department of Neuroscience and Pharmacology at the University of Copenhagen, Denmark, and an adjunct Professor of Neurology and Neurosurgery at McGill University in Montreal. Dr Gjedde said that the work he shared with Dr Hakim has been very fruitful for the development of stroke treatment in Denmark, where a number of publications have resulted, including several stroke papers and papers on the use of magnetic resonance scanning for very early diagnosis published in Danish journals and internationally.

11.7.4 Benefits to future research and research use

The results of this research, and ongoing and subsequent studies within the PI's laboratory and elsewhere, confirmed that damage caused by stroke changes from minute to minute and that the reversibility of ischaemic injury that was seen in rats is also present in people. Results from this research, in combination with the findings of other concurrent national and international research, destroyed the myth that the damage resulting from ischaemia is permanent; it is now known and accepted that damage can be reversed in a time-dependent fashion. The science has indicated that patients need proper treatment to save the surrounding cells from permanent damage and death. The PI stressed that this did not all happen because of the one grant.

In the early 1990s, nimodipine was in fashion and drug companies were interested in learning more about its potential as a neuroprotective drug for the prevention of consequences after stroke. This idea was abandoned after it became evident that, although there were often some effects in experimental animals, particularly in rats, they could not be replicated in other animals (Hakim was involved in tests in humans at the same time as the work funded by the case study grant). Nimodipine was not found to have a positive effect in humans as a neuroprotective drug. Researchers believe this is because of the blood–brain barrier in humans. Dr Hakim explained that researchers are still looking for neuroprotective drugs, although none have been found to date.

Future use of this research is largely restricted to stroke, although the scope does include risk factors for stroke, such as high blood pressure (HBP). The research team has been in communication with drug companies regarding medications for HBP. In addition, some of the subsequent research conducted by the PI has studied the cells that were dying as a result of ischaemia, which has yielded results that can be applied to numerous other conditions.

The team later received a grant from the MRC for work in which they proposed to monitor various brain transmitters in drug addiction and alcoholism using the binding techniques developed at MNI. This is still a very active line of research.

Dr Hakim has continued to work in the area of stroke, and his efforts have resulted in the creation of the CSN. Hakim led the application to establish the CSN in 1999. The team was successful and was awarded a term of seven years, with funding of Can$4.5 million per year. Hakim is CEO and Scientific Director of the CSN, which has brought together more than 100 scientists and clinicians to work collaboratively within a broad partnership that includes the academic research centres, the HSFC, industry and government. The CSN's research falls within four themes: 'Better Stroke Prevention', 'Optimizing Acute Stroke Care', 'Reducing Cell Death and Minimizing Stroke Damage' and 'Enhancing Brain Repair and Functional Recovery Post-Stroke'. In addition to research and partnership development around stroke, the CSN accepted the mandate of preparing the future generation of stroke researchers and caregivers. The CSN's ultimate goal is alleviation of the burden of stroke for the individual, their family and the nation.

In its first term, the CSN established a new approach to stroke research. Multidisciplinary, inter-institutional collaborations were formed to address major research challenges in the field of stroke and to ensure that the results of this research are translated into health and economic benefits. This approach produced a number of important research contributions.

Scientists from the CSN evaluated a potential role for stem cells in stroke, described the role of TRPM7 channels in imparting ischaemic vulnerability and developed a blocker for these channels, which is now being tested in phase I–II trials. Scientists from the CSN also showed calcium movement in previously unknown hemi-channels, which may activate inflammatory pathways. Based on a thorough review of the world literature, experts within the CSN have also determined the best practices in stroke rehabilitation, and these are currently being tested in clinical trials. The CSN also maintains a registry of stroke patients, which has allowed a number of correlations between premorbid physiological and social characteristics of patients and stroke outcomes. Finally, in its first term, the CSN put together a broad financial partnership that contributed to training 45 stroke scientists and clinicians. The network has just been awarded a second seven-year term, with an unprecedented 35 percent increase in budget, to continue its research and to establish and promote a Canadian Stroke Strategy (CSS). The strategy, devised by the CSN in partnership with the HSFC, is a plan that starts with the premise that the best stroke research must be moved into practice.

11.8 Stage 4 – secondary outputs

As stated earlier, one of the most important findings from the rat studies, together with other research, was that when the brain is deprived of its blood supply there is time during which one can intervene medically. This idea led to the realisation that when a patient recognises that they are having a stroke and seeks medical attention, damage resulting from the stroke can be minimised if they are given tissue plasminogen activator (tPA), a thrombolytic agent (clot-busting drug), in a timely fashion (Marler, 1995). It thus became paramount that the general public knew the signs and symptoms of stroke and when to seek treatment. Spearheading this movement in Canada, Dr Hakim, along with others, as organised by the HSFC, was involved in surveying the public to determine their level of awareness regarding the symptoms of stroke. The results of the survey indicated that the public's level of knowledge regarding the symptoms of stroke was poor. There was a need to educate the public, as well as to train paramedics and staff in emergency departments.

Before administering tPA, it is important to take an image of the brain via computed tomography (CT) to determine whether the stroke is a result of bleeding or a blocked artery. If the stroke is due to the latter, damage can be minimised if the patient is given tPA. Scanners for CT are expensive. As it was unfeasible at the time that all hospitals would acquire the machinery necessary for the timely brain images, paramedics needed to recognise patients and transport them to appropriate facilities. Staff at emergency departments needed to be trained on when and how to administer tPA, and technicians were required on staff at all hours to collect and interpret brain images. This involved a vast education effort by the CSN, which started in 2000, and was complemented by the Center for Stroke Recovery (CSR); both of these were preceded by the Ottawa Stroke Consortium for Applied Research (OSCAR), which was founded in 1994 and led by Dr Hakim.

Another challenge overcome by the efforts of OSCAR, the CSR and the CSN was the paramedic system. Previously, ambulances could not cross municipal lines, meaning that if a paramedic picked up a patient who was having a stroke, the ambulance was not allowed

to bring them to the appropriate facility (one with a CT scanner) if it was not in their municipality. The patient had to be brought to the nearest centre and was then transferred to an appropriate facility after further assessment. This meant precious time lost for the patient. This has since changed in Canada and is included in the Canadian best practice recommendations for stroke care (Lindsay et al., 2008).

11.9 Stage 5 – adoption by practice and the public

The CSN is committed to changing the delivery of stroke care by ensuring the most recent stroke knowledge is transferred to healthcare providers and used for patients. With respect to prevention, the CSS proposed a broad coalition aimed at setting up stroke prevention clinics across the country. In acute therapy, the CSS instituted a programme of national education – not only of the public but also of healthcare providers – thus bringing about a change in the delivery of acute stroke care through coordination of services and implementation of best practice. Thrombolytic therapy with recombinant tPA has been shown to be cost effective and safe. By 2003–2004, tPA came to be the expected treatment where hospitals had, or had access to, the appropriate imaging capacity. The latest version of the Canadian best practice recommendations for stroke care recommends that 'all patients with disabling acute ischemic stroke who can be treated within 4.5 hours after symptom onset should be evaluated *without delay* to determine their eligibility for treatment with intravenous tissue plasminogen activator' (Lindsay et al., 2008). Thrombolysis for stroke with tPA is now a standard of care in North America (Nadeau et al., 2006). This has resulted in very substantial improvement (sometimes 10-fold increases) in the rate of tPA administration in several Canadian sites (Hakim interview, 2008).

11.10 Stage 6 – broad health and economic outcomes

Stroke has large social consequences. Disability affects 75 percent of stroke survivors enough to decrease their employability (Coffey et al., 2000). Stroke can affect patients physically, mentally, emotionally and with some combination of the three. Some of the physical disabilities that can result from stroke include paralysis, numbness, pressure sores, pneumonia, incontinence, apraxia (inability to perform learned movements), difficulty carrying out daily activities, appetite loss, speech loss, vision loss and dementia. Coma or death can result if the stroke is severe enough, or in a certain location, such as some parts of the brainstem. Stroke also greatly affects family members and others close to the patient. An estimated 50 percent of women who care for stroke patients will become clinically depressed in the first year (Hakim interview, 2008).

Dr Hakim claimed that one of the biggest challenges was to convince governments to support a long-term commitment to stroke care. They were able to do so by offering solutions and economic analysis that showed ministers that the Canadian healthcare system could save millions of dollars. In a 2008 article published in *Stroke,* a Calgary-based study estimated that Canada's healthcare system could save up to Can$240 million a year in inpatient hospital costs if all stroke patients were treated in organised 'stroke units'. The study showed that length of hospital stay is reduced for stroke patients from an average of

19 days on a general hospital ward to 15 days in a stroke unit (a place in a hospital where stroke patients are cared for by an experienced and specially trained team). Reduced lengths of stay translate into savings of Can$240 million a year based on the 2008 rate of 50,000 clinical strokes in Canada every year. Supporting this study is Sally Brown, CEO of the HSFC, who was quoted as saying: 'If we implement proven stroke therapies and practices in every province and territory the savings in lives and dollars will be significant' (Canadian Stroke Network, 2008).

Assisting this overhaul in the healthcare system was anger from the patients who had not been treated with tPA. Patient groups supported the efforts to communicate the findings and ensure proper treatment for future patients. The PI also attributes the success of the CSS to getting patients behind the effort.

11.11 Payback

The payback framework describes the process from research to several categories of impact. The categories allow us to compare across different case studies. Table 11-3 shows, in point form and by impact category, some of the impacts, described more fully above, that have emerged from this grant.

Table 11-3 Payback

Payback category	Impacts from case study
Knowledge production	• Increased nimodipine binding to ischaemic tissue, which is an index of tissue vulnerability to ischaemic cell death, is initially reversible with prompt reestablishment of cerebral blood flow and is a sensitive indicator of early and reversible ischaemia induced cerebral dysfunction • 23 related publications
Research targeting and capacity building	• Dr Hogan completed his fellowship and continues to work in the area of stroke • Contributed to creation of Canadian Stroke Network in 2000 • Assisted in PI's ability to recruit students, further the reputation of laboratory and improve physical infrastructure • Contributed to teachings of students
Informing policy and product development	• Led to massive education programmes on stroke care for the public and healthcare practitioners • Inclusion in Canadian best practice recommendations for stroke
Health and health sector benefits	• Administration of tPA is now standard and can reduce or eliminate negative outcomes of ischaemic stroke
Broader social and economic benefits	• Savings to healthcare system

11.12 References

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CHAPTER 12 Genetic and cellular determinants of increased growth of vascular smooth muscle in spontaneously hypertensive rats

12.1 Overview of case study grant

The grant of interest to this case study, titled 'Genetic and Cellular Determinants of Increased Growth of Vascular Smooth Muscle in Spontaneously Hypertensive Rats', was funded by the Medical Research Council (MRC), from July 1990 to March 1993, with a total amount of Can$116,846. The team proposed to study the genetic and cellular determinants of increased growth (in the form of hyperplasia) of vascular smooth muscle in a genetic hypertension model. The rationale for this research was based around three points: 1) Folkow's hypothesis, which proposed that narrowing of vessels may be primarily involved in the pathogenesis of hypertension; 2) Hyperplasia of heart, kidney and vascular smooth muscle cells is present in the neonatal stage in genetic rodent models of hypertension; and 3) The increased growth of vascular smooth muscle from spontaneously hypertensive rats persists in cultured cells and is characterised by an enhanced response to certain growth factors, elevated expression of *c-fos* and *c-myc*, shortened transition from the G_0/G_1 phase to the S phase of the cell cycle and slower decline of specific growth rate at high cell density. The team proposed to study the hypothesis that two distinct intermediate phenotypes of increased proliferation of vascular smooth muscle cells in spontaneous hypertensive rats (ie shortened transition from the G_1 phase to the S phase and delay in contact inhibition of growth at confluency) are genetically linked with hypertension. These projects were intended to establish the relationship between growth abnormalities and genetic determinants of hypertension and ultimately to localise the defect(s) on the chromosomes of the animals.

This research was led by Dr Pavel Hamet and was conducted at the Centre de Recherche at the Hôtel-Dieu de Montréal.

12.2 Introduction to case study

Vascular smooth muscle refers to the particular type of smooth muscle found within, and composing most of, the walls of blood vessels. It contracts or relaxes to change both the volume of blood vessels and the local blood pressure, a mechanism that is responsible for redistribution of the blood within the body to areas where it is needed (ie areas with temporarily enhanced oxygen consumption). The main function of vascular smooth muscle thus is to regulate the calibre of the blood vessels in the body. Excessive vasoconstriction leads to hypertension, while excessive vasodilatation leads to hypotension.

One of the major mechanisms that leads to hypertension is narrowing of the lumen of a vessel, which results in increased resistance to blood flow. The narrowing of vessels may be due to increased contractility, but it may also result from an increase in the thickness of the vessel wall secondary to a higher number of cells in the vascular layer. This increased number of cells is called 'hyperplasia'.

The overall aim of the project titled 'Genetic and Cellular Determinants of Increased Growth of Vascular Smooth Muscle in Spontaneously Hypertensive Rats' was to evaluate the molecular and genetic basis underlying alterations in growth exhibited by cultured vascular smooth muscle cells (VSMCs) from spontaneously hypertensive rats (SHRs). Three underlying factors made this research plausible. Firstly, in accordance with Folkow's hypothesis, which proposes that narrowing of blood vessels may be a primary event in the pathogenesis of hypertension (Folkow, 1982), it was believed to be possible that hyperplasia of VSMCs could directly cause hypertension. Secondly, Hamet's team and others had found that hyperplasia of heart, kidney and vascular smooth muscle cells occurs by the neonatal stage of development in genetic models of hypertension in rodents (Hamet et al., 1982, and Kunes et al., 1987). Finally, Hamet's team had recently detected two abnormalities in the regulation of growth of VSMCs from hypertensive rats compared with that of normal rats, which involved a variety of parameters directly related to cell proliferation control, specifically the duration of the G_1 phase of the cell cycle and the rate of cell growth cessation at high density. The general hypothesis thus was that certain genes that control proliferation of VSMCs are affected in SHRs, resulting in altered proliferation of VSMCs that directly causes high blood pressure.

The goal of this project was to characterise the growth abnormalities and to establish their link to the pathogenesis of hypertension. The project was broken down into three studies:

1. In the first study, the team proposed to examine the cellular regulatory pathways that are responsible for abnormal regulation of the proliferation of VSMCs.
2. The second study intended to evaluate the role of transforming growth factor beta 1 (TGF-β1), a growth regulatory factor that the team found was expressed abnormally in cells cultured from hypertensive rats.
3. The third study planned to assess directly the genetic relevance of the two abnormalities in relation to the growth of VSMCs.

For these studies, the team used rat strains called 'recombinant inbred' and 'congenic', which allowed genetic analysis of the linkage between any abnormality and the disease.

These projects were expected to allow the team to establish the relationship between the growth abnormalities and genetic determinants of hypertension and ultimately to localise the defect on the chromosomes of the animals.

The principal investigator (PI) on this grant was Dr Pavel Hamet, professor of medicine. Hamet had received a doctorate in medicine in 1967 from Charles University, Prague, Czech Republic, a doctor of philosophy (PhD) degree in experimental medicine in 1972 from McGill University and a Certificat de Spécialiste de la Province de Québec in Endocrinology in 1974 from the Université de Montréal. He has been a fellow of the Royal College of Physicians and Surgeons of Canada since 1984 and a member of the Board of the Canadian Academy of Health Sciences since 2005. From the mid 1970s to 1990, Hamet was a director at the Clinical Research Institute of Montréal, working within the department of Laboratory Physiopathology of Hormone Action. In 1990, Hamet moved to a director's position at the Centre de Reserche Hôtel-Dieu de Montréal. He was awarded the 'Harry Goldblatt Award' by the American Heart Association in 1990 for his achievements in the field of hypertension.

12.2.1 The case study approach

This case study is based on two face-to-face interviews with Dr Pavel Hamet (the PI) and Dr Hadrava (a former student of Hamet's and a member of the research team) and one telephone interview with Dr Meloche (a co-applicant on the five-year follow-up grant). We also conducted a review of the original grant application, supporting documentation and the PI's curriculum vitae, as submitted to the Canadian Institutes of Health Research; documentary analysis of the scientific literature; and bibliometric analysis.

12.3 Stage 0 – topic/issue identification

The idea for this research project was derived from the PI's personal interests and previous work experiences, including:

1. the PI's persistent interest in hypertension
2. the PI's interest in primary prevention
3. the PI's previous research.

These three factors are elaborated on below.

12.3.1 The PI's interest in hypertension

Dr Hamet indicated that his interest in hypertension dates back to the beginning of his PhD thesis, which he wrote under the supervision of Dr Jacques Genest at McGill University while also doing a fellowship (also under Genest's supervision) at the Clinical Research Institute of Montréal (CRIM). Genest's research involved clinical studies in cardiovascular risk factors, the genetics of premature coronary artery disease and the translational biology of the formation of high-density lipoproteins.

The PI explained that it was common knowledge that increased proliferation of VSMCs was due to hypertension, but the mechanisms were unknown. Hamet's team first proposed that the increased proliferation is due to a primary genetic defect in an application to the

National Institutes of Health (NIH) in the United States in 1975. The NIH refused to fund the application, with the feedback that the hypothesis was naïve. Since then, various published studies have shown that there is a genetic component that leads to hypertension and thus increased proliferation of VSMCs.

12.3.2 The PI's previous research

In the application, it is written that the team's interest stemmed from the observation that not only the heart but also the kidneys display hyperplasia in newborn offspring of SHRs. Since 1986 the team have completed various studies examining this phenomenon in newborn SHRs. The first study, titled 'Enhanced DNA Synthesis in Heart and Kidney of the Newborn Spontaneously Hypertensive Rat', demonstrated enhanced incorporation of [3H]-thymidine after in-vivo injection of the titrated material into newborn pups within six hours of the birth and increased synthesis of new DNA in heart, kidney and aorta (Walter and Hamet, 1986). The team then organised an international study in which newborn genetically hypertensive rats (strains of SHRs) were compared with normotensive rats. This study demonstrated that hyperplasia in the heart and kidney occurs only in genetically hypertensive animals with essential hypertension (Pang et al., 1986). In their next study, the team established that newborn SHRs had an increased heart to body weight ratio compared with normotensive rats. Evaluation of the DNA and protein contents and their specific concentrations showed that the increased organ:body weight ratio of heart and kidney was an increment in the cellularity (Kunes et al., 1987). Together, these studies in newborn SHRs demonstrated that hyperplasia of the heart, kidney and aorta appears very early in life and may constitute primary hypertension. In order to further assess whether this influence was due to blood pressure, the team continued their study in vitro with cells derived from the aorta. With these cells, the team established that the increased proliferation continues in vitro and that the excess proliferation in SHRs is steadily expressed. In addition, the team concluded that the increased growth rate seemed to be due to hyper-responsiveness to growth factors in vitro (Hamet et al., 1988).

The more detailed study that followed established abnormalities in growth characteristics of aortic smooth muscle cells in SHRs (Hadrava, Tremblay and Hamet, 1989). The first abnormality observed was an increased initial growth rate. This was followed by a second abnormality, which was a slow decline in the growth rate of cells in SHRs. The combined effect was a significantly increased number of cells. Current studies within the laboratory at the time of the application used flow cytometry to establish that entry from the G_1 phase to the S phase of the cell cycle was shorter by four hours and was accompanied by an increment in expression of the *c-fos* and *c-myc* proto-oncogenes (Hadrava, Tremblay and Hamet, 1989) and that the slower decline of cell growth at high density is paralleled by abnormal expression of TGF-β1 and messenger ribonucleic acid (mRNA).[1]

These studies led the team to hypothesise that there are two distinct phenotypes of increased proliferation of VSMCs in SHRs: one in which there is a shortened transition

[1] Messenger ribonucleic acid (mRNA) is a molecule of RNA that encodes a chemical 'blueprint' for a protein product. mRNA is transcribed from a DNA template and carries coding information to the ribosomes for protein synthesis.

from the G_1 phase to the S phase and another in which there is a delay in contact inhibition of growth at confluency.

12.4 Interface A – project specification and selection

The overall objective of this study was to evaluate the integrity of cell regulatory pathways involved in controlling the transition between the G_0 phase and the S phase of the cell cycle and to evaluate the role of TGF-β1 in the abnormal growth of VSMCs through segregation studies using mRNA and antisense nucleotides in vivo and in vitro.

The proposal had two parts:

1. Abnormalities of the cell cycle were evaluated using cultured VSMCs. The correlation between altered cell growth and known modulators of cellular growth, such as cyclic nucleotides and calcium, was established. The activities of the enzymes involved in the metabolic pathways of these compounds were established. The group tested whether the production of TGF-β1 is increased in the cells of higher mitotic potential.
2. The PI planned to investigate whether the abnormally high hyperplasia of VSMCs is directly related to hypertension. For this goal, the applicant used a recently developed recombinant inbred strain of hypertensive rats. Analysis of the phenotype segregation was performed to determine whether the altered characteristic of cell growth segregates with a hypertensive trait.

The use of a series of recombinant inbred and congenic rat strains would allow genetic analysis of the linkage between any abnormality and the disease. The team proposed to undertake the following three experimental projects:

1. biochemical analysis of second messenger signalling systems in cultures of VSMCs from SHRs and normal rats to examine the cellular regulatory pathways responsible for normal regulation of proliferation of these cells, thereby evaluating the abnormalities of cell regulatory pathways involved in the shortening of the G_0–S phase transition in cultured VSMCs from SHRs
2. cell-culture experiments to elucidate the possible role of TGF-β1, a growth regulatory factor found abnormally expressed in VSMCs from SHRs, in the slower decline of VSMC growth seen in cells from SHRs cultured at higher density
3. genetic analysis based on recombinant inbred and congenic rat strains to establish (or disprove) genetic linkage between the cell growth abnormalities of the phenotypes of growth of VSMCs and expression of hypertension.

Experiments 1 and 2 were a continuation of ongoing research in Dr Hamet's laboratory. The team was planning to use a new type of in-vivo assay with antisense oligonucleotides within the terms of this proposal. This new type of antisense oligonucleotide gene therapy was being investigated by several groups in different areas, as discussed at the (then recent) Cambridge Conference in Boston in April 1991.

These projects were intended to establish the relationship between growth abnormalities and genetic determinants of hypertension and ultimately to localise the defect on the chromosomes of the animals. Specifically, the team intended to characterise the cellular regulatory pathways that lead to increased growth rate of VSMCs from SHRs, as well as the control of TGF-β1 putatively involved in the slower decline of the growth at confluency, and to establish a link between the hyperplasia of VSMCs and hypertension. In the long term, the team believed that the knowledge gained in this study could permit the establishment of strategies for preventative treatment of hypertension via control of VSMC hyperplasia.

Overall, the peer-review comments were positive, with three reviewers giving the application a high rating, one giving a medium-to-high rating and the fifth commenting that this application should be given a high priority with a recommendation to concentrate on the third project. Other comments suggested that this proposal presented a logical approach to the analysis of altered mitotic and growth characteristics of VSMCs and was based on solid preliminary data. Concern was raised over the limitations of the measurements because, at the time, little was known about the regulation of the mitotic cycle or TGF-β1. Other comments centred on the lack of proposed future research, stating that the findings from this study would be the first step and should lead to subsequent studies. Concern was also raised as to how Dr Hamet would be able to commit 25 percent of his time to this project, as proposed, given his other research commitments.

12.5 Stage 1 – inputs to research

12.5.1 Funding

The applicant applied for Can$65,627 for the period 1990–1991, but he received Can$43,817 after revising the initial proposal due to feedback from the review committee. An annual breakdown of the funds received, as described in the notification of award issued by the MRC, is given in Table 12-1. The PI had indicated in his application that the requested funds for the first year were to be allocated as shown in Table 12-2.

Table 12-1 Funding Amounts Requested

Period	Fiscal year	Amount (Can$)
1 July 1990–31 March 1991	1990–1991	43,817
1 April 1991–31 March 1992	1991–1992	58,423
1 April 1992–30 June 1993	1992–1993	14,606
Total		**116,846**

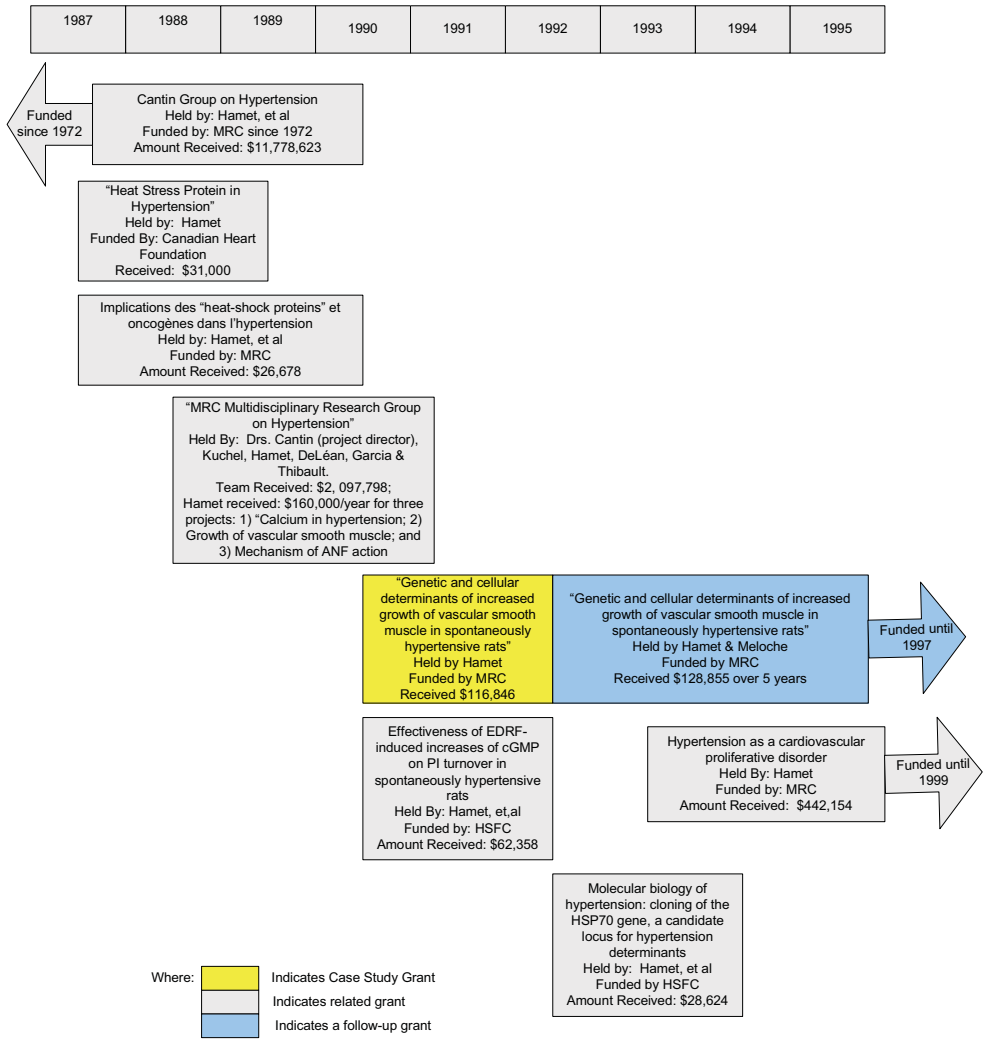
Table 12-2 Research Costs

Item	Amount (Can$)
Salary (two technicians at 50% of Can$33,067 each)	33,067
Animals	900
Expendables	27,960
Services (computer, statistician assistance)	1,600
Other (publication costs, travel, etc)	2,100
Total requested	**65,627**

Dr Hamet stated in his interview that if he had not been funded by the MRC he would have applied for funding from the NIH or industry.

Dr Hamet held various other grants through the years 1988 to 1993, as described in Figure 12-1. The grant of interest to this case study received renewal funding of Can$128,855 from the MRC for a five-year period from 1 July 1992 to 30 June 1997; Can$30,000 of this was for new equipment and the remaining funds were for operating fees.

Figure 12-1 Related peer-reviewed grants held by the PI from 1987 to 1995



12.5.2 Collaborations

The transgenic rats were provided through collaboration with Dr Detlev Ganten from Heidelberg. They were not available on the market. As stated by a reviewer: 'the most interesting part of the study has to do with the use of an inbred strain secured via collaboration with a laboratory in Prague. This highly inbred strain will undoubtedly be a powerful tool for segregation studies and phenotypic linkage assessment. Thus it is likely that the applicant will succeed in establishing the relationship between genetic loci for

abnormalities of VSMC growth and hypertension'. Animal inbreeding is known to be labour intensive and therefore expensive. Access to the developed inbred strain was a great facilitator for the project.

For the third experiment mentioned above, which involved genetic analysis, Dr Hamet collaborated with Jaroslav Kunes, PhD, a visiting professor from the Czechoslovak Academy of Science in Prague and the developer of the recombinant inbred strain, who had recently joined CRIM.

Dr Hamet also collaborated with Johanne Tremblay, PhD, associate director of the Laboratory of Molecular Pathophysiology at CRIM. Tremblay provided input into the experimental design of the first project described above – mainly in terms of the involvement of the calmodulin activator and other cell regulatory pathways of the cell cycle.

12.5.3 Facilities

The research was performed at the Centre de Reserche Hôtel-Dieu of Montréal. Dr Hadrava described the facilities as 'very good…open atmosphere'. Hadrava also thought that Hamet's laboratory had above-average resources in comparison with other laboratories he had worked in, supposing that Hamet's ability to sell himself, his network and his clinical and research experience was a reason for this, along with his industry funds. He concluded by saying that Hamet had a great span of networks that facilitated his ability to obtain funds.

12.5.4 Research team

Dr Hamet was thought of as a 'very productive investigator' who was 'fully capable in directing the proposed experiments'. He was also noted as a researcher with an extensive publication record in the area of hypertension and had published a number of articles directly related to the proposal (MRC, 1990). His application indicated that he would spend 25 percent of his time working on this project (Hamet, 1990).

Two technicians – Carole Long and Monique Poirier-Dupuis – spent 50 percent of their time each on the projects of this grant. Long was in charge of the tissue culture facilities in the laboratory. She was also responsible for initial plating, cell maintenance, cell determinations, thymidine incorporation and cytofluorometry. Poirier-Dupuis had been working with Hamet since 1975 as a polyvalent technician. She was responsible for determining all cell regulatory pathways, as well as for the hybridisation studies conducted with the TGF-β1 probe.

Steve Pang, a postdoctoral researcher and co-author on many of the publications, worked in the laboratory for about two years. Vratislav Hadrava, who also came from the Czech Republic, had just finished his medical degree and was looking to start a PhD. He was given Hamet's name and joined the research team when he found that his interest in haematology was closely related to Hamet's work on proliferation and the cell cycle.

Dr Sylvain Meloche, a junior co-applicant on the follow-up grant, was awarded the MRC Centennial Fellowship in 1992. Meloche had significant training in molecular pharmacology and biology and had knowledge in the area of cell activator pathways from

his recent curriculum vitae. His previous laboratory experience involved work on the basic mechanisms of cell growth.

12.6 Stage 3 – primary outputs from research

Throughout this study the team aimed to provide preliminary answers as to whether or not hyperplasia is a cause of hypertension. It was acknowledged that the work conducted via the case study grant was a first step that was expected to lead to subsequent studies in which the key working hypothesis of a relationship between growth of VSMCs and the development of high blood pressure would be tested directly.

Hamet claimed that many of the team's publications on proliferation and hypertension were the first in this domain.

12.6.1 Knowledge production

The PI identified 29 publications as directly related to this grant; however, this list included nine papers published prior to funding of the case study grant. In an attempt to discuss the outputs from the specific case study grant and the subsequent body of work, we have randomly selected a sample of five publications, published from 1990 onwards, from the list provided by the PI. The publications we will describe are as follows:

1. Hamet, P., V. Hadrava, U. Kruppa and J. Tremblay, 'Transforming Growth-Factor Beta-1 Expression and Effect in Aortic Smooth-Muscle Cells From Spontaneously Hypertensive Rats', *Hypertension*, Vol. 17, 1991, pp. 896–901.
2. Tremblay, J., V. Hadrava, U. Kruppa, T. Hashimoto and P. Hamet, 'Enhanced Growth-Dependent Expression of TGF-Beta-1 and HSP70 Genes in Aortic Smooth-Muscle Cells from Spontaneously Hypertensive Rats', *Canadian Journal of Physiology and Pharmacology*, Vol. 4, 1992, pp. 565–572.
3. Hamet, P., 'Proliferation and Apoptosis of Vascular Smooth Muscle in Hypertension', *Current Opinion in Nephrology and Hypertension*, Vol. 4, 1995, pp. 1–7.
4. Hamet, P., D. deBlois, T.V. Dam, L. Richard, E. Teiger, B.S. Tea, S.N. Orlov and J. Tremblay, 'Apoptosis and Vascular Wall Remodeling in Hypertension', *Canadian Journal of Physiology and Pharmacology*, Vol. 74, 1996, pp. 656–667.
5. Teiger, E., T.V. Dam, L. Richard, C. Wisnewsky, B.S. Tea, L. Gaboury, J. Tremblay, K. Schwartz and P. Hamet, 'Apoptosis in Pressure Overload-Induced Heart Hypertrophy in the Rat', *Journal of Clinical Investigation*, Vol. 97, 1996, pp. 2891–2897.

The first article clearly builds on the team's previous finding that cultured VSMCs have an increased response to growth factors and that VSMCs from SHRs grow to a greater density than control cells (Hamet et al., 1991). With these findings in mind, the team investigated the relationship between cell density and expression of the marker gene *c-fos* and TGF-β1 in cells from SHRs and a control group. This paper concluded that contact inhibition of VSMCs from SHRs at a higher cell density is accompanied by earlier expression of *c-fos*

and preceded by exaggerated expression of TGF-β1 at a high cell density. These results suggested abnormal feedback control (autocrine stimulation) of this growth factor and its involvement in altered contact inhibition of VSMCs from SHRs.

The second paper studied the expression of genes putatively involved in regulation of growth of VSMCs (Tremblay et al., 1992). One such gene, *TGFB1*, is a bifunctional modulator of cell growth whose action is dependent on cell density. The accumulation of *TGF-B1d* messenger RNA was enhanced in growing cells from SHRs at every density studied as early as 24 hours after inoculation, with further increases at later times. Expression of the proto-oncogenes *c-fos* and *c-myc*, which had been implicated in the G_1/S phase, was compared between SHRs and Wistar-Kyoto (WKY) rats, as was expression of *hsp70*. The team concluded that VSMCs from SHRs respond more to mitogenic stimulation and environmental stress such as heat than VSMCs from WKY rats. Heat-inducible *hsp70* mRNA was observed at higher levels in cells from SHRs than WKY cells. This paper concluded that a defect in feedback regulation of TFG-β1 and expression of *hsp70* could be involved in the hyperproliferation and altered density-dependent inhibition of VSMCs from SHRs.

The third paper is an overview summarising the research on proliferation and death of VSMCs in hypertension and the findings obtained throughout the grant of interest to this case study, as well as outputs of subsequent studies supported through the wider research programme (Hamet, 1995). This paper highlights that:

- there is supporting evidence that at least some vascular, renal and myocardial hyperplasia is implicated in the pathogenesis of hypertension
- proliferation of VSMCs is driven by other cell types and involves the proto-oncogenes *c-myc* and *c-fos*
- the distinct characteristics of growth factors (such as TFG-β1) in hypertensive- and normotensive-derived cells can offer new therapeutic opportunities, although further studies were then needed to evaluate the potential of antihypertensive therapy in modulating the activity of both extrinsic and intrinsic growth factors
- shortening of the cell cycle (as witnessed in VSMCs from SHRs) occurs in the transition from the G_1 phase to the S phase and is of particular interest as therapeutic interventions may be directed at this part of the cell cycle
- apoptosis (cell death that is initiated from the interior of the cell and characterised by cell shrinkage) and not necrosis (where cells are damaged from the exterior) may contribute to the pathogenesis of hypertension and, furthermore, may cause loss of myocytes, thus the involvement of this process in aging of the cardiovascular system should be examined.

In 1996, Teiger et al. demonstrated a phase of apoptosis (or programmed cell death) during the first seven days after pressure overload (Teiger et al., 1996). Pressure overload induces cardiac growth in the rat, which implies hypertrophy of cardiac muscle cells and proliferation of non-muscle cells. The team confirmed that an increase in pressure induced apoptosis, mainly in the cardiomyocytes. Some apoptosis was also observed in the basal state of non-muscle cells. The team concluded that cardiac hypertrophy is initiated by a

wave of apoptosis of cardiomyocytes and therefore may be involved in the pathogenesis of heart remodelling. Cardiac hypertrophy was thus thought to offer a new target for therapeutic intervention.

Bibliometric analysis was conducted on 18 of 29 publications identified by the PI as directly related to the case study grant. Nine publications were excluded from the bibliometric analysis as they presented preliminary findings and were published prior to the case study grant.[2] Two other publications were excluded from the analysis because they were not primary research publications (ie articles, reviews or notes). Of the 18 included articles, only 16 were included in the citation analysis because two were not indexed in the Web of Science. Table 12-3 outlines the findings of the bibliometric analysis.

Table 12-3 Publication output and impact

Number of journal articles:	18
Number of articles included in citation analysis:	16
Total number of citations (all papers):	849
Aggregate relative citation impact:	1.98 (Class IV)
Self-citations:	17%

Distribution of publications across relative citation impact classes:

	Class I (uncited)	Class II (>0 and <0.8 citations)	Class III (0.8 to 1.2 citations)	Class IV (1.2 to 2.0 citations)	Class V (>2.0 citations)
Number of publications	1	6	2	2	5
Proportion of total output	6%	38%	13%	13%	31%

Most highly cited publication[3]:	Teiger, E., T.V. Dam, L. Richard, C. Wisnewsky, B.S. Tea, L. Gaboury, J. Tremblay, K. Schwartz and P. Hamet, 'Apoptosis in Pressure Overload-Induced Heart Hypertrophy in the Rat', Journal of Clinical Investigation, Vol. 97, 1996, pp. 2891–2897
Times cited:	251

12.6.2 Dissemination

Dr Hamet stated that it was a priority to publish in reputable journals and that he is 'very involved in the transfer of knowledge'. The team also disseminated findings through students, networks and conferences such as the annual meeting of the International Society on Hypertension, at which Hamet was a keynote speaker. The team also gave poster presentations nationally and internationally. Members of the research team said that Hamet encouraged them to send in abstracts with preliminary results, as well as final papers. Hadrava mentioned that these publications and presentations were important in

[2] Of the nine publications that were indirectly linked to this grant, all were indexed in Web of Science, receiving 182 citations in total and giving a relative citation impact of 0.69. Six publications were in relative citation impact Class I, one in Class II and two in Class IV. The self-citation rate was 25 percent.

[3] Citation count extracted April 2009.

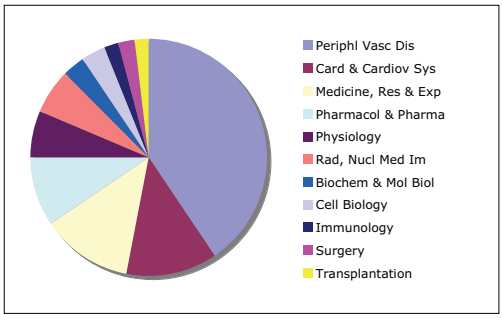
advancing his ability to talk to other researchers, advancing his ideas and promoting himself as a young researcher.

The team disseminated findings to patient groups via the Canadian Heart Foundation, to policymakers via Hamet's position as a research director and directly to decisionmakers within pharmaceutical companies. Hamet also explained that the team disseminated findings to health practitioners and those involved in health management at the provincial and federal level. The team also made presentations to students during 'Student Day', when students were welcome to tour the laboratory.

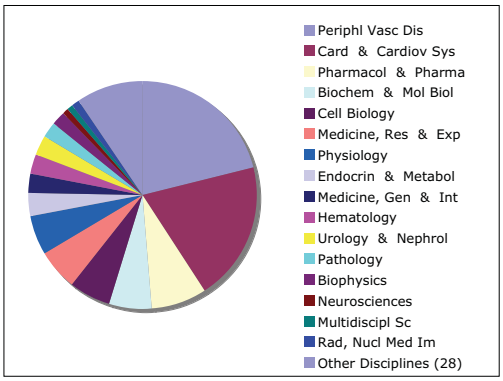
The bibliometric analysis also investigated knowledge diffusion, which found that Hamet and his team most commonly publish in the areas of vascular disease. Their work is most commonly cited by those working in vascular disease, cardiovascular surgery and cardiovascular systems and by those from the United States and Japan.

Figure 12-2 Knowledge diffusion: fields of research of output (a), fields of citing papers (b) and countries of citing papers (c)

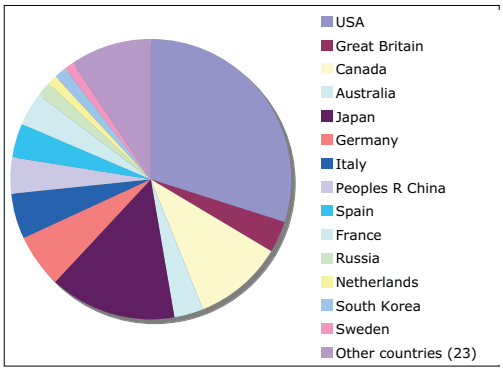
(a)



(b)



(c)



12.6.3 Training and capacity building

Dr Hamet continues to have a very successful research career. He was the Director-Founder of the Centre Hospitalier de l'Université de Montréal (CHUM) from 1996 to 2006. He currently holds a Canada research chair on predictive genomics and is Chief of Gene Medicine Services Research Centre at CHUM, a member of Endocrinology Services at CHUM and Director of the Laboratory of Molecular Medicine at CHUM. He is also a tenured professor in the Department of Medicine at the Université de Montréal, adjunct professor in the Department of Medicine at McGill University and visiting professor at the

First Faculty of Medicine at Charles University, Prague, Czech Republic. Hamet has authored and co-authored more than 450 scientific publications and holds several international patents. Of these patents, Hamet claims some are partially related to the case study grant. He serves on many national and international boards including the Institute of Circulatory and Respiratory Health of the Canadian Institutes of Health Research (CIHR).

Dr Hamet receives financial support from major funding institutions, including the CIHR, National Institutes of Health (USA), Canadian Foundation for Innovation and Valorisation-Recherche Québec, and from the pharmaceutical industry. Active in many societies, Hamet is President-Elect of the International Society of Pathophysiology. He was also President of the Canadian Hypertension Society and General-Secretary of the International Society of Hypertension. He has received many prizes, including the Distinguished Scientist Award of the Canadian Society for Clinical Investigation and the Achievement Award of the Canadian Cardiovascular Society in 1996. In 2001, he received the prestigious Wilder-Penfield award from the Quebec government. In February 2005 Hamet was appointed as an honorary member of the Czech Medical Academy and in October of the same year he received the Michel Sarrazin Prize from the Club de Recherches Cliniques du Québec for his contributions to the advancement of biomedical research. In addition, he received the Canadian Hypertension Society/Novartis Distinguished Service Award.

Dr Hadrava came to Canada in 1985 after obtaining a medical degree in the Czech Republic. He opted to do a PhD under Dr Hamet's supervision, as he wanted to complement his medical training with research experience. Hadrava claimed that his involvement in this project was at the beginning of his professional life. He said that his work on growth factors and receptors and how they relate to the cell cycle was applicable to his post-doctoral fellowship, in which he was studying neural transmitters and how they relate to receptors and influence neuronal activity. After his post-doctoral appointment, Hadrava completed the Canadian medical examination with the expectation that he would apply for a residency. He said he did not want to stay in the field of basic research and wanted to make use of his medical degree. He applied for a residency position via a residency matching programme but was unsuccessful in finding a position. He has also applied to positions in industry and Dr. Hamet provided him with valuable references. Dr Hadrava has worked at Pfizer since 1995 in Medical Affairs. Currently, he is no longer doing research. His career has progressed towards management in Clinical Research Operations, Medical and Regulatory Affairs. He now focuses on regulatory work, acting as a conduit between Pfizer Global Research and Development and Health Canada. His group is responsible for Pfizer's interactions with Health Canada, providing Health Canada with information about Pfizer's drugs in order to obtain regulatory approval. In addition, his group relates questions from Health Canada to the Pfizer researchers. Dr. Hadrava has been selected on several occasions as a member of National Advisory Committees to Health Canada and CIHR (National Placebo Initiative, Pharmacovigilance of Health Products, Drug Safety and Effectiveness Network). He benefits from and uses both his past research knowledge in cellular and molecular pathophysiology and his clinical training.

Stephen Pang, who was a PhD student on the case study grant, is currently at Queen's University, where he is a professor and Head of the Department of Anatomy and Cell Biology. His laboratory is interested in determining the role of the atrial natriuretic peptide (ANP) system in the control of arterial blood pressure and cardiovascular growth. Using a mouse model of ANP gene disruption, he is currently studying the interaction between the ANP and renin–angiotensin systems and their role in salt-induced cardiac hypertrophy. In addition, he also examines the mechanism of cardiac growth and differentiation during early postnatal development and experimental hypertension in the hope of developing a novel strategy to treat cardiac hypertrophy (Queen's University, 2010).

Dr Sylvain Meloche, the co-applicant on the five-year follow-up grant, claimed that, as a new investigator, collaboration on this grant gave him an additional source of funding that helped him establish his research career. Meloche had no specific publications emerge from his participation. He subsequently moved to another institute, as his interest was not in hypertension, which was Hamet's main focus. Meloche now works for IRIC (Institute for Research in Immunology and Cancer) and is a full professor with the Department of Pharmacology of the Université de Montréal Faculty of Medicine. His current research focuses mainly on the signalling mechanisms that control cell proliferation – that is, the processes by which extracellular factors, such as hormones, signal cells to multiply, differentiate or commit suicide (apoptose).

Meloche said that he did not learn any new techniques. He already had experience with the techniques he applied to this project, which are transferable across disease areas because the same basic mechanisms apply for the growth or cell proliferation of any cell type and because growth or cell proliferation is abnormal in many diseases. Cancer is probably the best example, but the same applies in the field of cardiovascular disease. Hypertension involves the disorders of these cell mechanisms in the vascular smooth muscle. Meloche also said that the techniques he was using then are part of his research programme now. He indicated that the case study grant was a stepping stone in his career. It was mutually beneficial, as he also brought skill and experience to Dr Hamet's team.

Dr Hamet claimed that, overall, this research, as well as the ongoing research at CRIM, had positive effects on the team's reputation and their ability to recruit to the laboratory, as evident by the stream of international students who chose to train at CRIM rather than any other centre worldwide. Hamet believes this is a testimony to the value and reputation he has as a researcher. Hamet's international students were primarily from Japan and Europe.

As a result of a successful research programme, including the case study grant, Dr Hamet has been the recipient of multiple awards from the Canadian Foundation of Innovation, an agency that funds research infrastructure. In addition, Hamet and his team were able to form new collaborations for subsequent research projects, such as the Specialized Centers of Research (SCOR) programme, which was funded from 1996 to 2006 by the NIH, in which the team conducted further studies on the molecular genetics of hypertension. Hamet's laboratory received more than Can$2 million throughout the decade for his human studies. Four other PIs were also involved in this project.

12.6.4 Benefits to future research and research use

Dr Hamet and his team were the first group to understand the genetic aspects of the proliferation of VSMCs. The work completed through this grant contributed to the knowledge that hypertension is a multifactorial disease and that the proliferation of VSMCs is part of the process. It also contributed to general cell biology, in that researchers now know that TGF-β1 is involved in contact inhibition of proliferation.

Dr Hamet reflected that he is often approached at international conferences by other researchers who claim they have followed and are interested in his research. The last time this had happened was a week before our interview at a symposium in the Czech Republic, when Hamet met a German researcher who said that he had been following Hamet's work for 40 years and was inspired by it.

Other researchers worldwide (in the United States and France) were doing similar and related work. Dr Hamet also reported that Dr Edward Frohlich[4] was using his team's results in his studies.

12.7 Stage 4 – secondary outputs

No patents are directly related to the case study grant. Dr Hamet said in our interview that 'the application is really going to come from the work we are doing now', which is aimed at developing personalised medicine. In other words, Hamet's work is now focused on being able to predict what kind of treatment individual patients will need based on their own genetic profile. Being able to do so would, theoretically, enable practitioners to use genetics to understand who may have a predisposition to certain cardiac events, such as myocardial infarction or atrial fibrillation, and thus allow them to intervene to prevent such events through possible drug therapy and lifestyle changes.

Due to his involvement in genomics and personalised medicine, Dr Hamet was a member of the Canadian Biotechnology Advisory Committee. This committee undertook an examination of the Canadian Biotechnology Strategy in the context of developments in Canada and globally since the strategy's inception, as a basis for providing advice to the Government of Canada on a national policy framework going forward (Canadian Biotechnology Advisory Committee, 2006).

12.8 Stage 5 – adoption by practice and the public

This work has not produced findings that are at a stage to be adopted into practice or by the public.

[4] Dr Edward D. Frohlich is an international expert on the treatment of hypertension. He is an Alton Ochsner Distinguished Scientist and the Vice-President for Academic Affairs, Alton Ochsner Medical Foundation in New Orleans.

12.9 Stage 6 – broad health and economics outcomes

To date, this research has not led to any widespread health improvements or economic outcomes.

12.10 Payback

The payback framework describes the process from research to several categories of impact. The categories allow us to compare across different case studies. Table 12-4 shows, in point form and by impact category, some of the impacts, described more fully above, that have emerged from this grant.

Table 12-4 Payback

Payback category	Impacts from case study
Knowledge production	• 20 peer-reviewed articles identified by the PI as stemming from the case study grant • Findings presented at various meetings nationally and internationally
Research targeting and capacity building	• Knowledge transfer within laboratory to students and post-doctoral researchers • 1 PhD obtained • Techniques shared among team members and taught to international students attracted by research programme
Informing policy and product development	• Currently working with industry
Health and health sector benefits	• None to date
Broader social and economic benefits	• None to date

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CHAPTER 13

The study of the role of the regulatory gene, *Mlx1*, in cardiac development and disease, and the creation of animal models for myocardial dysfunction

13.1 Overview of case study grant

In 1993, Professor Richard Harvey (the principal investigator (PI)) received a grant in aid of Aus$60,000 over two years from the National Heart Foundation of Australia (NHFA) to study 'The Role of the Regulatory Gene, *Mlx1*, in Cardiac Development and Disease, and the Creation of Animal Models for Myocardial Dysfunction' (grant reference: G92M3633). The research project was conducted at the Walter and Eliza Hall Institute of Medical Research in Victoria (the WEHI), where the PI had access to high-quality facilities and expertise in an environment in which ambitious research such as this was encouraged.

This project looked at the development and specification of heart tissue at the earliest stages of embryonic life. The purpose was to study the genes and proteins that regulate and coordinate the heart genetic programme in order to understand how that process goes wrong in heart disease later in life. A particular focus was the *Mlx1* gene (now called *Nkx2-5* gene) that was expressed earlier than any other heart gene characterised at that time. This gene was believed to function as part of the development of the heart muscle lineage.

The project increased the understanding of the role of a key gene in heart development and opened up a new research field, which in turn opened up new research and collaboration opportunities and continues to influence the direction of the PI's research even today. The project was also very important in establishing the PI as an independent investigator in his own right, opening up avenues for further funding (particularly from the National Health and Medical Research Council (NHMRC)).

The project also developed a genetically modified 'mouse model' to provide a tool for further investigation of heart muscle problems. In addition to influencing the adoption of this technique for research and clinical purposes (eg genetic screening tests for congenital heart disease), this new capability helped establish the credentials of the Institute in a highly specialised field and contributed to the growth and dissemination of expertise in the relatively new technique of genetically modified 'knockout' mice within the WEHI.

13.2 Introduction to the case study

13.2.1 Overview

The research for this grant focused on the genes involved in heart development and more precisely those that are responsible for driving the development of heart tissue at the earliest stages of embryonic life. The grant used a mouse model system to examine heart development.

The main focus was on a gene the research team had discovered and named *Mlx1* (now known as *Nkx2-5*), which is part of a family of genes known as homeobox genes. Homeobox proteins regulate the expression of other genes and are often involved in important developmental processes.

It was thought this gene was important in controlling the development of heart muscle (myogenesis) and there was suggestive evidence that it regulated the expression of the genes that produced myofilaments, the part of muscle cells that provide the contractive force. The grant aimed to test the function of the *Nkx-2.5* gene in normal heart development, as well as in familial cardiomyopathies (heart muscle disease) and in ventricular hypertrophy (thickening of the heart muscle), which are contributing factors to common cardiovascular genetic disorders such as hypertropic cardiomyopathy (HCM)[1].

It was believed that the greater knowledge of the role of the gene in the early development of the heart would lead to more understanding of the causes of life-threatening heart diseases such as cardiac hypertrophy, in which one or both of the heart's pumping chambers are enlarged. It was also recognised that there was a need for animal models of cardiac disease with broad clinical significance, and so an integral part of understanding the function of *Nkx2-5* was to create mouse strains carrying mutations in the *Nkx2-5* gene using gene targeting technology.

In the years preceding this grant, the Molecular Biology Unit at the WEHI (where the PI worked at the time) had been exploring, as part of their ongoing research into the causes of cancer, the role of the *Hlx* homeobox gene in regulating early development with a view to understanding how genes guide the development of different cell types and control their survival. By 1992 they had begun using transgenic mice to 'delineate the biological effects of key regulatory genes', and by 1993 they had narrowed their search down to a small number of putative 'master genes', including the 'unusual' *Nkx2-5* gene.

The starting point for the discovery of the *Nkx2-5* gene was to find in mice a gene similar to that found in fruit flies. This gene, called *tinman*, plays a key role in the development of the heart in fruit flies. The research team took the risk of assuming that genes similar to the *tinman* gene existed in mice and humans. The punt paid off and it turned out that there are a number of genes similar to *tinman* in mammals and they cloned three of them; two of them were previously uncharacterised.

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[1] Population-based clinical studies suggest that HCM affects up to one in 500 of the general population, making it the most common cardiovascular genetic disorder known and the most common structural cause of sudden cardiac death in people younger than 35 years of age (see Maron et al., 1995).

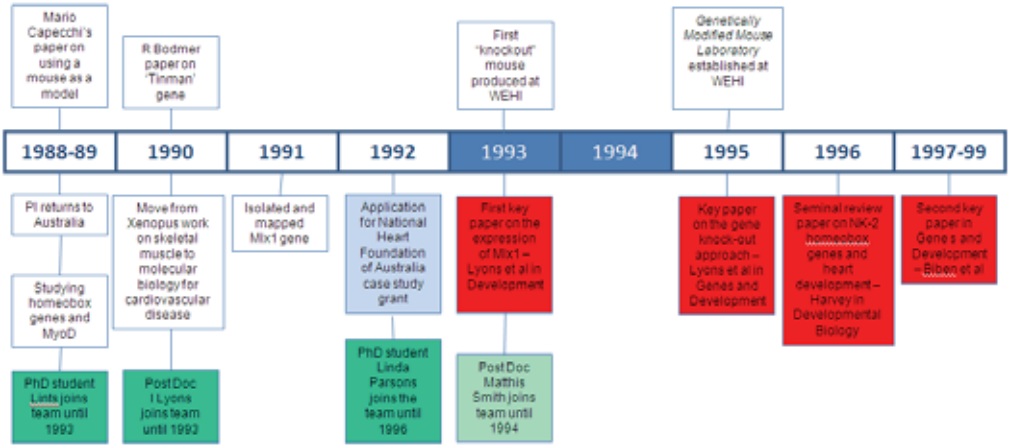
By the time the grant application was made to the National Heart Foundation of Australia, the PI had identified the *Nkx2-5* gene but had not been able to study the 'expression' of this gene in order to fully understand its role and function in controlling the development of heart muscle.

At the time, the 'knockout' mouse provided the earliest generated heart phenotype using the new gene targeting technology and as a result attracted significant interest. The ability to produce 'knockout' mice was still fairly new at this time, both for the WEHI and in Australia, and there were only one or two other laboratories in the United States looking at heart morphogenesis using molecular tools, but none were as advanced as the PI's research at the time of the grant, particularly in relation to this gene.

Indeed, the method itself (the introduction of specific gene modifications in mice by using embryonic stem cells) was still very new having been developed in the late 1980s by Capecchi, Evans and Smithies (who would go on to win the 2007 Nobel Prize for Medicine for this pioneering work that dramatically increased the speed and accuracy of genetic research using mice).

Figure 13-1 illustrates the timeline of key events relating to the grant. Key developments in the wider research field are shown above the line and grant-specific events below the line. Clear knowledge outputs from the grant are shown in red and clear training outputs in turquoise. Where the impact of the grant on particular outputs is debated, a lighter shade has been used. The years coloured blue correspond to those in which money on the grant was supplied.

Figure 13-1 Timeline of key events relating to the grant



13.2.2 The case study approach

The case study based on this research grant involved a combination of: a review of documentation for the grant, one face-to-face interview with the PI on the project, interviews with other investigators that were on the team, a review of the curriculum vitae of the PI and documentary analysis of key citing papers, publications and conference abstracts arising from it.

13.3 Stage 0 – topic/issue identification

There were five key interrelated influences on the PI's identification of the research topic and decision to pursue the study:

1. timing
2. valuable PI skills developed in another area of research
3. funding and research environment in Australia
4. other research
5. capacity to pursue novel opportunities.

13.3.1 Timing

The PI, Professor Richard Harvey, had recently returned to Australia after being awarded a Queen Elizabeth II fellowship and was accepted as a senior postdoctoral fellow and laboratory head at the WEHI in Melbourne. 'People used to say it was a super postdoc position. It was a fabulous time and the initial support from the National Heart Foundation [of Australia] allowed me to obtain other grants and develop autonomy and independence and hire new people' (Harvey interview, 2007).

13.3.2 Valuable PI skills developed in another area of research

While new to cardiovascular disease (CVD) research, Professor Harvey was a basic researcher with a focus on developmental biology and had an excellent track record with previous experience in muscle development and the Xenopus (frog) model.

Professor Harvey had trained in Australia and overseas in biology and genetic engineering and became interested in developmental biology. His overseas training included a postdoctoral fellowship in France (in industry) and he then studied at Harvard University for three years looking into the early embryology of the frog system. He was looking at the patterns of gene expression in the early embryo with a particular focus on skeletal muscle, working with Xenopus techniques. When he returned to Australia he continued the work he had been doing in the United States. He had already published in prestigious journals including multiple papers in the *Proceedings of the National Academy of Sciences*, two in *Development* and *Genes & Development* and a paper in *Cell*, as well as others listed in the funding application. Harvey said, 'It was the beginning of an interesting era of development. I had just came from a postdoc where molecular techniques were being applied to frogs to study how the cells divided and how they participate in a particular pattern process; and…many of the patterning ground rules were laid down before the molecular era. It was a good example of how molecular techniques could extend our understanding of development and open doors to create a new field' *(*Harvey interview, 2007).

13.3.3 Funding and research environment

Professor Harvey said that the career opportunities and career structure in the middle years of his career beyond the first one or two postdoctoral degrees were a bit vague. He had completed postdoctoral studies in France and Harvard in the United States of America

with a solid track record and returned with existing funding; however, there was a need to establish himself in the funding environment of Australia.

Some model organisms, including the frog system, were less established and accepted in Australia, and at the same time it became clear to Professor Harvey that as biomedical science funding in Australia was driven mostly by the NHMRC, it was important to have a strong health outcomes association to his work. It was also a period of reduced availability of funding in Australia as a result of the economy being in recession, which the General Manager of the WEHI acknowledged in a letter to the National Heart Foundation of Australia in October 1992: 'In these difficult economic times, resulting in very few new grants being possible, it is encouraging to know that fundamental research projects and outstanding young Australian scientists, are still able to gain funding' (Brumby correspondence, 1992).

Harvey said, 'The frog system was not a popular one in Australia but is strongly featured in the history of embryology. Each of the model systems that we use for the study of embryonic development has its strengths and weaknesses. The frog has been very powerful because it lays very large eggs that are very robust and you can apply "cut and paste" techniques to combine different regions of the embryo artificially, and do certain other manipulations on the embryos that are impossible in other systems. However, my work was very basic in nature and it became clear to me in the Australian research culture it would be difficult to excel working on basic patterning mechanisms in the frog system because there wasn't an understanding of the relevance of this approach and this model' (Harvey interview, 2007).

There was also an element of risk to the project that made it difficult to pursue other funding avenues at time when there was already a shortage of research funds, as Professor Harvey's PhD student, Linda Parsons, explained; 'At the time, the National Heart Foundation [of Australia] provided a really unique opportunity for funding...there is no doubt at this particular point in time the National Heart Foundation [of Australia] provided funding on a really key project at a really critical time. It was unclear at that particular point in time that the NHMRC would fund what was thought to be a 'reasonably risky' project because we hadn't actually established that we could handle the technology and that we could produce something at the end of it' (Parsons interview, 2009).

As described by Professor Harvey above, in the first few years after his return to Australia he took a difficult decision to stop the Xenopus work on skeletal muscle and move into the molecular biology of cardiovascular development and disease. It was apparently crystallised by Mario Capecchi's paper in 1989, in which Capecchi described technology for using the mouse as a genetic model for studying human genetic disease. It was also relevant as the WEHI was already experienced in mouse breeding. Harvey said, 'That was a critical moment for me. I fully released my engagement with the frog system. Political and cultural factors meant there was a need to switch to a more acceptable model. It was a survival instinct. As much as my inclination was to study basic pattern mechanisms in the embryo I recognised that I needed to study something medically relevant and ensure its relevance to the funding agencies in this country' (Harvey interview, 2007).

13.3.4 Other research

Professor Harvey's shift to cardiovascular research happened through discovery of the gene called *Nkx2-5*. In addition to studying this class of homeobox genes, the research team had also been studying the key regulatory transcription factor for skeletal muscle development, *MyoD*. This had made them very aware of the long-term assumption that there were close links between cardiac and skeletal muscles in terms of their genetic regulation. It was turning out not to be true, in that *MyoD* and related genes were not expressed in heart development.

That created an opportunity and they noticed a paper from Rolf Bodmer (1993) describing a developmental gene from the fruit fly, *Drosophila melanogaster*, called *tinman*. It fitted exactly the sort of gene they wanted to be working on in the mouse and is a key regulatory transcription factor gene of a class that had been associated with other developmental processes. Harvey said, 'In some sense, this was a risky leap because the literature had considered the hearts of different organisms to be the result of convergent evolution…so to look at a paper describing the *Drosophila* gene did not necessarily mean we would find that gene connected to heart development in mice. So we were either profoundly wise or very stupid' (Harvey interview, 2007).

It took some time to see if they could isolate genes from mice that were homologous and highly related to the *Drosophila* gene, *tinman*. They found two genes, neither of which had been described or categorised. Out of that came the gene *Nkx2-5*.

By the time the application for funding had been made, the research team had found the gene but had not characterised it at all. In fact, the first aims of the grant were to determine the expression pattern. Harvey said, 'It was an exciting moment and that is basically where we were when we wrote this grant. We knew we had the gene, we knew it was expressed in the heart…and everything afterwards would be interesting and [we were] prepared to change our lives to focus on this project' (Harvey interview, 2007).

13.3.5 Capacity to pursue novel opportunities

As will be discussed later, Professor Harvey had secured additional funds in the form of a National Institutes of Health (NIH) grant based on his work at Harvard, had been awarded a Queen Elizabeth II fellowship and at the same time had some initial financial support and ongoing project and infrastructure support from the WEHI. This combined with the case study funding from the National Heart Foundation of Australia at this stage of his career provided, what Harvey believes, was the flexibility and security to explore novel opportunities. Harvey said, 'It can make a nice difference [having funding provided at the beginning of someone's career] if only to give you a bit of flexibility, because I can remember the moment it started, a student came up to me and said 'look at this paper' and we discussed it and decided that he should do a screen. To have such flexibility is so good, and then once you had a germ of an idea and know you were onto something and then to formalise it in a way of a grant to give you security over a period of time, even a modest amount of time. We were perhaps much less ambitious at that stage of our career as well. I am probably more ambitious in terms of what our needs are now...and we need bigger lumps of cash and get frustrated when we don't get it. At that stage I was finding my feet' (Harvey interview, 2007).

13.4 Interface A – project specification and selection

The priorities of the project were to identify the broad regulatory mechanisms active in the development of heart muscle (myocardium) and, with these priorities in mind, to analyse the development and performance of the heart in mutant mice.

The application for funding was a first in many ways. It was the first application for Professor Harvey in Australia and the first application for him in the new research area of CVD. It was also his first application with the National Heart Foundation of Australia, and Harvey noted at interview for the case study that it was an ambitious basic research application by a young researcher with a model that was not well established and little preliminary data going to a largely clinically based panel. Harvey said, 'I am very grateful for the grant…at this point it was an important grant for me and taking a risk on a project for which there was no precedent and coming from an agency that would have had mostly cardiologists on the review panels' (Harvey interview, 2007).

All three reviewers of the grant application gave very positive reviews. All three rated the application as excellent and recommended funding the project, including one reviewer stating the following: 'Such investigators should be highly encouraged' (Grant application reviewer, 1992).

It was described as an outstanding project and it was noted that the identification of gene regulatory molecules in the heart lay at the cutting edge of research into the molecular mechanisms of heart function. It was recognised that it had the potential of identifying the much sought after regulatory gene controlling cardiac muscle development.

It was stated that the project proposed to employ many 'state-of-the-art' techniques, and special note was made of the gene targeting technology to be used to attempt to generate gene 'knockout' animals as models of cardiac disease. While the specific role of the *Nkx2-5* gene was obscure at that time, the reviewers felt it was likely that results of considerable significance would emerge from this work.

The proposed approach was also described as well thought out and, while the techniques were sophisticated and the work was to be time consuming, it was felt to be feasible given the considerable expertise in molecular biology being brought to bear and the requisite skills being possessed by the investigators. The request in the application for funds to cover the salaries of a postdoctoral fellow and research assistant was viewed as realistic and appropriate due to the work being so labour intensive.

While no changes were noted in the grant documentation or recalled by Professor Harvey in terms of the proposed approach, one reviewer commented on the ambitiousness of the proposal and what could be realistically accomplished within the timeframe of the grant. The reviewer suggested that 'clearly' not all aspects could be initiated within the first year as listed in the timetable in the grant application. The reviewer was not convinced that the gene knockout approach was appropriate during this granting period and suggested that it may be prudent to postpone it, which would then remove the need for a research assistant with these skills. Another reviewer also noted the suggestion that the possibility of the applicant's institution absorbing the cost of equipment and maintenance be raised at interview.

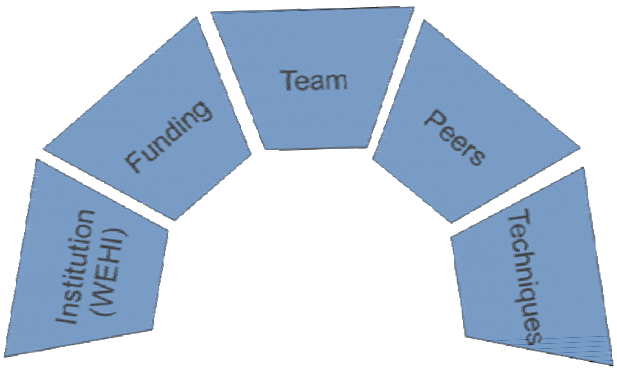
Professor Harvey specifically recalls the benefit of the grant application process involving an interview. It gave him an important opportunity to present himself and his application for basic research funding to a predominantly clinical panel. Harvey said, 'When you face an interview it sometimes goes well and sometimes it doesn't. I can't remember details of that interview except that I was on my own. I was a young scientist not clinically trained and knew nothing about cardiology and had to justify this (Harvey interview, 2007).

Other funding avenues were not explored. However, Professor Harvey strongly believed that another fund provider would have had to be found had the National Heart Foundation of Australia not awarded the grant. He said, 'We never have back-up plans. It is not the way it works. We cobble things together. It would have gone ahead one way or another presumably and we got a fairly large grant not long after this from the Human Frontiers Science Program (Harvey interview, 2007).

13.5 Stage 1 – inputs to research

Figure 13-2 indicates the key inputs to this case study.

Figure 13-2 Key inputs



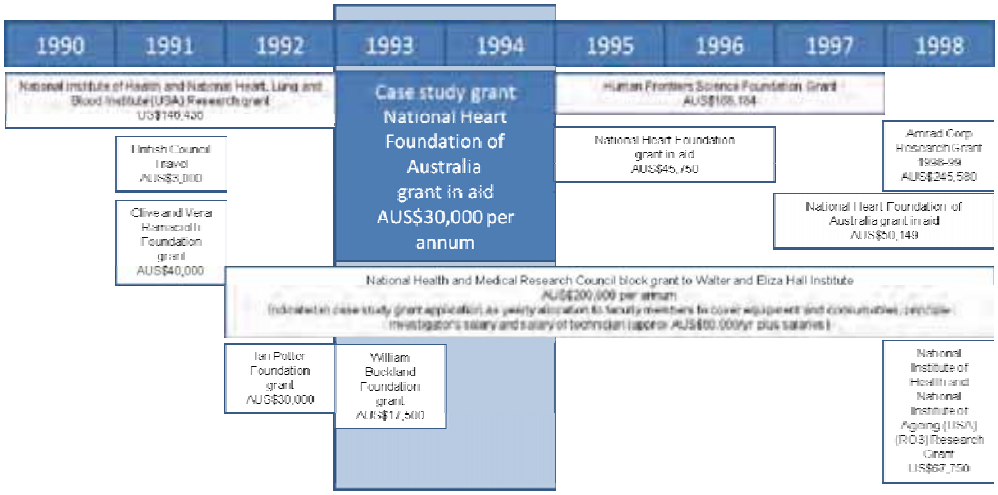
13.5.1 Facilitators

As already noted, prior to the grant, Professor Harvey had access to funding that included: an NIH grant he won based on the work from Harvard; the Queen Elizabeth II fellowship; and the financial, infrastructural and intellectual support of the WEHI in his position as a senior postdoctoral fellow and laboratory head. The WEHI is an internationally recognised institution with an important scientific research reputation. Harvey said, 'I was lucky enough to have a bit of support from WEHI at that time, which was extraordinary because most institutes in Australia operated on the basis that you live and die by your grants…There were people very supportive of the new initiative…they were prepared to give it all a go, and so it was a very good environment and lots of good techniques and lots of resources and equipment there, and they also supported me to get involved in this gene targeting technology and additional funds were made available because it was a complicated and delicate endeavour' (Harvey interview, 2007).

After the initial grant funding submission it was also confirmed that the funding of the salary for postdoctoral fellow for the project was to be covered by an Australian Research Council (ARC) Fellowship that had been awarded.

However, there was not specific funding to investigate the expression patterns and gene regulation and to develop the mouse models. The case study funding from the National Heart Foundation of Australia provided this important specific funding to facilitate the project. The funding was for Aus$60,000 over the two years from 1993 to 1994, which was reduced from the initial funding bid for Aus$100,000 each year for three years due to the factors described above, but mainly precipitated by a general shortage of funding available for research grants at the National Heart Foundation of Australia at that time.

Figure 13-3 A funding map for the grant; this shows major inputs to the PI's laboratory over the period from 1990 to1998.



The grant application makes reference to a number of collaborations for the project. Prior to the case study project, there had been collaborations in: mapping the *Nkx2-5* gene to the t-complex of chromosome (ch) 17; mapping the chromosomal location of the human *Nkx2-5* gene using chromosome in situ hybridisation; and targeting *SCL* and *NSCL* genes from which chimeras were derived and bred for germline transmission. There had also been the establishment of a collaboration with another researcher at the Royal Prince Alfred Hospital in Sydney, who was working with at least six to seven kindreds (extended families) whose disease status has been documented and where further kindreds were being collected, with the aim of first establishing whether there is disease linkage with the *Nkx2-5* gene, and then, if established, to examine the nature of *Nkx2-5* mutations at the DNA sequence level. In addition, there were important references to other research that informed the project development.

Interest and support from peers was motivating, as well as competition from another research group (in the United States) coming to their attention and providing an urgency for the project.

13.5.2 Knowledge, expertise and techniques

Professor Harvey's previous postdoctoral experience provided important developmental and molecular biology expertise and an established track record in muscle development and gene modelling.

His team developed the new approach and conducted the research that formed the foundations for the funding application. It started with a doctor of philosophy (PhD) student who was essentially the discoverer of the gene. He performed the screen that is believed to have led to the finding of gene, while Professor Harvey performed the experiment that led to the understanding of the heart expression pattern.

They were very quickly joined for the grant project by a postdoctoral fellow who had impeccable references and came from one of the best genetics laboratories in the world, where he had participated in the successful quest for the testis-determining gene and was skilled in molecular biology. His job was to investigate the *Drosophila tinman* gene in mice. He was later replaced by another postdoctoral fellow from the WEHI, who had experience in transgenic technology and was given the task of setting up the transgenic models for the gene targeting. Harvey said, 'We were the first two or three groups to set it up in Australia after the first paper in 1990' (Harvey interview, 2007).

It was indicated in the grant application that the research assistant was employed full-time as a blastocyst injector, a 'demanding and necessary step in the gene targeting technology', and was highly trained in many areas of embryo handling. Professor Harvey also indicated in his grant application that she was highly sought after for her skill.

There was also valuable mouse breeding expertise available at the WEHI, which began exploring new transgenic techniques around the same time that Professor Harvey undertook his research. The availability of expertise in this cutting-edge technology allowed Harvey to take the somewhat brave and risky step of including the development of a 'knockout' mouse for the new gene in his grant proposal.

13.5.3 Space, consumables, equipment and personnel

Professor Harvey was provided office and laboratory space at the WEHI. He shared an office with another young colleague and had one or two bench sides and shared two benches with this colleague.

The personnel commitment to the project included 67 percent of Professor Harvey's time, a postdoctoral fellow, a research assistant and technician, and a PhD student. Professor Harvey was very hands on during this process but as laboratory head he also had a directorial role.

13.6 Stage 2 – research process

In summary the grant research involved further studying expression patterns of *Nkx2-5* and its regulation through the use of mouse models and a gene knockout approach.

Professor Harvey's intention was to further determine the expression patterns of *Nkx2-5* in early mouse embryos before they developed heart muscle using radioactive probes for the gene. He further planned to look in man to see whether *Nkx2-5* was expressed under

conditions of myocardial hypertrophy, exploring the possibility of a regulatory role for the *Nkx2-5* protein and in particular the *troponin T* gene. The possible role of *Nkx2-5* protein in disease was to be pursued in familial cardiomyopathies if collaborative work in mapping the human chromosomal location of the *Nkx2-5* gene was successful. The effects of preventing expression of *Nkx2-5* were to be investigated through the creation of a 'knockout' mouse mutant using embryonic stem-cell and gene-targeting technology.

As foreseen by one of the reviewers, not all of the aims were achieved within the timeframe of the project. In fact some of the work proposed has only recently been published.

Understanding the expression patterns was considered most important and, therefore, it was the first aim. It was completed in great detail and successfully achieved. Unfortunately, it was not possible to test whether *Nkx2-5* was important in familiar cardiomyopathies because of the focus on the embryology and because the antibodies necessary to test for *Nkx2-5* had not been purified. They performed some work on the *Nkx2-5* protein. There was also the creation of mutant mice.

The other aims related to the role of this gene in adult-onset disease or familial disease. The research team discovered the chromosomal localisation in the mouse gene, which provided an approximate location for the human gene, so a separate team was able to identify the location of the causative gene in some dominantly inherited families. Harvey said, 'And so we made it easier for them and they went straight to that gene sequence…We weren't involved in collective families and human genetics at that time but our work clearly made it a simple task for them to identify that gene. So it turns out that mutations in *Nkx2-5* account for probably one to a few percent of specific categories of congenital heart disease and the mutations that have been found are now about 30, and this has led to another side of the work where people try to understand what goes wrong at the cellular and molecular level when the gene is mutated. Our article published this year [2007] has made a great contribution to that and some work in progress has also turned up other magical things' (Harvey interview, 2007).

13.7 Stage 3 – primary outputs from research

13.7.1 Knowledge production

While not all of the aims were met in the grant period, the research was successful, with the key achievements being analysis of gene expression and *Nkx2-5* protein in a mouse model system and the development of the transgenic mouse. Harvey said, 'By using this knockout mouse funded by the grant, we have been able to make connections in a way that very few others have. Genetics is one of the most powerful techniques for understanding molecular pathways and knowing how pathways impact on cells and the structure the heart. That was the key aim that was realised' (Harvey interview, 2007).

Table 13-1and Figure 13-4 below provide analysis of the publication output of this grant.

Table 13-1 Publication output and impact of directly related publications[4]

Number of journal articles:	2
Number of articles included in citation analysis:	2
Total number of citations (all papers):	936
Aggregate relative citation impact:	10.83 (Class V)
Self-citations:	6%

Distribution of publications across relative citation impact classes:

	Class I (uncited)	Class II (>0 and <0.8 citations)	Class III (0.8 to 1.2 citations)	Class IV (1.2 to 2.0 citations)	Class V (>2.0 citations)
Number of publications					2
Proportion of total output					100%

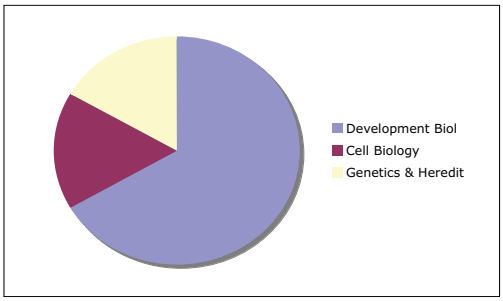
Most highly cited publication[5]:	Lyons, I., L.M. Parsons, L. Hartley, R. Li, J. Andrews, L. Robb and R.P. Harvey, 'Myogenic and Morphogenetic Defects in the Heart Tubes of Murine Embryos Lacking the Homeobox Gene *Nkx2-5*', *Genes & Development*, Vol. 9, 1995, pp. 1654–1666
Times cited:	528

[4] In addition, 38 publications were indirectly linked to this grant. 37 of these publications were indexed in World of Science and received 1,824 citations in total, giving a relative citation impact of 1.88. They were spread across the five relative citation impact classes, with one in Class I, 12 in Class II, five in Class III, five in Class IV and 14 in Class V. Their self*citation rate was 18%.

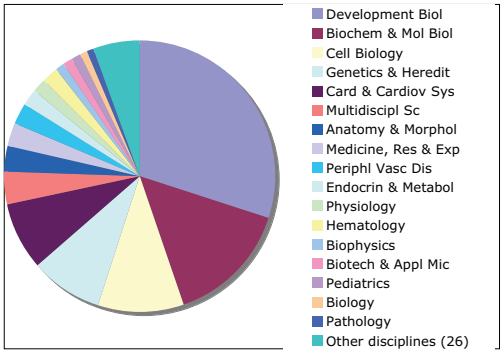
[5] Citation count extracted April 2009.

Figure 13-4 Knowledge diffusion: fields of research of output (a), fields of citing papers (b) and countries of citing papers (c)

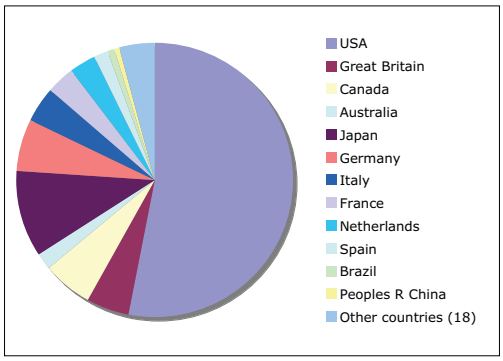
(a)



(b)



(c)



Some key papers arose from the project and very quickly, partly due to the competition from another team in the United States conducting research in the same area at around the same time.

The first paper, by Lints et al. (1993), focused on the expressions of *Nkx2-5* and was published in *Development*. This paper described the distribution of gene expression through the body during development of the heart (and other tissues) and concluded that '*Nkx-2.5* expression will therefore be a valuable marker in the analysis of mesoderm development and an early entry point for dissection of the molecular basis of myogenesis in

the heart'. It has been cited 399 times. The article on the gene knockout approach, by Lyons et al. (1995), was published in *Genes & Development* and described the expression of the gene when it is disrupted to create mutations using transgenic techniques. The article concluded that '*Nkx2-5* is essential for normal heart morphogenesis, myogenesis, and function' and that 'this gene is a component of a genetic pathway required for myogenic specialization of the ventricles'. It has been cited 450 times. Harvey said, 'They were the major ones that really stem from this grant directly. They were the papers that put us on the map' (Harvey interview, 2007).

As early as 1994 the significance of Professor Harvey's findings had been noted by his peers at the WEHI; the WEHI Annual Report (1993-94) stated that: 'Remarkable technologies have been developed by which a single gene can now be added to mouse chromosomes, creating a 'transgenic mouse', or a single gene destroyed, yielding a 'knockout' mouse. A flurry of findings from our 'knockout' mice this year have clarified the role of three homeobox genes, genes that guide development. Two have proven to be vital for the survival of the embryo: one (*Nkx-2.5*) governs the proper architecture of the heart, while the other (Hlx) is essential for the development of the liver. Studies such as these suggest that certain homeobox genes serve as 'master' regulators that supervise how an organ is made'.

After that, a seminal review paper was written and published in *Developmental Biology* (Harvey, 1996). It has been cited 299 times and laid out the discovery and implications of the discovery to the evolution of hearts. Harvey said, 'I am very proud of that because it has been referred to many times and basically rewrote the textbook notion that the hearts of different species are a result of convergent evolution. It puts a fine point on terminology' (Harvey interview, 2007).

Another paper that was important was published in 1997 in *Genes & Development* (Biben and Harvey, 1997).

These were the initial key papers that came from the grant and a further 40 papers have been identified by Professor Harvey as following on from the case study research project.

The project supported the generation of new research areas and the shaping of mouse genetic technology in Australia at the time in terms of the use of 'models' for research.

13.7.2 Benefits to future research

Capacity building and career development

The research project further established the reputation of Professor Harvey and particularly in this area of research. He was hands on in relation to the research during the grant period. He is still highly active in basic research at the Victor Chang Cardiac Research Institute, Sydney, and is highly regarded in the research community.

According to the former head of the WEHI (and Australian of the Year in 2000), Sir Gustav Nossal, Professor Harvey's 'outstanding' developmental biology research into homeobox genes was the reason that Harvey was offered the Sir Peter Finley Professorship at the Victor Change Institute and University of New South Wales in 1996 (where he relocated together with four staff from his laboratory). Professor Nossal provides the following summation of the impact of Harvey's work in his recent book, which chronicles

the evolution of the WEHI under his 31-year management: 'Richard Harvey's work on homeobox genes in the mid-1990s took the story of genetic regulation into developmental biology of tissues apart from the lymphoid system. He found that when the homeobox gene *Nkx2-5* is "knocked out", embryos died at 8.5 days of gestation because the heart did not develop properly. In contrast, when the homeobox gene *Hlx* was knocked out, a severe defect in liver development was noted and mouse embryos died at day 15 of gestation, that is three-quarters of the way through pregnancy. Proper development of the intestines is also disrupted. Harvey has done distinguished work on the molecular mechanisms whereby these genes exert their action. He has also shown that the genes are extraordinarily conserved, in evolutionary terms, with homologous genes being active in the fruit fly. Because of this outstanding work, Harvey was offered the Sir Peter Finley Professorship of Heart Research at the Victor Chang Cardiac Research Institute in Sydney in 1998 (Nossal, 2007).

Collaborations for other studies and interest in the gene by a number of other people has resulted since the grant project. Professor Harvey's curriculum vitae shows that he collaborates widely with people around the world. He believes that collaboration is particularly important as this encourages further capacity building, establishes a critical mass, assists with further funding and generates more research (even in other 'loosely' related areas).

The project provided an important track record for further funding as set out below, particularly in relation to the NHMRC, and the Human Frontiers Science Program grant was specifically noted as a direct outcome of this research. There have also been a further two National Heart Foundation of Australia grants.

The research and resulting papers have been indicated as very important to the career development of one of the postdoctoral fellows and to the PhD student on the project. One is in industry based on the transgenic expertise and the other is in stem-cell research. Two others in the team are no longer in research (including the postdoctoral fellow on the original application).

Targeting of future research

Professor Harvey has advised that the case study research was new at the time (an important opportunity), that everything he has done since has pretty much been derived from the work and he is still working on it. The grant research has continued to feed Harvey's team, while at the same time providing some spin-off research for other teams. Harvey said, 'We have just published our strongest paper this year in *Cell* and it keeps throwing up interesting things. We use it as a vehicle for further study' (Harvey interview, 2007).

There was the creation of a new research area in regard to the identified gene, and this moved on to the study of all associated genes, mechanistic studies and studies in higher order mammals, including humans.

The transgenic mouse used in this study was one of the first applications of knockout technology in Australia and has since been used in further studies. In fact, they are still using the transgenic mouse and still publishing outcomes. This provided the WEHI with the confidence to invest in the transgenic technology. In 1995, a new Genetically Modified

Mouse Laboratory was established at the WEHI, which provided genetically modified mice and gene-targeting services for research laboratories at the WEHI and other Australian institutions, and the dissemination of this new technique was made a priority. The WEHI annual report for 1995–1996 stated: 'Over the past few years we have established a functional gene targeting core facility at the WEHI and have generated eleven genetically-modified mouse strains in collaboration with five Units. The experimental use of these animals has so far resulted in a number of publications... Over the next year this technology will be increasingly used in new targeting strategies. The dissemination of gene targeting skills is a major aim. The laboratory has provided advice to fourteen scientists from Australian and New Zealand institutions and on occasion has entered into collaborations, with the aim of transferring the techniques to a key person within that institute' (WEHI Annual Report, 1995-96).

This early commitment to the new transgenic technology enabled the WEHI to become a market leader, which laid the foundation for future commercial successes in this field, including the establishment of the highly successful private company Ozgene by the former head of the Genetically Modified Mouse Laboratory at WEHI.

Importantly, the case study project provided the preliminary data to ensure 'fundability' of future research by similar mechanisms.

13.8 Interface B – dissemination

The dissemination approach was 'academic' and largely limited to publications. Professor Harvey was also invited to present his work at a number of conferences and other scientific meetings.

When they first started the research it was a new field and so they built connections through the muscle field and cardiac field and then into the cardiology field. Financial support for dissemination was limited to that available from WEHI as the supporting institution.

13.9 Stage 4 – secondary outputs

13.9.1 Policymaking

The influence on policy was limited. It could be argued that the success of this project helped to firm up the idea of using animal models for development (and possibly other areas of research), but this would have been part of a broader body of work.

It certainly did lead to new areas of research that would be of influence, such as looking for gene defects related to the developmental genes studies. Some of these mutations have now been established as screening tools for congenital heart defects in families with a history of the disease.

13.9.2 Product development

The relationship to product development is a lot clearer, as the project contributed to the establishment of a knockout facility by raising the profile and providing credibility to this

emerging technology. As mentioned earlier, the WEHI established a profile as experts in this area and established their Genetically Modified Mouse Laboratory as a core service that is still in use today and has spawned a number of collaborations and commercial ventures in recent years.

13.10 Stage 5 – adoption by practice and public

It is difficult to attribute exactly how much the use of transgenic mice in Australia and the resultant facility was influenced by this project, as transgenic mice have become a standard technique (and part of a worldwide trend).

Downstream application to clinical practice has also occurred in genetic screening in congenital heart disease, but this has been very recent. There is also potential for further application in relation to the study of 'risk' genes for cardiovascular disease.

13.11 Stage 6 – final outcomes

As indicated earlier, the research contributed to the establishment of a transgenic mouse laboratory at WEHI and contributed to development of prenatal genetic testing.

The production of genetically modified mice and rats is a highly specialised and lucrative business (eg 'knockout' mice can be worth up to Aus$100,000 each). Genetically modified mice and rats are currently the only animal models that allow researchers to mimic human conditions of genetic disorders on a molecular level and represent the most sophisticated and valuable tools in functional genomics and drug target validation. Thanks to the early success of Professor Harvey and others, the WEHI, Australia, is home to a number of companies that specialise in transgenic mice and testing techniques, including Ozgene – a multi-million dollar business started by the former head of the WEHI's Genetically Modified Mouse Laboratory. Ozgene now exports 80% of its services and has the world exclusive licence for the proprietary TranzEmbryo™ lentiviral technology (which was co-developed with Tranzyme Inc in Alabama, USA), as well as having alliances with the Jackson Laboratory (USA) and Oriental Yeast Company (Japan).

In the past decade the WEHI has also established a number of commercial spin-off ventures specialising in diagnostic and drug development that also leverage their in-house transgenic capabilities.

13.12 Additional observations

The PI was truly motivated to share his research and to collaborate, which is apparent in his later work and from comments by his colleagues on this project at the WEHI. Parsons said, 'He enjoys the synergy of collaborations and I think he encourages that in his postdocs... he creates an environment where it's certainly okay for you to go and interact with people and collaborate with them' (Parsons interview, 2009).

This led to the broader studies in humans and others that led to the prenatal genetic testing.

There appears to have been some possible barriers to translation of the research more broadly, with the focus on adding to the basic research being knowledge based. There was identification of the potential for the knowledge and techniques to be applied elsewhere in basic research but not more broadly. There may have been potential for mentoring and support for broader dissemination and greater recognition of the broader implications and potential.

The initial investment in the project is still being realised, with Professor Harvey continuing to be associated with the National Heart Foundation of Australia.

13.13 Summary of case study impacts

The payback framework describes the process from research to several categories of impact. The categories allow us to compare across different case studies. Table 13-2 shows, in point form and by impact category, some of the impacts, described more fully above, that have emerged from this grant.

Table 13-2 Payback

Payback category	Impacts from case study
Knowledge production	• Peer-reviewed publications • Citations
Research targeting and capacity building	• Identification of a new gene in heart development spurned a new research field • Broader application in development research and related fields of research • Large number of research collaborations for the PI • Increased research capacity
Informing policy and product development	• Transgenic facility • Genetic tests
Health and health sector benefits	• Limited but better understanding of mechanisms is likely to lead to further understanding of genetic defects related to congenital heart disease and possibly will have broader application to cardiovascular disease (CVD) and CVD risk • Health benefits related to genetic testing and better understanding of the developmental process
Broader social and economic benefits	• Helped to establish expertise in transgenic technology, which translated into the establishment of a knockout facility and commercial ventures such as the commercial transgenic facility in Australia, Ozgene

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CHAPTER 14 Effect of simulated stroke on developing astrocytes

14.1 Overview of case study grant

The grant of interest to this case study, titled 'Effect of Simulated Stroke on Developing Astrocytes', was funded by the Heart and Stroke Foundation of Canada (HSFC) for two years from July 1993 to June 1995, with an amount of Can$101,000.

Some neurons die within minutes of a stroke, while others take several days to die, causing permanent brain damage. Although little can be done for neurons that die immediately following a stroke, it was believed to be possible to rescue the neurons that take several days to die. Doing so required an understanding of the mechanisms that cause this delayed neuronal death. In the brain, cells known as astrocytes function to support the neurons. Through this grant application, the team proposed to determine how hypoxia–ischaemia affects astrocytes at different stages of maturation. Astrocytes subjected to hypoxia–ischaemia were compared with neurons that were not exposed to any insult in order to determine how such dysfunctioning astrocytes affect the survival and function of neurons.

This study was led by Dr Leif Hertz and two co-applicants, Dr Bernhard Juurlink and Dr Jerome Yager. The work was conducted at the University of Saskatchewan.

14.2 Introduction to case study

Strokes are commonly associated with elderly people, but they can occur in any age group and occur frequently in premature babies. Many affected infants will suffer permanent brain damage, manifested as cerebral palsy, mental retardation and seizures, as a consequence of nerve cell death. Some neurons die within minutes of the stroke, others take several days to die. Although little can be done for the neurons that die within minutes of a stroke, researchers thought that it should be possible to rescue the neurons that take several days to die. Doing so would require an understanding of the mechanisms that cause this 'delayed neuronal death'.

Astrocytes exist within the brain, where their function is to support neurons. Specifically, astrocytes are characteristic star-shaped glial cells in the brain and spinal cord. They perform many functions, including biochemical support of endothelial cells that form the blood–brain barrier, provision of nutrients to the nervous tissue and a principal role in the

repair and scarring process of the brain and spinal cord following traumatic injuries (Wikipedia contributors, 2010, 'Astrocytes'). The supportive functions of astrocytes change as they mature. The research team believed that many neurons die following a stroke because of injury to astrocytes, so the objective of this research was to better understand the effect stroke has on immature and mature astrocytes and how astrocyte injury leads to neuron death.

Through this grant, the principal investigator (PI) wished to test the hypothesis that astrocytes play a key role in the susceptibility of the brain to anoxic–ischaemic injury (where anoxic refers to a lack of oxygen and ischaemic refers to a lack of blood supply) and that the mechanism by which astrocytes are affected during these insults changes with brain maturation. The specific objectives of the study were:

1. to determine whether mature and immature astrocytes die because of the same or different mechanisms – the team proposed to evaluate this by focusing on energy charges, redox potentials, cellular pH changes, cytosolic free calcium changes and free radical related activity both during the insult and while astrocytes were recovering from a limited insult
2. to determine whether functional disturbances in astrocytes recovering from an insult interfere with the ability of the cells to regulate the composition of the extracellular milieu and to support survival of cerebral neurons.

The basis for this proposal was that there are profound differences in vulnerability of brain to hypoxia–ischaemia at different ages. Evidence had indicated that some of these differences are related to changes in astrocyte function with maturation. Preliminary work from the PI's laboratory supported these suggestions. The research team had demonstrated that mature astrocytes suffer more damage during reperfusion following hypoxia than during the hypoxic insult (Sochocka et al., 1992), that astrocytes exposed to ischaemia have less ability to support cerebral neuron survival than control astrocytes (Hertz and Peng, unpublished observations) and that immature astrocytes are more susceptible to substrate deprivation in the presence of oxygen than in the absence of oxygen, whereas the reverse is true of mature astrocytes (Juurlink, Hertz and Yager, 1992).

Leading up to 1993, although the vast majority of studies in the area of cerebral ischaemia had focused directly on the neurons, evidence was accumulating that astrocytic impairment was of functional importance and that primary effects on astrocytes may lead to delayed neuronal death. This is because glutamate accumulates to neurotoxic levels within the extracellular space during hypoxia–ischaemia (Rotham and Olney, 1986) due to a combination of enhanced release and failure of the normally active energy-dependent neuronal and glial re-uptake mechanisms (Drejer et al., 1985). Astrocytes accumulate neuroactive compounds like potassium and glutamate from the extracellular fluid. The accumulated glutamate is partly metabolised by the astrocytes. Because these compounds do not easily cross the blood–brain barrier, and because neurons have no pyruvate carboxylase activity, there can be a shortage of glutamate and gamma aminobutyric acid (GABA). Therefore, unless another anaplerotic enzyme is found in neurons (such as malic enzyme), they cannot perform a net synthesis of glutamate through the tricarboxylic acid cycle (TCA), since pyruvate carboxylase is found almost exclusively in the astrocytes.

It thus was evident that astrocytes play a critical role in the maintenance and integrity of the electrical and biochemical environment of the brain and are intricately involved in survival of neurons. Astrocytes were recognised as being at least partly responsible for maintenance of the acid–base homeostasis of the brain, uptake of excitatory amino acids and potassium, scavenging of free radicals and supplying neurons with trophic factors and citric acid cycle intermediates (Tanaka, Araki and Masuzawa., 1992). Some of the team's previous work indicated that some astrocyte functions were important to the physiologic and pathophysiologic adaptations of the brain to hypoxia–ischaemia and were age dependent. For example, experimental data suggested that perinatal animals and presumably newborn humans respond differently to similar hypoxic–ischaemic insults compared with adults. Important among these differences was the observation that newborns are capable of surviving hypoxic–ischaemic insults much longer than adults. Furthermore, the neuropathologic consequences of such insults differed in distribution and extent of damage. Reasons for the differences in vulnerability to hypoxia–ischaemia at different ages were not known, although data at the time pointed to several mechanisms encompassing developmental differences.

Additional work conducted previously by the PI and his colleagues demonstrated that moderate hypothermia has a beneficial effect on ischaemic damage of astrocytes not only when applied during the insult but also when applied exclusively following the insult, therefore indicating a potentially clinical significant result.[1] Hypothermia was thus thought to be a useful modulator of injury. These observations could have been used to test the hypothesis of this grant, as it is possible during hypothermic ischaemia to precipitate depletion of adenosine-5'-triphosphate (ATP), which would allow for the critical testing of the first hypothesis.

The team believed that the results from this study could help develop strategies for medical therapies in the treatment and prevention of stroke-related neuronal death and complications specific to different ages.

This study intended to delineate the role that astrocytes play in neuronal cell death following hypoxic–ischaemic insults in newborns as well as adults. This information was expected to have a direct influence on the development of agents or therapeutic interventions specific for patients with strokes, whether such individuals are premature infants or elderly people.

14.2.1 The case study approach

The findings presented in this case study are based on the original grant application, an interview with the PI, the PI's curriculum vitae, documentary analysis of the scientific literature and bibliometric analysis of publications the PI identified as relevant to this grant. Unfortunately, despite numerous efforts via email and telephone, the authors were unable to interview the two co-applicants, or other non-PIs, for corroboration. Thus efforts have been made to reconcile the PI's statements regarding the science through the literature. Other comments made regarding impacts, the research climate, and so on, have been taken at face value.

[1] Appendix 8.3 of grant application.

14.3 Stage 0 – topic/issue identification

The idea for this project arose from the PI's scientific interest, his previous work and ongoing collaborations. These three factors are elaborated on below.

14.3.1 The PI's scientific interests

The PI states that he was always interested in astrocytes, which are a type of glial cell. There was little general scientific interest in astrocytes at the time because people associated them with glue (they are also known as astroglia, which translates to nerve glue). They were not thought to have a terribly interesting function. Hertz's interest in astrocytes was piqued in Denmark in the 1960s, when he and his colleagues found that energy metabolism in astrocytes was stimulated by elevated potassium concentrations.

In Denmark, Hertz was working on cultured cells in a biochemistry laboratory in which work was limited by a lack of tissue culture expertise. This instigated his move to the Department of Anatomy at the University of Saskatchewan in 1974, where he and his colleagues were able to establish the necessary cultures, including astrocytes and neuronal cultures. The team learned about the metabolism of astrocytes, observing that they have some enzymes that neurons do not – for example pyruvate carboxylase. This means that a neuron cannot make glutamate and must depend on astrocytes to do so.

14.3.2 Collaborations

Various collaborations led the PI to follow this research path. In 1975, when he joined the Department of Anatomy, Hertz met and started to collaborate with Dr Juurlink. Juurlink, a co-applicant on the grant of interest, was well established in performing cell-culture techniques.

The PI started researching glutamate and astrocytes in the 1980s. At that time, his research was not related to stroke but was basic function research. He started working within the area of stroke as a result of collaboration with Dr Shuaib, who was very interested in stroke and was the driving force in setting up the Saskatchewan Stroke Research Center, a coalition of researchers interested in stroke, which was funded by the Saskatchewan Health Board.

14.3.3 Building on previous findings

After joining the Department of Anatomy and establishing the cell cultures, the team looked at glutamate uptake under different conditions and found that astrocytes maintain uptake even when under hypoxic conditions for some time. When the basic functioning of astrocytes was understood, the PI began to develop an interest in stroke – an obvious successive possible avenue of research, as it was possible to mimic stroke in astrocytes. Thus, it was a natural fit to work with Dr Shuaib.

In addition, a key publication in the early 1980s by Fred Plum indicated that if a stroke is relatively mild, selected neuronal cell death may occur (Plum, 1983). If an infarct occurs, then all cells die, including astrocytes. This reinforced the fact that astrocytes behave differently to neurons after stress and thus further supported the work being done by the PI and his co-applicants to continue studying astrocytes.

14.4 Interface A – project specification and selection

The application was written by the PI, Dr Hertz, and two co-applicants, Dr Yager and Dr Juurlink. The team proposed to determine how hypoxia–ischaemia affects astrocytes at different stages of maturation. Astrocytes subjected to hypoxia–ischaemia were combined with neurons that had not been exposed to any insult in order to determine how such dysfunctioning astrocytes affect the survival and function of neurons. The application was submitted to the HSFC in response to an open call for applications.

All studies were performed using an established model of hypoxic–ischaemic brain damage in the immature rat via primary cultures of astrocytes, glutamatergic neurons and GABAergic neurons. GABAergic neurons are those that secrete GABA as their primary neurotransmitter. GABA is an inhibitory neurotransmitter that inhibits whichever neurons its binds to. It is the primary inhibitory neurotransmitter in the brain. It is the converse of glutamate, which is the primary excitatory neurotransmitter in the brain. Newborn rats were used for preparation of *in-vitro* primary cultures. Pregnant animals were needed for preparation of the cerebral cultures. Specifically, experiments were designed to study:

- the effects of substrate deprivation in the presence or absence of oxygen (the latter simulating ischaemia) on cellular functions of 'young' (one week old) and 'old' (five week old) astrocytes in culture; the following measurements were made:
  - cell death, using mainly the lactate dehydrogenase (LDH) release method (Juurlink and Hertz, 1993)
  - energy exchange, by examining cellular contents of adenosine nucleotides: ATP, adenosine diphosphate (ADP) and adenosine monophosphate (AMP)
  - mitochondrial membrane potentials, using [123]-rhodamine
  - cytosolic redox potential, by examining the ratio of effluxed lactate to effluxed pyruvate
  - mitochondrial redox potential, by examining the ratio of acetoacetate to hydroxybutyrate
  - cytosolic free calcium, using a cytofluorometric method with a dye indicator
  - intracellular pH, using a cytofluorometric method with a dye indicator
  - free radical activity, using the T-bar method, as well as the [123]-dihydrorhodamine method
  - intracellular glutathione levels, by an enzymatic method
- the effects of injury of young and old astrocytes on their ability to accumulate glutamate and to support neuron survival; the first part of the experiment was determined by measuring the uptake of glutamate into astrocytes during and following injury; in latter experiments, surviving neurons were counted or stained immunocytochemically to identify injured neurons.

Metabolic fluxes, including carbon dioxide production from labelled precursors, were measured in the cells and in released glutamate and GABA. These experiments required

precise techniques. All of the methods were well established, except for the measurement of intracellular calcium, which proved to be very complicated. The PI reflects that this was not easy to set up and required one of the team members to go to another laboratory in Salt Lake City to learn the technique. The team did not publish findings using this method.

Overall, the review committee acknowledged the important and somewhat neglected area of enquiry and the 'extremely prolific' applicants who had 'substantial track records'. Criticism focused on the 'several methodological deficiencies', largely due to the description of the methods being brief, yet including a number of techniques that the reviewers thought might suggest a lack of focus. It was also noted that expression of *c-fos* is not necessarily a marker of injured cells, and its evaluation 24 hours after the insult was felt, by the reviewers, to be too late to see a past effect, which usually occurs much earlier. All reviewers commented on the lack of experimental data, as well as providing an evaluation of the pitfalls of the in-vitro assays.

The PI recalled that the HSFC had two peer reviewers come out to the laboratory and that both were very positive about the application. The PI also received support from Dr Antoine Hakim, a well-established and well-renowned stroke researcher, and discussed the application with members of the Saskatchewan Stroke Research Center.

14.5 Stage 1 – inputs to research

14.5.1 Funding

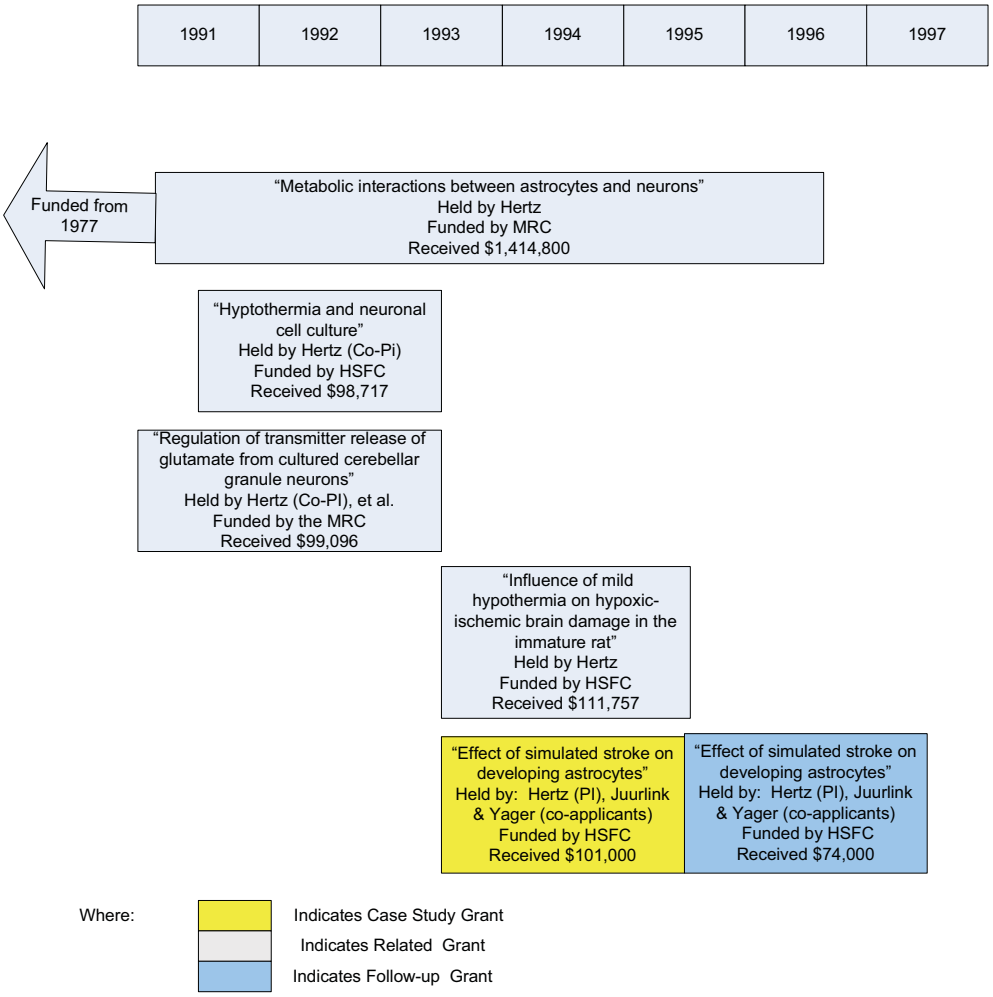
The HSFC grant of interest was funded for two years from 1993 to 1994 with an amount of Can$101,000. The applicants did not receive the requested funding of Can$158,377. Table 14-1 shows a breakdown of the proposed budget.

Table 14-1 Research Costs

Fiscal year	1993-1994 (Can$)	1994-1995 (Can$)
Technician (salary and benefits)	41,418	43,489
Equipment (filters to adapt cytofluorometer to measure intracellular pH)	600	--
Experimental animals (newborn and pregnant mice)	5,000	5,500
Materials and supplies	18,700	20,570
Other (anatomy facilities, travel and publication costs)	11,000	12,100
Total	**76,718**	**81,659**

Figure 14-1 outlines the other projects with which the PI was involved and other sources of funds within his laboratory for the time period covering the case study grant plus and minus two years.

Figure 14-1 Related funding awarded to the PI from 1991 to 1997



The HSFC initially approved renewal funding for the three years following the case study grant, although this funding was cut off in the last year, which led Dr Hertz to take a sabbatical and then to retire in 1998.

The PI said that it was not extremely difficult to get funding at the time, although he noted that he did not have enough money to do all that he wanted. The production costs for the large amount of cultures needed for this study were high, especially because of the strict requirement for aseptic technique and conditions. Had he not been funded by the HSFC, the PI said he would not have applied elsewhere; he would have focused on other things instead.

14.5.2 Facilities

This research was conducted within the Departments of Pharmacology, Anatomy and Paediatrics of the College of Medicine at the University of Saskatchewan. The PI said that his laboratory was relatively well equipped and that his team received some equipment from the stroke group. He also claimed that space was not a problem.

The team had access to photographic facilities (but not supplies), an ultraviolet microscope (that would otherwise have cost Can$5/hour), some dishwashing and autoclaving facilities, animals and media for a few of the experiments.

14.5.3 Research team

The PI came to the University of Saskatchewan in 1974. He was a professor in the Department of Anatomy until 1979, after which he moved to the Department of Pharmacology, where he proceeded to lead an effective and active cell-culture laboratory for almost 20 years. His strength on the team was in relation to the biochemical–metabolic aspects. He was regarded as an 'experienced and well renowned cell culture expert. [His contributions to the field were] viewed as a great benefit…' (Heart and Stroke Foundation of Canada, 1992).

Two co-applicants were listed on the grant: Dr Jerome Yager and Dr Bernhard Juurlink. Yager, a clinician, was an assistant professor in paediatrics at the Royal University Hospital in Saskatchewan and a member of the stroke research team. He was concurrently investigating hypothermia, perinatal hypoxic ischaemia and astrocytes. Juurlink, a biologist, worked in the Department of Anatomy at the University of Saskatchewan and was experienced in cell-culture techniques. He had a research interest in the survival of motor neurons and ischaemic events in cell cultures. His contribution to the team was in relation to the developmental-cell biological aspects of the studies. As one reviewer mentioned in the Scientific Review Committee Report, there was 'no doubt that these investigators have the necessary resources, experience and enthusiasm to perform…these studies. This is a longstanding collaboration which has been very fruitful' (Heart and Stroke Foundation of Canada, 1992).

Drs Juurlink, Yager and Hertz were all members of the Saskatchewan Stroke Research Center at the university. It was written in the application that Hertz and Juurlink were to dedicate approximately 35 percent of their time to the centre.

The grant proposal indicated that two technicians would be involved. One, Mrs Dunlop, a part-time technician, was said to be a highly skilled tissue-culture technician who would prepare and maintain most of the cultures used in the project. The other, Mr Reichert, a full-time technician, was tasked mainly with performing the biochemical analyses on the cultures and assisting with setting up the cultures. He had already started to set up the cultures when the application was drafted.

14.6 Stage 3 – primary outputs from research

The experiments conducted through the case study grant demonstrated that relatively brief periods of ischaemia can markedly interfere with the ability of astrocytes to support neuronal survival. The research also confirmed that young astrocytes survive better with substrate deprivation. These findings are elaborated on more thoroughly in the following section.

14.6.1 Knowledge

The PI identified the following publications as directly related to the case study grant; the paragraphs following the list of publications describe the main findings from these articles:

1. Juurlink, B.H.J. and L. Hertz, 'Ischemia-Induced Death of Astrocytes and Neurons in Primary Culture: Pitfalls in Quantifying Neuronal Cell Death', *Developmental Brain Research*, Vol. 71, 1993, pp. 239–246.
2. Shuaib, A., E. Sochocka, R. Ishaqzay, L. Hertz and W.E. Code, 'Protective Effects of Hypothermia During Ischemia in Neuronal Cell Cultures', *Neurochemical Research*, Vol. 18, 1993, pp. 663–665.
3. Sochocka, E., B.H.J. Juurlink, W.E. Code, V. Hertz, L. Peng and L. Hertz, 'Cell Death in Primary Cultures of Mouse Neurons and Astrocytes During Exposure to and 'Recovery' from Hypoxia, Substrate Deprivation and Simulated Ischemia', *Brain Research*, Vol. 638, 1994, pp. 21–28.
4. Huang, R. and L. Hertz, 'Effect on Anoxia on Glutamate Formation From Glutamine in Cultured Neurons: Dependence on Neuronal Subtype', *Brain Research*, Vol. 660, 1994, pp. 129–137.
5. Yager, J., G. Kala, L. Hertz and B.H.J. Juurlink, 'Correlation Between Content of High-Energy Phosphates and Hypoxic-Ischemic Damage in Immature and Mature Astrocytes', *Developmental Brain Research*, Vol. 82, 1994, pp. 62–68.
6. Huang, R. and L. Hertz, 'Neuroprotective Effect of Phenylsuccinate, an Inhibitor of Cytosolic Glutamate Formation From Glutamine, Under Anoxic Conditions But Not During Exposure to Exogenous Glutamate. *Neuroscience Letters*, Vol. 183, 1995, pp. 22–26.
7. Hertz, L., J.Y. Yager and B.H.J. Juurlink, 'Astrocyte Survival in the Absence of Exogenous Substrate: Comparison of Mature and Immature Cells', *International Journal of Developmental Neuroscience*, Vol. 13, 1995, pp. 523–527.
8. Juurlink, B.H.J., E. Schultke and L. Hertz, 'Glutathione Release and Catabolism During Energy Substrate Restriction in Astrocytes', *Brain Research*, Vol. 710, 1996, pp. 229–233.
9. Huang, R., E. Sochocka and L. Hertz, 'Cell Culture Studies of the Role of Elevated Extracellular Glutamate and K^+ in Neuronal Cell Death During and After Anoxia/Ischemia', *Neuroscience Biobehavioural Review*, Vol. 21, 1997, pp. 129–134.
10. Juurlink, B.H.J., S.K. Thorburne and L. Hertz, 'Peroxide-Scavenging Deficit Underlies Oligodendrocyte Susceptibility to Oxidative Stress', *Glia*, Vol. 22, 1998, pp. 371–378.
11. Hertz, L., 'Astrocytic Amino Acid Metabolism Under Control Conditions and During Oxygen and/or Glucose Deprivation', *Neurochemical Research*, Vol. 28, 2003, pp. 243–58.

In the first article listed above, Juurlink and Hertz demonstrated that both astrocytes and cerebellar granule cell neurons die during an ischaemic insult only when there is complete loss of mitochondrial membrane potential (Juurlink and Hertz, 1993). This was

determined by comparing the ability of mitochondria to sequester [123]-rhodamine with the ability of cells to exclude propidium iodide. The authors also demonstrated that loss of cellular lactate dehydrogenase (LDH) in astrocyte cultures correlates directly with propidium iodide uptake, ie cell death, and inversely with uptake of [123]-rhodamine. Thus, both LDH loss and [123]-rhodamine uptake can be used to quantitatively measure astrocyte cell death in culture; however, this is not the case with neuronal cultures. It was demonstrated that even in highly enriched cultures of cerebellar granule cell neurons, in which astrocytes make up less than ten percent of the total culture protein, about 40 percent of total culture LDH was in the astrocytes. This was also the case with [123]-rhodamine sequestration. The authors concluded that caution must therefore be used when using the LDH technique to determine the proportion of neurons that have died in such enriched neuronal cultures.

In the second paper listed above, the authors described a study that was designed to look at the protective effects of hypothermia in cultures of cerebellar granular (glutamatergic) and cortical (GABAergic) neurons (Shuaib et al., 1993). The team used LDH release into the medium as an indicator of neuron damage. Experiments were all done in sister cultures, with groups of six cultures at two temperatures (37°C and 32°C). The duration of ischaemia was three hours in cerebellar granular neuronal cell cultures and six hours in cortical neurons. Release of LDH was measured immediately after the insult. The team observed that hypothermia protected both granular and cortical neurons, which suggests that, similar to the findings with astrocytes, the protective effects of hypothermia are evident in neuronal cell cultures from the cerebellum and cerebral cortex.

In 1994, the team studied the effects of hypoxia, substrate deprivation and simulated ischaemia (combined hypoxia and substrate deprivation) on cell survival during the insult itself and during a 24-hour 'recovery' period in primary cultures of mouse astrocytes and cerebral cortical neuronal–astrocytic co-cultures (Sochocka et al., 1994). Cell death was determined by release of the cytosolic high molecular enzyme LDH, as well as morphologically (using retention of staining with [123]-rhodamine and lack of staining with propidium iodide as an indicator of live cells). Glutamate concentrations were measured in the incubation media at the end of the metabolic insults. Astrocytes were very resistant to hypoxia but less resistant to simulated ischaemia; under both conditions, the glutamate concentrations in the media remained low. Cerebral cortical neurons were almost equally susceptible to damage by hypoxia and simulated ischaemia, although hypoxia had a faster deleterious effect on some of the neurons and simulated ischaemia during a long-term insult (nine hours) killed all neurons, whereas a non-negligible neuronal subpopulation survived nine hours of hypoxia. Neuronal cell death after long-term hypoxia (but not after simulated ischaemia) was correlated with high concentrations of glutamate in the incubation media. After certain insults, most notably relatively short-lasting simulated ischaemia (three hours) in neurons (which caused no increased cell death during the insult), a large release of LDH during the 'recovery' period was observed.

In 1995, Hertz, Yager and Juurlink published their findings from studies in which astrocyte cultures prepared from newborn mouse neopallium were grown for either one or three weeks (representing, respectively, immature and mature astrocytes) and then exposed to deprivation of substrate (glucose and amino acids) for up to 48 hours (Hertz, Yager and Juurlink, 1995). Cultures that had been deprived of metabolic substrates for 24, 30, 36 or

48 hours were examined for LDH efflux into the medium (an indicator of cell death) and ATP content. Significant cell death in mature astrocytes began after 30 hours of incubation in the substrate-deprived medium – when ATP had fallen to about ten percent of its initial value. Immature astrocytes survived on a substrate-free medium for 48 hours before there was any indication at all of cell death, and this corresponded to a time when ATP values had fallen to five percent of the initial values. These findings were compared to previous observations during simulated ischaemia (substrate deprivation plus anoxia) when there was a faster cell death and when cell death occurred at higher ATP levels.

In the mid-1990s, it was known that extracellular concentrations of glutamate and potassium ions increase during vascular insults in the brain, such as a stroke (Huang, Sochocka and Hertz, 1997). It was also well known that an increase in glutamate contributes to the death of neuronal cells at an earlier time than they would have succumbed to energy deprivation (Huang and Hertz, 1994). The origin of the released glutamate was unknown and cannot easily be studied in the brain in vivo. Huang, Sochocka and Hertz (1997) had shown through cell culture studies that the neuronal rate of formation of glutamate from glutamine is substantially increased during anoxia, especially in glutamatergic neurons. This increase is further enhanced in the presence of excess potassium ions. Phenylsuccinate, a compound that decreases formation of glutamate from glutamine in glutamatergic neurons, counteracts the increase in glutamate formation and, by doing so, improves cell survival (Huang and Hertz, 1995). Astrocytes in neuronal-astrocytic co-cultures were found to protect against anoxic neuronal damage to some extent by accumulating glutamate and thus keeping the extracellular glutamate concentration lower than in isolated neuronal cultures (Hertz, 2003).

All 11 articles identified by the PI were included in the bibliometric and citation analysis. The results of this analysis are shown in Table 14-2.

Table 14-2 Publication output and impact[2]

Number of journal articles:	11
Number of articles included in citation analysis:	11
Total number of citations (all papers):	323
Aggregate relative citation impact:	0.79 (Class II)
Self-citations:	26%

Distribution of publications across relative citation impact classes:

	Class I (uncited)	Class II (>0 and <0.8 citations)	Class III (0.8 to 1.2 citations)	Class IV (1.2 to 2.0 citations)	Class V (>2.0 citations)
Number of publications	1	7	1	2	
Proportion of total output	9%	64%	9%	18%	

Most highly cited publication[3]:	Juurlink, B.H.J. and L. Hertz, 'Ischemia-Induced Death of Astrocytes and Neurons in Primary Culture: Pitfalls in Quantifying Neuronal Cell Death', *Developmental Brain Research*, Vol. 71, 1993, pp. 239–246
Times cited:	78

The PI also referred to one of his recent publications, which reviews the science at a cellular level of cerebral ischaemia from the 1980s to the present (Hertz, 2008). This article states that 'it is now recognized that we need to protect not only neurons but the whole network of brain cells (astrocytes, oligodendrocytes, microglia, etc) affected by cerebral ischemia'. The article carries on to say that although astrocytes may live longer during energy deprivation, they will eventually perish, mainly as a result of compensatory mechanisms during reperfusion. Astrocytic demise, in turn, damages the neurons due to insufficient glutamate uptake. If severe, it leads to cell death of all cell types. Hertz highlights that, under some conditions, including perinatal brain ischaemia, pre-ischaemic hyperglycaemia can be neuroprotective, while hyperoxia after the insult is damaging both in the mature and immature brain.

14.6.2 Dissemination

Dissemination activities focused largely on publications and meetings. The PI attended mainly international meetings, as the audience was bigger and broader (Dr Hertz did not remember anyone outside his research network doing similar research in Canada at the time, although Americans were then involved in research in this area). Hertz recalled being invited to meetings but not as a keynote speaker. His students who participated in the studies, Rong Huang and Liang Peng, also conducted poster presentations at various conferences and meetings. Presentations were aimed at academics and other researchers, and although non-academics may have attended, they were not the primary audience. Dr

[2] In addition, three publications were indirectly linked to this grant. Two of these publications were indexed in Web of Science and received 21 citations in total, giving a relative citation impact of 1.32. One publication was uncited and the other was in relative citation impact Class IV. The self-citation rate was ten percent.

[3] Citation count extracted April 2009.

Hertz's research on astrocytes is central to his systematic review on the cellular mechanisms involved in cerebral ischemia (Hertz, 2008).

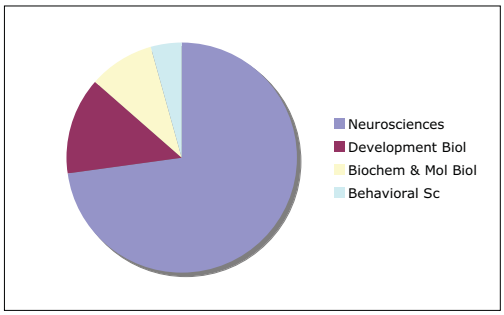
Dr Hertz feels that papers have become the most effective method of dissemination since the 1990s, when literature searches became more common due to the internet. Hertz credits the internet for making 'a big difference for writing and the accessibility of papers'. Prior to the internet, researchers would have had to consult electronic files or books in the library.

This research also fed into the training of students at the graduate and undergraduate level. Although Dr Hertz left the university in 1996 on medical leave and then retired in 1998, Juurlink continues to do research and train students. The first reference listed above has been cited in a chapter on 'Neural Cell Culture Techniques', written by Juurlink and Walz, in a textbook titled *Cell Neurobiology Techniques* (Boulton, Baker and Bateson, 1999).

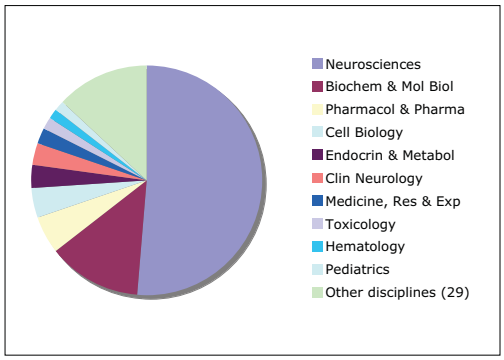
The bibliometric analysis also investigated knowledge diffusion. Hertz and his team most commonly published and cited in the area of neuroscience, with most citations being by those in the United States and Germany.

Figure 14-2 Knowledge diffusion: fields of research of output (a), fields of citing papers (b) and countries of citing papers (c)

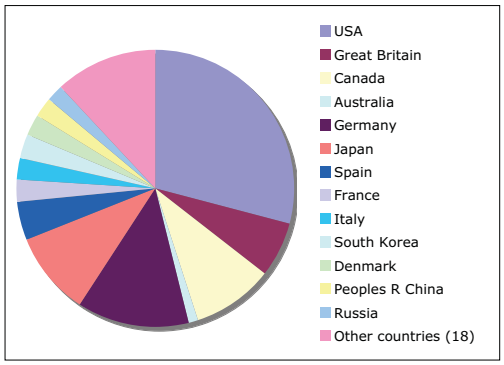
(a)



(b)



(c)



14.6.3 Training and capacity building

Dr Hertz believes that his research group was the only group in Canada studying simulated ischaemia and non-neuronal cells in the late 1980s and early 1990s. This has since become a much larger field of research, as evident by the growth in literature reporting research on glial cells. Such research was an emerging field in the late 1980s and is now quite prominent.

As noted above, at the onset of this research project, one member of the team went to another laboratory in Salt Lake City to learn the technique for measuring intracellular

calcium. In turn, the rest of the team was exposed to the technique. In addition, various scientists from other laboratories come to Saskatchewan to learn the techniques being used in Dr Hertz's projects.

The research conducted through the case study grant involved two doctoral students, Huang and Peng. Both finished their degrees. Peng is now a professor at the China Medical University in the Department of Clinical Neurology. Dr Hertz said that Peng is still using many of the same cell culture techniques and is still studying astrocytes.

Dr Hertz believed that this research may have helped in the ability to recruit researchers to the University of Saskatchewan, because he received 'quite a few' applications. However, as he left the university in 1996, he is unaware of any lasting influences. Hertz did think that the research he, Juurlink and Yager conducted had a positive impact on his group and their reputation, because he was offered space within the laboratory of Dr Anthony Hakim, a prolific Canadian stroke researcher in Ottawa, upon his retirement from the University of Saskatchewan.

14.6.4 Benefits to future research and research use

As stated previously, Dr Hertz retired shortly after this grant. He became a professor emeritus of the University of Saskatchewan in 1998 and has continued to publish. Hertz is still considered an expert in cerebral ischaemia. In 2003, he accepted a position as a visiting professor at the College of Basic Medical Sciences, China Medical University. He continues to attend international meetings and has written a review article for *Neuropharmacology* (Hertz, 2008).

Dr Yager is now Director of the Laboratory for Perinatal Brain Research at the University of Alberta. He has been consistently funded by the HSFC and Canadian Institutes of Health Research (CIHR), as well as the Hospital for Sick Children Foundation in Toronto and others. Research in his laboratory focuses on perinatal asphyxia and the development of models that reflect human newborn disease, their prevention and outcomes. His most recent focus has been on the effects of age on stroke injury and patient outcomes. Yager and his team have developed a model of stroke that spans age groups from birth to late adulthood. They have found that, contrary to general wisdom, the newborn brain is actually more susceptible to injury than the older brain but is much more capable of functional recovery. Yager is currently looking into underlying pathophysiologic reasons as to why this might be the case and is trying to determine whether specific genetic triggers might allow for this to occur in the newborn but not the adult. Other areas of ongoing interest are the determination of neuroprotective agents that are safe and useful in both the mother and foetus. In this regard, Yager's team has developed models of intra-uterine growth restriction and hypoxia to better reflect the 'real world' of the infant. They are now testing a host of neuroprotective agents that will be safe to the mother and foetus but that will provide enhanced endogenous antioxidant capabilities to prevent cerebral injury.

Dr Juurlink remained at the University of Saskatchewan for a number of years following the case study research, conducting further research on the management of oxidative stress responses in the body with an emphasis on the cardiovascular and nervous systems. He is now a Professor of Anatomy and Cell Biology at Alfaisal University in Saudi Arabia,

pursuing the same line of research. Dr Hertz has not been involved with Juurlink since 1995.

Dr Hertz believes the work conducted through this grant and the follow-up grant did help his co-applicants generate new ideas and obtain further funding. This grant helped create a long-term collaboration with Peng and another new collaboration with Dr Dienel from the Department of Neurology at the University of Arkansas.

14.7 Stage 4 – secondary outputs

There have been no secondary outputs of this research such as a new policy, drug or device development. However, Dr Hertz believes that clinical effects should be witnessed within the next decade, as researchers now understand more about what factors are detrimental to astrocytes and the role these cells play in cerebral function.

This research contributed greater understanding of glial cells in the context of stroke. Fifteen years ago, practitioners only looked at neurons, but guidelines now recognise the importance of the whole network of brain cells (Meairs et al., 2006). It is now known that a lot of the damage occurs after reperfusion and that there is calcium-mediated damage in astrocytes, which in turn affects the survival of neurons. Hertz believes that these findings should have clinical importance for how people are treated after stroke.

14.8 Stage 5 – adoption by practice and the public

The results obtained through this research grant and the subsequent funding have not changed practice or had an impact on the public.

14.9 Stage 6 – broad health and economic outcomes

This research has not had an effect on society through improved population health, spin-off companies, employment of people, sale of products by industry or otherwise.

14.10 Payback

The payback framework describes the process from research to several categories of impact. The categories allow us to compare across different case studies. Table 14-3 shows, in point form and by impact category, some of the impacts, described more fully above, that have emerged from this grant.

Table 14-3 Payback

Payback category	Impacts from case study
Knowledge production	• 11 related peer-reviewed articles • Papers and posters presented at various international meetings
Research targeting and capacity building	• Knowledge transfer within laboratory to students and postdoctoral researchers • Two PhDs obtained • Techniques taught to students and visiting researchers • Work cited in textbook
Informing policy and product development	• Importance of glial cells now discussed in guidelines
Health and health sector benefits	• Not applicable
Broader social and economic benefits	• Not applicable

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CHAPTER 15 The effects of alcohol consumption on blood pressure, plasma lipoprotein cholesterol fractions and sympathetic nervous system function in man

15.1 Overview of case study grant

This case study examines the evolution and impacts of the National Heart Foundation of Australia (NHFA)-funded clinical research grant awarded to Professor Laurence Howes for the period of 1988–1989, entitled 'The Effects of Alcohol Consumption on Blood Pressure, Plasma Lipoprotein Cholesterol Fractions and Sympathetic Nervous System Function in Man' (grant reference G2328).

This project aimed to further investigate the underlying mechanisms of the relationship between alcohol consumption and various biological responses by examining the effects of 4–5 days of regular alcohol consumption on blood pressure, cholesterol levels and sympathetic nerve activity. At the time this study was conducted, it was understood that excessive alcohol consumption accounted for up to 30% of hypertension in the Australian community. There was also evidence at the time from population-based studies that regular alcohol consumption increased the levels of high-density lipoprotein (HDL) cholesterol; however, the mechanisms by which alcohol impacted both blood pressure and HDL cholesterol levels were unknown.

Prior research had shown a rise in blood pressure with regular alcohol consumption, whereby blood pressure had been recorded in the conventional way by a clinician in a clinical setting. This case study grant research proposed a different and more modern approach to blood pressure monitoring – ambulatory blood pressure monitoring (ABPM) – a more recent development that was gaining favour in clinical research. The advantage of ABPM, whereby blood pressure measurements were taken at regular intervals throughout a 24-hour period, was that it eliminated a potential variable associated with conventional clinical blood pressure measurement of 'measurement anxiety', now commonly acknowledged as the 'white-coat effect'. No previous research had been conducted on the effects of regular alcohol consumption on ambulatory blood pressure measurements. As such, results of this case study granted research contradicted earlier findings, such that ambulatory blood pressure (ABP) did not increase in response to regular alcohol consumption, although it became more variable. In addition, results from this prospective,

controlled clinical research contributed to a higher level of evidence that regular alcohol consumption led to an increase in plasma HDL cholesterol.

The study itself was small and had little direct impact; however, the results were cited by, and replicated by, other key researchers, thus augmenting the study's credibility. This body of work was pioneering in its use of ABPM to measure blood pressure responses to regular alcohol consumption, and its findings added to the momentum of a change in perception about the relationship between alcohol consumption, blood pressure and cardiovascular health. It is now understood, more than 20 years later, that regular moderate alcohol consumption may be beneficial to cardiovascular health.

15.2 Introduction to case study

15.2.1 Scientific background

At the time of the grant application, research had shown excessive alcohol consumption to be a preventable cause of hypertension that may account for up to 30% of hypertension in the Australian community (Saunders, 1987). Previous research using clinical blood pressure assessment had shown that acute alcohol consumption had little effect on blood pressure (Howes and Reid, *Clinical Science*, 1985), while 4–7 days of regular drinking of alcohol produced a consistent rise in blood pressure (Potter and Beevers, 1984; Howes and Reid, *British Journal of Clinical Pharmacology*, 1985; Howes and Reid, *Journal of Hypertension*, 1986; Howes et al., *British Journal of Clinical Pharmacology*, 1986; and Malhorta et al., 1985). These studies measured blood pressure at 1–2 time points in the clinic. It had also been shown that, in addition to increasing blood pressure, regular alcohol consumption also increased plasma HDL cholesterol levels (Maserei et al., 1986), something which could, in part, counteract the increase in blood pressure by reducing the risk of atheroma formation. Little was known about the mechanism underlying these opposing effects of cardiovascular risk factors, but research suggested that it was possible that both effects could be mediated by changes in sympathetic nervous system function.

Prior research had also investigated the relationship between alcohol consumption and nervous system function and how this may affect blood pressure. Sympathetic adrenal effects are ultimately elicited by the neurotransmitter noradrenaline (NA) and the hormone adrenaline, and these catecholamines play a central role in regulation of blood pressure. Their release is under control of the central nervous system and is finely modulated by several subclasses of pre- and post-synaptic receptors. NA is a neurotransmitter that is released naturally by the nerve cells and has the physiological effect of increasing blood pressure by increasing vascular tone through alpha-adrenergic receptor activation. Research had shown that acute alcohol consumption appeared to reduce NA clearance (Eisenhofer et al., 1983) and seemed to decrease the formation of the neuronal NA metabolite 3,4-dihydroxyphenylethylglycol (DHPG) (Howes and Reid, *Clinical Science*, 1985), suggesting that the reduction of NA clearance was a result of diminished neuronal uptake. It had also been discovered by Howes that NA clearance was reduced following several days of regular drinking, although DHPG levels were unaltered (Howes and Reid, *British Journal of Clinical Pharmacology*, 1985). A reduction in neuronal uptake was expected to increase plasma NA levels and increase NA availability at post-synaptic receptors such as the alpha-

adrenoceptors on the smooth muscle of the blood vessel walls, causing vasoconstriction and thus having the effect of raising blood pressure. Professor Howes' intention was to further investigate this mechanism.

Subsequent research had supported this theory by showing that cardiovascular responsiveness to infusions of NA (Howes and Reid, *Journal of Hypertension*, 1986) was reduced following regular alcohol consumption, suggesting post-synaptic adrenoceptor downregulation. However, it was also possible that regular alcohol consumption had specific effects on adrenoceptors and that different adrenoceptor subtypes altered in response to alcohol in different ways. For example, basic research with rats had shown that regular alcohol administration decreased alpha-1 receptor numbers in brain and liver (Lee et al., 1983, and Ciofalo, 1980) and that numbers of cardiac beta receptors increased or remained unchanged (Segal and Mason, 1982, and Bannerjee et al., 1978).

Evidence at the time also suggested that adrenoceptors played a role in the modulation of plasma lipoprotein cholesterol fractions. It had been shown in clinical research that alpha-1 agonists could decrease plasma low-density lipoprotein (LDL) cholesterol levels and increase HDL cholesterol levels (Cutler, 1983), while the selective beta-2 agonist terbutaline was reported to increase plasma HDL cholesterol levels (Hooper et al., 1981). These concepts led to the hypothesis that a reduction in NA clearance due to decreased neuronal uptake, in conjunction with reduced alpha-1 receptor responsiveness but enhanced beta-2 receptor responsiveness, may therefore explain the increase in plasma HDL levels that accompany regular alcohol consumption and lead to a rise in blood pressure that is due more to an increase in cardiac output than a rise in peripheral resistance. If this was the case, then increased blood pressure due to excessive alcohol consumption may therefore be most appropriately treated with cardioselective (beta-1) beta blockers, as long as the relatively minor effects of these drugs on beta-2 receptors did not lead to a substantial reduction in plasma HDL cholesterol levels. This had clinical relevance if it were to be proven and applied within public health.

New research was also emerging at this time in relation to blood pressure assessment, highlighting the reliability of 24-hour ABPM to establish the true average daily blood pressure in both hypertensive and normotensive patients. Prior to this, the standard approach had been to take blood pressure measurements in a clinical setting at 1–2 time points throughout the day. This newer method (ABPM) of measuring blood pressure serially and non-invasively during customary daily activities over a 24-hour period had been used relatively little in the research but had much potential to better quantify the effects of alcohol on blood pressure more reliably. It was newly recognised to be a superior method of assessing blood pressure as it removed the variable of 'emotional stress' induced by having blood pressure taken in a clinical setting – now widely acknowledged as the 'white-coat effect'. Indeed, average blood pressure levels recorded by ABPM were shown to be approximately 10mmHg lower than blood pressure readings determined by a physician in a clinical setting (Waeber et al., 1984). A major study emphasising the 'white-coat hypertension' phenomenon was published in the *Journal of the American Medical Association* by Pickering et al. in 1988. Although Professor Howes did not refer directly to Pickering's work in his proposal, years later, Pickering (a prominent American researcher in the field) cited Howes' pioneering use of ABPM in relation to alcohol affects on blood pressure in a paper (Pickering, Schwartz and James, 1995).

Although several crossover studies (Potter and Beevers, 1983, Howes and Reid, *Clinical Science*, 1985, and Howes and Reid, *Journal of Hypertension*, 1986) had shown that 4–7 days of regular moderate alcohol consumption produced an increase in blood pressure, as these studies had used clinical blood pressure monitoring, there was interest in the effect on ABP, as this had not yet been explored. There was also evidence that the rise in blood pressure that is observed following a period of moderate drinking may depend on the age, sex and prior alcohol exposure of the individual and, in particular, whether they are hypertensive or normotensive (Malhotra et al., 1985, and Howes, 1985).

Consequently, the following hypotheses were developed to be tested within the research project:

1. that regular alcohol consumption increases mean 24-hour home blood pressure
2. that regular alcohol consumption alters the reactivity of adrenoceptor subtypes in different ways (reduced alpha responsiveness and increased beta responsiveness)
3. that regular alcohol consumption alters plasma HDL cholesterol levels after a relatively short (four-day) period
4. that the rise in blood pressure that accompanies regular alcohol consumption and changes in HDL cholesterol levels are related to a reduction in NA clearance
5. that the magnitude of rise in blood pressures, alterations in reactivity of adrenoceptors and the reduction in NA clearance is greater in hypertensive patients than in normotensive patients.

15.2.2 PI's background

The PI responsible for this research was Professor Laurence Howes, who was at the time a senior lecturer and honorary physician at Austin Hospital (Victoria, Australia).

His early background involved undergraduate and postgraduate training at Austin Hospital, where he completed his doctor of philosophy (PhD). He spent two years of this time as a National Health and Medical Research Council (NHMRC) Neil Hamilton Fairly Fellowship recipient at the University of Glasgow in 1984 and 1985. Following this, he was Professor of Medicine, Pharmacology and Physiology at the University of New South Wales for 12 years.

Professor Howes is currently Professor of Pharmacology and Therapeutics at Griffith University, since the commencement of the School of Medicine in 2004.

Throughout his career, Professor Howes has practiced successfully as a clinician and has been involved in much research in relation to hypertension, cardiovascular health and nervous system function. He has authored chapters in numerous textbooks and has authored or co-authored almost 200 research papers published in reviewed journals. His body of work has contributed significantly to medical science, and Howes is listed in *Who's Who in Australia* (from 1995) and *Who's Who in the World* (from 1998) for his contributions to medical research and education.

15.2.3 The case study approach

The case study based on this research grant involved a combination of: face-to-face interviews with the PI (no further members of the team were available), a review of the original grant application and supporting documents including assessor comments, and documentary analysis of various papers produced by the PI from this research.

15.3 Stage 0 – topic/issue identification

Professor Howes said, 'I had already done quite a lot of work on the cardiovascular effects of alcohol...and there was a lot of interest because alcohol increased blood pressure and we had shown this was associated with the decreased reactivity of noradrenaline and also an increase in plasma noradrenalin levels due to a reduction in uptake. So we wanted to go ahead and follow that further, and we had a model whereby we gave normal volunteers alcohol for a period of seven days and their blood pressure went up, pretty much similar to what had been shown in population studies' (Howes interview, 2009).

The topic was identified largely in response to work Professor Howes had himself previously undertaken, although work by other groups, including groups in Australia funded by the National Heart Foundation of Australia (such as Ian Puddey in Perth, Western Australia), was also an influence.

The research was clinical, and although Professor Howes was a practising clinician at Austin Hospital at the time, he believes this did not influence the topic identification.

As the only person at Austin Hospital working in this area at the time, colleagues had little influence either. The following examines how the research topic was identified:

- PI's previous work
- research by others
- relevance to health benefits
- a new research area

15.3.1 PI's previous work

Prior to the grant application, Professor Howes had been involved in a significant amount of research in the area of cardiovascular effects of alcohol. The grant application references eight publications by Howes relevant to the research proposal, and a further 13 publications by others are referenced as relevant to the proposal. This research experience led to the development of the hypotheses at the heart of this grant application. The committee spokesperson noted in his report: 'Committee overall was impressed by Howes and his response to questions and his knowledge of the subject. Ability to do the project not questioned' (Grant-in-Aid Report of Interview, 1987)

Professor Howes' body of work included the following observations:

- Acute alcohol consumption has little effect on blood pressure (Howes and Reid, *Clinical Science*, 1985), while 4–7 days of regular alcohol consumption produced a consistent rise (Howes and Reid, *British Journal of Clinical Pharmacology*, 1985).

- Acute alcohol consumption appeared to decrease the formation of the neuronal NA metabolite DHPG (Howes and Reid, *Clinical Science*, 1985).
- After several days of regular drinking, NA clearance was reduced but DHPG levels were unaltered (Howes and Reid, *British Journal of Clinical Pharmacology*, 1985).
- Regular alcohol consumption reduced cardiovascular responsiveness to infusions of NA and to sympathetic autonomic reflexes (Howes and Reid, *Journal of Hypertension*, 1986).
- The rise in blood pressure following a period of moderate drinking may be influenced by age, sex and prior alcohol exposure of the individual, in particular whether they are hypertensive or normotensive.

At the time of the grant application, Professor Howes was the recipient of a National Heart Foundation of Australia grant for alterations in sympathetic function during the treatment of cardiac failure.

Professor Howes was also aware of the increasing popularity of measuring 24-hour ABP as a means of establishing true average daily blood pressure in hypertensives and for the assessment of antihypertensive drugs. He was aware of a knowledge gap in terms of the effects of regular alcohol consumption on 24-hour ABP readings, something that would help to determine the effects of alcohol on mean 24-hour blood pressures, blood pressure variability and the temporal relationship between alcohol intake and changes in blood pressure.

15.3.2 Research of others

At interview, Professor Howes mentioned that while his own work was the largest driver of topic identification, the work of other groups did have an influence, including those also conducting research funded by the National Heart Foundation. Publications of several groups were referenced and listed in the grant application, but at interview the only name mentioned was that of Ian Puddey and his colleagues, who were based in Perth at the time.

15.3.3 Relevance to health benefits

Professor Howes was aware of a good degree of interest in further understanding the relationship between alcohol and blood pressure. The previous body of work had shown that alcohol consumption had the effect of increasing blood pressure, but the mechanism by which this worked was unknown at the time and, according to one of the assessors, 'the possible mechanisms for an effect of alcohol on blood pressure certainly warrant further investigation' (Grant-in-Aid Assessor Report, 1987).

15.3.4 A new research area

There were two main parts to this research: exploration into the mechanism of how alcohol affected blood pressure and, perhaps more significantly in the eyes of Professor Howes, taking a closer look to see what happened to ABPM in response to alcohol intake. The latter was important, 'because we knew quite a few things associated with a rise in clinical blood pressures but not necessarily ambulatory blood pressures' (Howes interview, 2009).

15.4 Interface A – project specification and selection

Professor Howes was named on the application as the PI and Dr E. Conway as an associated senior investigator. The grant was prepared by Howes alone; at that stage in his career, securing funding for himself was an important part of his role.

According to Professor Howes, there were two key components of the research: to further investigate the mechanism underlying the effect of alcohol on blood pressure, an area where little work had to date been undertaken, and to take a closer look to see what happened to ABPM, as research to date had focused on clinical blood pressure. Howes noted that large population studies had previously demonstrated that alcohol consumption increased HDL cholesterol levels but no prospective randomised controlled trial had been carried out to show this.

As such, four research aims were listed in the application:

1. to determine the effects of regular, moderate alcohol consumption on 24-hour ABP recordings in normotensive subjects and in untreated patients with mild to moderate hypertension
2. to determine the effects of regular alcohol consumption on cardiovascular reactivity to selective adrenoceptor agonists in normotensive subjects and patients with hypertension
3. to determine the effects of four days of regular alcohol consumption on plasma lipoprotein cholesterol fractions (total, HDL and LDL cholesterol)
4. to compare the effects of moderate alcohol consumption on plasma NA clearance and spillover rates in normotensive and hypertensive people and determine whether a correlation exists between changes in NA clearance on spillover and changes in blood pressure or HDL cholesterol.

The original application sought three years of funding, but in the approval process this was reduced to two years. A review of assessor reports shows that the budget was raised as a concern; indeed, it was the major concern with the application. The assessor questioned the time allowed for different levels of staffing involvement, commenting: 'This appears to be overgenerous for 5–7 patients per year!' (Grant-in-Aid Assessor Report, 1987). The assessor was also concerned at the time forecast for technical officer involvement, which was the major budget item: 'With the stated support staff, the project would probably be completed in 1–2 years and a reasonable level of technical support to carry out the tasks as listed may be 0.5 time for two years' (Grant-in-Aid Assessor Report, 1987).

Professor Howes could not recall any further changes to the original project specification; however, assessors made several comments and raised questions during the assessment process, and review of papers suggests some further changes were made.

An assessor report indicates that the background to the research was adequately argued and presented in support of the overall project aims, although the assessor also stated that Professor Howes has 'selectively quoted from the work of Eisenhofer and not considered a possible centrally mediated effect of alcohol on pressor responsiveness' (Grant-in-Aid Assessor Report, 1987).

An assessor suggested that some of the definitions of patient groups be 'tightened' to include a diastolic blood pressure (DBP) measurement; 'classification according to DBP looks more realistic' (Grant-in-Aid Assessor Report, 1987). The purpose of 24-hour urine collections on day four was questioned and seen to be imprecise given the number of patients. A suggestion was made that it might be better to match the patients according to body mass index (BMI) rather than body weight and that the ABPM should be obtained two to three times during the study rather than the single time proposed to increase the accuracy, but the latter was not implemented by Professor Howes. The same assessor further indicated that it was not clear from the application how studies 1–3 would fit together.

A second assessor reviewing the lipid aspects of the study in particular concluded that 'the proposed study has identified an important question relating to the effect of alcohol on serum lipoproteins and is worthy of funding' (Grant-in-Aid Assessor Report, 1987); however, several of the study design details were questioned. It seems that these questions were raised at interview and satisfactorily answered by Professor Howes.

The panel recommended the grant be funded, with a score of 3.25 out of 5.

It is possible that this application was also submitted to the NHMRC, as was commonplace at the time; if this was the case, the outcome would have been negative, because, in the event, the funding was awarded by the National Heart Foundation of Australia. Had the application with the National Heart Foundation not been successful, Professor Howes believes that it is unlikely the research would have happened at all.

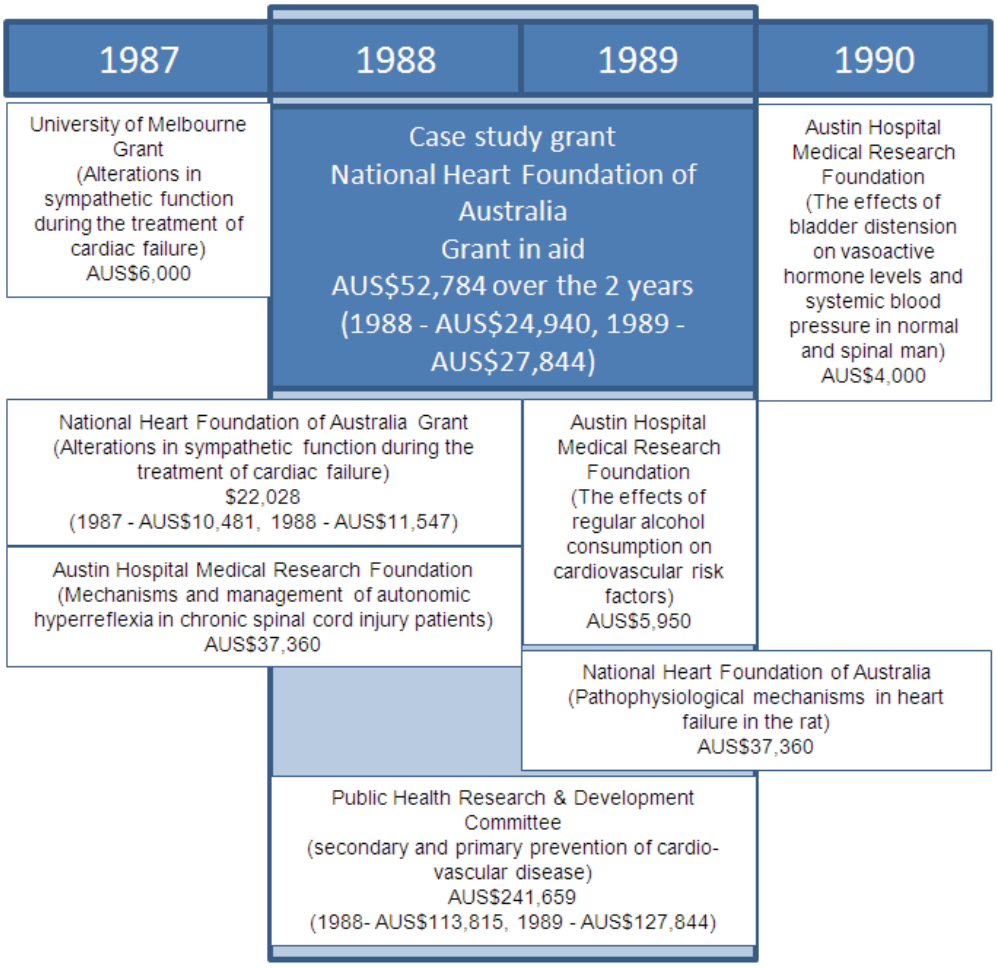
15.5 Stage 1 – inputs to research

15.5.1 Financial

The grant application requested around Aus$31,000 per year for three years (Aus$30,467 for year one, Aus$30,959 for year two and Aus$30,843 for year 3). However, this was reduced to a two-year period, with Aus$24,940 provided in 1988 and Aus$27,844 in 1989. Funding from the grant paid for a technical officer of Grade 1 for two years and Aus$2,000 of maintenance/consumables. Aus$2,000 for equipment was also requested for the first year. Professor Howes reported in interview that the funds from the National Heart Foundation were sufficient to cover the costs of the study.

At the time of the application, Professor Howes was a recipient of two other grants and was applying for another with NHMRC, as shown in Figure 15-1 below. While coming into his group, the grant from the Austin Hospital Medical Research Foundation was in fact for an unrelated stream of research, but one that has been quite widely cited.

Figure 15-1 A funding map for the grant; this shows major inputs to the PI's laboratory and sources identified in the grant application



The following resources were available to Professor Howes at the time of application:

- one full-time research nurse and two part-time research nurses to assist with data collection.
- five Copal ABP recorders, modified from commercially available recorders
- one Braun infusion pump; ideally the study required two
- a high-performance liquid chromatography with electrochemical detection (HPLC-ECD) system. including automatic sample injection and integrated recorder
- Dynamap (Critikon) automatic blood pressure recorders to be used for blood pressure measurements during agonist and ([3H]-) NA infusions
- electrocardiographs for recording heart rates during isoprenaline infusions.

The research took place at Austin Hospital in Victoria, Australia, one of the largest teaching hospitals in Australia with a sound reputation. Austin Hospital provided

laboratory space and helped with the recruitment of study participants. However, Professor Howes noted that at the time he was the only person at Austin Hospital working in this area, and it is assumed that while the reputation of the institution was valuable to the conduct of clinical research, it was perhaps of less importance from a collaborative and intellectual point of view.

15.5.2 Study recruits/samples

With the study being clinical research, there was a need for a patient sample. Groups of 11 and 12 normotensive, male volunteers aged 18–65 years were involved in the components of the study and were recruited with the help of Austin Hospital.

15.5.3 Knowledge, expertise and techniques

As noted previously, Professor Howes had extensive research experience in this field, and the research built directly on his prior work, taking in new approaches that were proving popular, ie use of ABPM in favour of a typical clinical approach.

Dr Conway was named on the grant application as an associated senior investigator; at the time, he was a senior research officer at Austin Hospital. According to Professor Howes, Conway was a scientist, rather than clinician, and as such had little involvement in the research.

Other people from Austin Hospital were involved in the course of the research. In particular, C.J. O'Callaghan and P.A. Phillips are named as co-authors on the publications produced from the grant. O'Callaghan was the Registrar at Austin Hospital at the time and Phillips a physician there. They assisted in the collection of the data and provided intellectual input in analysis and writing papers. As Professor Howes noted, 'We all helped each other out on other people's projects' (Howes interview, 2009).

Henry Krum was also noted in the application as an investigator associated with the project. As a clinician, he helped with the clinical studies and the recruiting of volunteers.

Professor Howes was himself a clinician; in particular he had extensive experience in clinical trials in the field of cardiovascular medicine.

Key to the research in regards to techniques was the use of ABPM. The research also followed a random-order crossover design: in one study period participants consumed a specified amount of alcohol between specified hours and in the other study period they abstained from all alcohol. In either study period, the same volume of fruit juice and calorie content was consistent.

15.5.4 Space, equipment and consumables

Laboratory space was provided at Austin Hospital where the trials were taking place.

Copal recorders modified for ambulatory use were used to record blood pressures at specified times during the study period to a maximum of 14 recordings a day (this was the limit for these machines).

Consumables were provided for by the grant. This included: vodka; alkaline cell batteries; HPLC columns; noradrenaline; preparation costs for alpha-methyl-NA; assay kits for HDL and LDL cholesterol; and chemicals and drugs.

15.6 Stage 2 – research process

The project proceeded broadly as planned, and even though only two years of funding was approved, the research was completed in the revised time; however, only the ABP and lipid studies were completed. The infusion studies originally proposed were not conducted, but this was not a consequence of the funding cuts, rather this was because initial findings from the ambulatory monitoring disproved the hypotheses that alcohol increased blood pressure. Previous research supporting this hypothesis had used clinical blood pressure monitoring – a less reliable method of measurement due to the associated 'white-coat effect'. The disproving of this hypothesis meant that there was little value in continuing with the infusion studies, instead the team concentrated on looking at other biomarkers of the effects of alcohol with respect to cardiovascular disease (CVD). Indeed, an assessor commented that 'aim number three regarding the acute effect of alcohol on plasma lipoprotein fractions has probably been adequately dealt with in the literature' (Grant-in-Aid Assessor Report, 1987). Studies concerning noradrenaline plasma kinetics were also not conducted as the team ran out of time; Professor Howes conducted research into this himself outside of this grant, working in collaboration with people in Glasgow when he was there, around the same time as this grant. With hindsight, Howes could not recall why this was included in the original grant because they already had data on this.

In the ABP study, 11 healthy males aged 18–65 years were recruited. They either consumed 1g/kg alcohol per night for four days or abstained from alcohol for four days in line with the randomised crossover study design. The group not receiving alcohol were matched for calorie intake by receiving glucose dissolved in water. The alcohol or glucose was consumed between 4pm and midnight each night. On the final day, ahead of the alcohol/glucose administration in the evening, the patients were fitted with an ABP monitor at 9am to record blood pressure hourly for 24 hours. Systolic blood pressure, DBP and heart rate (minimum, mean, maximum and coefficient of variation) were measured over this period. Spacelabs 90209 and Colin ABPM-630 recorders were used, with the same recorder used for both phases of an individual subject. Body weights were recorded when the blood pressure recorders were fitted.

In the study concerning HDL plasma concentrations, 12 healthy males aged 18–65 years were recruited. They consumed 1g/kg alcohol per night for four days or abstained from alcohol for this period in a randomised crossover study, with at least four days separating each study phase. Those abstaining from alcohol were given an isocaloric glucose substitute to match caloric intake in this period. On the final morning of each phase, venous blood was collected from the subjects after 15 minutes of supine rest. Cholesterol, triglyceride, uric acid and gamma-glutamyl transpeptidase (gamma-GT) were then tested.

Also in regard to research process, the scale of the research conducted was smaller than that proposed, with sample sizes reduced in actual numbers and through sample design. The research proposed to conduct the studies with normotensive and hypertensive patients; however, in the event, only normotensive patients were studied. The research had commenced with studying normotensive patients and there was the intention of then studying hypertensive patients, but the team ran out of time in this grant period, according to Professor Howes, in part through being distracted by other work. However, Howes noted that the hypertensive element of the proposal was returned to later on, when a year

or so later he undertook population studies across all ranges of blood pressures, including hypertensive patients – this later work was funded by the department not the National Heart Foundation.

The number of patients studied was also smaller than proposed, with actual sample sizes of 11 and 12 as opposed to the original 20 patients. Professor Howes recalled this was due to the unforeseen power and reliability of the ABPM approach, which meant that the initial numbers proposed were not required.

It would also seem that the inclusion criteria for patient demographics were widened in the study compared to what was proposed in the grant application. The research plan in the grant application specified subjects were to be aged 20–50 years; however, a publication reported that subjects were 18–65 years (Howes, Krum and Phillips, *Clinical and Experimental Pharmacology and Physiology,* 1990).

15.7 Stage 3 – primary outputs from research

There were two key findings from the research:

1. Regular, moderate alcohol consumption did not increase ABP, although it became more variable; this was in contrast to studies using clinical measurements, which showed a rise in blood pressure with regular alcohol consumption.
2. Regular alcohol consumption did lead to an increase in plasma HDL cholesterol.

This research reinforced the value of conducting ambulatory rather than clinical blood pressure monitoring and initiated a change of perceptions about the relationship between alcohol consumption, blood pressure and cardiovascular health.

15.7.1 Knowledge production

Table 15-1 and Figure 15-2 illustrate the publication output attributed to the case study grant application, its impact and the extent of the knowledge diffusion.

Table 15-1 Publication output and impact of directly related publications

Number of journal articles:	3
Number of articles included in citation analysis:	3
Total number of citations (all papers):	22
Aggregate relative citation impact:	0.61 (Class II)
Self-citations:	5%

Distribution of publications across relative citation impact classes:

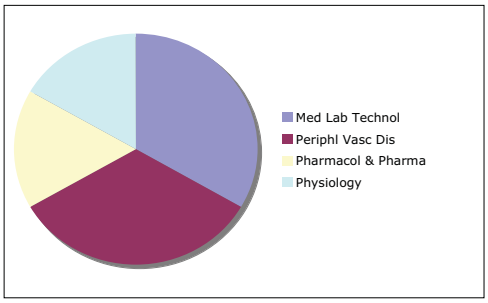
	Class I (uncited)	Class II (>0 and <0.8 citations)	Class III (0.8 to 1.2 citations)	Class IV (1.2 to 2.0 citations)	Class V (>2.0 citations)
Number of publications		2			1
Proportion of total output		67%			33%

Most highly cited publication[1]:	Howes, L.G., H. Krum, C.J. O'Callaghan and P.A. Phillips, 'Twenty-four Hour Ambulatory Blood Pressure Profiles Following Regular Alcohol Consumption', *American Journal of Hypertension*, Vol. 5, No. 10, 1992, pp. 771–772
Times cited:	10

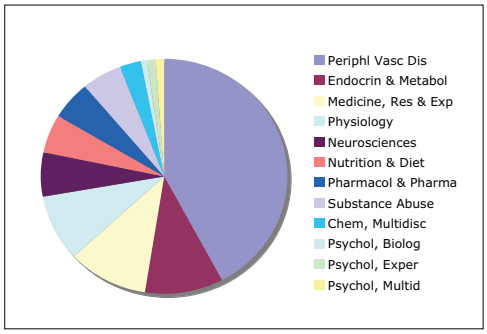
[1] Citation count extracted April 2009

Figure 15-2 Knowledge diffusion: fields of research of output (a), fields of citing papers (b) and countries of citing papers (c)

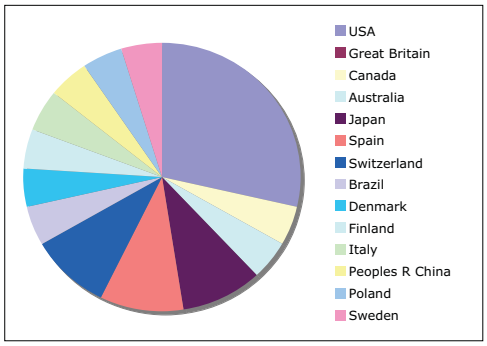
(a)



(b)



(c)



As indicated in the analysis above, the research produced three peer-reviewed articles; two concerning the effect of regular alcohol consumption on blood pressure and one concerning the effect on HDL in plasma:

- Howes, L.G., H. Krum and P.A. Phillips, 'Effects of Regular Alcohol Consumption on 24 Hour Ambulatory Blood Pressure Recordings', *Clinical and Experimental Pharmacology and Physiology*, Vol. 17, 1990, pp 247–250.
- Howes, L.G., H. Krum and P.A. Phillips, 'Effects of Four Days of Regular, Moderate Consumption of Ethanol on High-Density Lipoprotein Cholesterol Concentrations in Plasma', *Clinical Chemistry*, Vol. 36, No. 9, 1990, p. 1692.

- Howes, L.G., H. Krum, C.J. O'Callaghan and P.A. Phillips, 'Twenty-four Hour Ambulatory Blood Pressure Profiles Following Regular Alcohol Consumption', *American Journal of Hypertension*, Vol. 5, No. 10, 1992, pp. 771–772.

In the first paper, Howes et al. (*Clinical and Experimental Pharmacology and Physiology*, 1990) demonstrated that short-term regular alcohol consumption did not have a significant effect on blood pressure when compared with a similar period of matched calorie intake. However, results indicated that DBP variability was increased with alcohol consumption, suggesting that cardiovascular reactivity is increased with alcohol consumption without a sustained pressor effect. These findings were in contrast to the results of previous studies of the effects of several days of alcohol consumption, which had shown a rise in clinic blood pressure following the alcohol phase of treatment. Howes et al. proposed that the results may differ due to the different measurement approaches used and that it may be the stress of having blood pressure measured in a clinic that caused a rise in blood pressure while a relatively brief period of alcohol consumption does not in fact produce a sustained rise in blood pressure. However, Howes et al. acknowledged that a further difference in approach may account for the results – notably that, in contrast to previous studies, this research had used glucose supplementation during the control phase to match calories provided by alcohol. They noted that this calorie loading may have produced an increase in blood pressure similar to that produced by several days of alcohol consumption and, if so, potentially the rise in blood pressure observed in response to alcohol consumption may have in fact been due to caloric content alone. Howes et al. concluded a need for further studies of the effects of alcohol and calorie loading on cardiovascular reactivity using 24-hour ambulatory monitoring, in particular to determine whether similar results would be achieved if glucose was not administered during the control phase. They also noted a value on conducting ABPM studies comparing blood pressures between populations consuming and not consuming alcohol to determine whether a sustained pressor response occurs with long-term alcohol consumption.

The research demonstrated an increase in HDL cholesterol with regular alcohol consumption. The second paper (Howes et al., *Clinical Chemistry*, 1990) reports on studies concerning the lipid component of the research programme. The research was interested in the time course of HDL cholesterol in plasma in relation to changes in alcohol consumption, something the authors believed had not yet been adequately determined. The paper reports that results demonstrated that HDL cholesterol increased significantly after four days of regular alcohol consumption and accounted for an increase in total cholesterol that almost achieved statistical significance ($p=0.066$). Concentrations of LDL cholesterol remained unchanged, as did concentrations of triglyceride, uric acid and gamma-GT and the plasma haematocrit. The data were seen to demonstrate two points: changes in plasma cholesterol associated with increased alcohol consumption occur rapidly, and changes in HDL cholesterol in plasma are a more sensitive marker of short-term alterations in alcohol consumption than triglycerides, uric acid or gamma-GT, building on the work of Maserei et al. (1986).

The third publication was a letter to the editor (Howes et al., 1992). It reported that, in contrast to observations made by Abe et al. (1990), four days of regular alcohol consumption did not influence 24-hour blood pressure profiles in normotensive subjects. The authors hypothesised that the difference in results could be due to differences in study

design. The current study used only normotensive patients, while the study by Abe et al. observed hypertensive patients; furthermore, the current study used a random-order cross-over design, while Abe et al. used an uncontrolled single-order design. The conclusion reached was that short-term alcohol consumption does not produce sustained increases in blood pressure compared with a similar period of matched energy intake but rather increases blood pressure reactivity, which could be an effect of having blood pressure measured in a clinic.

15.7.2 Benefits to future research and research use

Capacity building and career development

According to Professor Howes, the research did not contribute to any PhDs and no research students came out of the grant. It did, however, help Howes to achieve a Merck Sharpe & Dohme grant looking at left ventricular mass, as Merck Sharpe & Dohme had a cardiovascular research fund at the time.

Professor Howes was a senior lecturer at the time of the research. In 1992 he moved to Sydney and continued the work on alcohol and other areas. He is now Professor of Pharmacology and Therapeutics at Griffith University since the commencement of the School of Medicine in 2004. He is also a pre-eminent consultant physician at the Gold Coast Hospital. He is listed in *Who's Who in Australia* and *Who's Who in the World* for contributions to medical research and education.

Krum is still working, although his focus now is in heart failure and he does a lot of work with the National Heart Foundation.

Targeting of future research

As a result of the case study grant research, Professor Howes focused his work more on population studies using ABPM rather than clinic blood pressure. He subsequently published a paper in the *American Journal of Hypertension* (O'Callaghan et al., 1995), in which it was shown that, similar to crossover studies in normal volunteers, when you look at the range of people drinking different quantities of alcohol in the community, their ABPs did not change but clinic blood pressures were higher, which suggested that alcohol was having a 'white-coat effect'. With the Merck Sharpe & Dohme grant, Howes also went on to measure left ventricular mass and showed there was no change in left ventricular mass associated with alcohol consumption. This indicated that alcohol was increasing the response to stress of measuring blood pressure in the clinic scene without really having an impact on average blood pressure over 24 hours.

Professor Howes felt that this research alone did not cause a change in direction; however, his results were replicated by others and, as a group, the findings caused a change in direction. Howes noted that 'The observation itself and the fact it was published [made this research of interest to others]. People who were interested in the area cottoned on to it' (Howes interview, 2009). In other words, eventually it would have flowed on to the medical world. People did indeed 'cotton on', and the studies were replicated by others, gaining momentum as this occurred.

The research helped to change the direction in which the relationship between alcohol consumption and heart disease was viewed. It started to change people's perceptions about

the relationship between alcohol consumption and blood pressure and the importance that it had from a cardiovascular perspective, and it started people looking at other potential mechanisms. Moderate alcohol consumption is associated with a good outcome despite apparently increasing blood pressure, and this research really showed this was most likely because moderate alcohol consumption was not associated with a significant increase in blood pressure. However, heavy alcohol consumption is associated with an increase in blood pressure, and others have gone on to look at other mechanisms for that and have shown that adverse effects on lipids at high doses probably account for the adverse effect.

The research helped to show the value of measuring ABP in preference to clinical measurements at a single point in time. This approach was replicated by other groups following this study. Groups that went on to use the same or very similar methods included Ian Puddey's group in Perth, Western Australia, and a group in Japan.

Although the level at which the papers from the case study grant research have not been cited may be expected from a pioneering piece of research, an article by Aguilera et al. (1999) cites two of the papers from this case study, and they are the earliest papers cited on ambulatory monitoring linked to alcohol, which suggests that Howes' work might indeed have been the first in this field. This is further supported by the fact that Pickering, as a key American author in the field (including a major study cited over 700 times in relation to 'white-coat hypertension', Pickering et al., 1988), cited Howes' pioneering use of ABPM in relation to alcohol's effects on blood pressure in Pickering, Schwartz and James (1995), although Pickering does not seem to refer to it directly again in some of his later important articles. A systematic review in this field on the effect of daily alcohol intake on blood pressure also shows Professor Howes' studies as the earliest (but not the largest) to use the ambulatory approach (McFadden et al., 2005).

Nowadays, ABPM is commonplace in clinical trials and forms part of the National Heart Foundation recommendations on measuring blood pressure.

The National Heart Foundation's *Guide to Management of Hypertension* (2008) notes that 'The possibility of raised BP in response to the assessment itself ('white coat' hypertension) should be considered and ruled out' and that 'Treatment decisions should be based on ambulatory BP, where available, because end-organ disease and cardiovascular event rates correlate more closely with ambulatory BP than clinic measurements'. Although the work of Professor Howes specifically is not referenced, this study contributed to the body of knowledge.

15.8 Interface B – dissemination

Professor Howes presented this body of research at a variety of conferences but could not recall specifically which studies were presented. Beyond publications and presentations, there was no further dissemination. More potent was other researchers picking up on this study and replicating the results to give the findings momentum.

15.9 Stage 4 – secondary outputs

The above-mentioned advice in the National Heart Foundation of Australia guide, in relation to monitoring blood pressure, seems to be general rather than being linked specifically to attempts to assess the impact of alcohol on blood pressure. It is possible in the future that some guidance linked to the specific issue of monitoring blood pressure related to alcohol consumption could partly draw more specifically on the findings from Professor Howes' stream of work. As noted previously, most studies examining the relationship of alcohol consumption and increased blood pressure are population studies rather than trials.

The systematic review by McFadden et al. (2005) identified 834 abstracts but only nine of those met the authors' inclusion criteria and five of these involved Professor Howes, including two from the case study grant research and a follow-on study article (O'Callaghan, 1995). While all the studies by Howes and colleagues were smaller than the largest of the studies, one of the clear messages from the review was the difference between the studies using conventional blood pressure monitoring and those using ABPM. The review suggested that their findings 'may have important implications for interpreting studies measuring the effect of alcohol on blood pressure as well as for regular clinical care' (McFadden et al., 2005). Any impact from the review would be most likely to come through the findings being used to inform a guideline related to this.

The results have not contributed to drug development.

15.10 Stage 5 – adoption by practice and public

The project itself did not specifically lead to any guideline developments or changes and Professor Howes was not asked to be involved with guideline production. Guidelines from the NHMRC would have existed at the time and would have changed as a result of this and other research; being a small study the results gathered momentum when replicated by others. This research 'was one of the bricks in the wall' (Howes interview, 2009) that led to it being more clinically accepted to make recommendations on alcohol consumption to hypertensive patients and to general thinking about the cardiovascular impacts of alcohol consumption. At the time, the thinking was that alcohol consumption increased blood pressure and certainly if you had hypertension you should not have alcohol. Today, the idea that small amounts of alcohol are okay but that large/excessive amounts of alcohol are deleterious and to be avoided is quite accepted. The case study grant research helped to show and reinforce this.

ABPM is now used as a more accurate instrument to determine blood pressure than one-off measurements in the clinic. The following reasons to use ABPM have been established since the Howes study (McGrath, 2002):

- to exclude 'white-coat' hypertension
- end-organ damage is more closely correlated with ABP than with clinic blood pressure readings

- ABP may be a better predictor of cardiovascular events and mortality than clinic blood pressure readings
- patients with hypertension whose nocturnal (sleep) blood pressure remains high (<10% lower than daytime average) may have a worse prognosis
- ABP provides a 24-hour profile, allowing assessment of clinic effects, drug effects, work influence, etc.

15.11 Stage 6 – final outcomes

Although the case study grant research has had no direct impact, it was part of body of work on informing understanding of and recommendations concerning alcohol consumption and cardiovascular health. The research helped to change the direction in which the relationship between alcohol consumption and heart disease was viewed. It started to change people's perceptions about the relationship between alcohol consumption and blood pressure and the importance that it had from a cardiovascular perspective. Moderate alcohol consumption is now associated with a good outcome, and this research really showed that this was most likely because moderate alcohol consumption was not associated with a significant increase in blood pressure. However, heavy alcohol consumption is associated with an increase in blood pressure, and others have gone on to look at other mechanisms for that and have shown that there are adverse effects on lipids at high doses, which are likely to account for the adverse effects.

Similarly, the case study grant research has been a small part of a body of work that provides understanding of and recommendations concerning ABPM, which has been found to correlate better with disease-oriented outcomes, such as left ventricular mass, retinopathy and microalbuminuria, than office measurement (Verdecchia et al., 1999, and Ozdemir et al., 2000). ABPM also has a better correlation with several patient-oriented outcomes. A cohort study of 1,076 patients found that an elevation in ABPM was a better predictor of cardiovascular events and overall mortality than office measurements. Another cohort study of 1,464 patients found that ABPM was linearly related to stroke risk and more predictive of a cerebrovascular event than screening blood pressure over an average of 6.4 years (Ohkubo, 2000). In a randomised parallel-group trial, 419 untreated patients were followed up using either ABPM or conventional office measurements to initiate and adjust antihypertensive therapy (Staessen et al., 1997). When compared with standard office measurement, management with ABPM led to less-intensive antihypertensive drug therapy without loss of blood pressure control. Evidence from other studies indicates that ABPM can be useful for risk stratification of patients in whom the diagnosis of hypertension is not clear (Khattar, Senior and Lahiri, 2001).

15.12 Other impacts

It was important that other studies followed this one, because, prior to this work, there had been a number of other studies showing something different – hence the subsequent studies were important validation of the new thinking. All of this became the body of work

that really influenced future thinking. Professor Howes said, 'I don't think it had an enormous impact at the time. Like all these things, people say 'oh yes' and then more studies come out and the impact slowly grows' (Howes interview, 2009).

15.13 Summary of case study impacts

The payback framework describes the process from research to several categories of impact. The categories allow us to compare across different case studies. Table 15-2 shows, in point form and by impact category, some of the impacts, described more fully above, that have emerged from this grant.

Table 15-2 Payback

Payback category	Impacts from case study
Knowledge production	• Three related peer-reviewed articles
Research targeting and capacity building	• No PhDs • PI achieved funding from Merck Sharpe & Dohme • PI took approach of ABPM into population studies • Contributed to body of knowledge supporting 'white-coat effect' of clinical blood pressure monitoring • Contributed to change in perceptions of relationship between alcohol consumption, blood pressure and cardiovascular health
Informing policy and product development	• No direct impact
Health and health sector benefits	• No direct impact but part of body of work informing understanding of and recommendations concerning alcohol consumption and cardiovascular health.
Broader social and economic benefits	• No direct impact

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CHAPTER 16 Electrophysiologic properties of cardiac myocytes in cardiomyopathy

16.1 Overview of case study grant

The grant of interest to this case study, titled 'Electrophysiologic Properties of Cardiac Myocytes in Cardiomyopathy', was initially funded for three years starting in 1991 by the Heart and Stroke Foundation of Canada (HSFC), after which it received a three-year renewal. This was a new project, led by Dr Susan Howlett, which aimed to explain the cellular mechanisms of heart disease, specifically cardiomyopathy, by studying a strain of genetically cardiomyopathic hamsters and normal controls. This research was conducted at Dalhousie University in Halifax, Nova Scotia, at a time when the PI was beginning her career in research and starting her lab at the university. The research team used an animal model of heart disease and investigated the electrical currents in isolated, single heart cells at several well-defined stages of the disease. These studies focused on the ability of individual normal and diseased cells to handle calcium – an ability that seemed to be impaired in many forms of heart disease. In the long term, the results of these studies were meant to provide information to aid in the identification and development of drug therapy.

16.2 Introduction to case study

Heart disease is a major cause of disability and death in North America. Many types of heart disease, such as heart muscle disease (cardiomyopathy) and ischaemia (a shortage of the blood supply to an organ), lead to necrosis (premature death caused by external factors such as toxins or trauma) of the myocardial (heart muscle) cells. Many researchers believed that calcium, which is essential for the healthy function of the heart, may build up to abnormally high levels in diseased heart cells (Wagner et al., 1989, and Weisman and Weinfeldt, 1987), triggering a series of events that culminates in myocardial cell death thought to be secondary to intracellular calcium overload.

A concentration gradient across cell membranes favours entry of calcium. Internal calcium concentrations in heart cells fluctuate, as compared with those in the extracellular fluid, which remain constant. High levels of intracellular calcium have a toxic effect on cells, so myocardial cells must allow entry of calcium but without allowing calcium overload. This balance is accomplished through several cellular mechanisms. By 1990, it had been well

established that the cardiac action potential consists of changes in membrane permeability to calcium, sodium, potassium and chloride ions. However, the mechanisms that predispose cells to accumulate calcium were not well understood.

Throughout several years leading up to this proposal, the principal investigator (PI) Dr Susan Howlett, had been investigating possible causes of cardiomyopathy in a unique strain of genetically modified cardiomyopathic (CM) hamsters. These hamsters develop a progressive, hypertrophic form of cardiomyopathy that results in congestive heart failure (CHF) and premature death (Jasmin and Proschek, 1982, and Jasmin and Proschek, 1984). This model had a number of important parallels with human heart disease; the animals had been used as models of cardiomyopathy resulting from unknown causes in the early stages and as models of hypertrophy and CHF in the later stages (Jasmin and Proschek, 1982). These animals provided a spontaneous and reproducible model with which to study basic mechanisms in the development of cardiomyopathy and CHF. Howlett thought that the intercellular calcium overload seemed to contribute to myocardial necrosis in this CM model, although much of the evidence at the time was indirect. The mechanisms involved had not been fully studied. Treatment with calcium antagonists was seen to prevent the development of cardiomyopathy.

The team had recently used [3H]-nitrendipine, a radiolabelled form of the dihydropyridine (DHP) calcium antagonist nitrendipine that bonds to the calcium channel of the cardiac sarcolemma, to show that neither the number nor the properties of high-affinity DHP binding sites change in cardiomyopathy (Howlett and Gordon, 1987, and Howlett, Rafuse and Gordon, 1988). However, these results did not rule out defects in the function or regulation of calcium channels in cardiomyopathy that may cause increased calcium influx and culminate in calcium overload and cell death. If calcium influx is enhanced in CM heart cells, Dr Howlett hypothesised that this would be reflected in an increased amplitude or change in the kinetics of the slow inward current. The pool of available calcium channels is regulated by a number of systems. Abnormalities in at least two of these systems – the beta-adrenoceptor-cyclic adenosine monophosphate (cAMP) system (Karliner et al., 1981) and the myocardial angiotensin II system (Carafoli, 1987, and Hirakata et al., 1990) – had been reported in cardiomyopathy and were thought to increase calcium channel availability. Whether these abnormalities enhance slow inward current and lead to calcium overload had not yet been investigated. Howlett thought that if intracellular calcium overload was linked to the development of cardiomyopathy, then it could be hypothesised that the arrhythmogenic transient inward current, which is promoted by increased intracellular calcium (January and Fozzard, 1988), should be more readily induced in the CM heart cells.

This case study investigates the grant that aimed to elucidate cellular mechanisms of heart disease, specifically cardiomyopathy, by studying a strain of genetically CM hamsters together with normal controls. The work was led by Dr Susan Howlett, who had obtained her doctor of philosophy (PhD) degree in the area of muscular dystrophy in 1985 from Memorial University in Newfoundland after finishing an undergraduate degree in neuroscience. Howlett went on to complete four years of postdoctoral work in pharmacology at the University of Alberta in Edmonton before she was appointed assistant professor in the Department of Pharmacology at Dalhousie University, where she began

cardiovascular and electrophysiological research. From 1990 to 1994, Howlett was a recipient of the HSFC's research scholar award.

16.2.1 The case study approach

This case study is based on two face-to-face interviews with the PI, Dr Susan Howlett, and the technician who worked on the project, Peter Nicholl; a telephone interview with Dr John Sapp, who was a student in Howlett's laboratory at the time; a review of the original grant application and supporting documentation; a review of the PI's curriculum vitae; documentary analysis of the scientific literature; and bibliometric analysis.

16.3 Stage 0 – topic/issue identification

The idea and proposed methods for this project were derived through a series of factors including:

1. previous work conducted by the PI
2. gaps in current state of knowledge regarding age-related heart disease
3. proximity to the breeder of experimental animals.

These three factors are elaborated on below.

16.3.1 Previous work

Hereditary cardiomyopathy has similarities to other types of inherited human disorders such as muscular dystrophy. The particular hamsters that Dr Howlett used for her studies are prone to muscular dystrophy. There are different models and types of muscular dystrophy in people. One form, known as Duchenne muscular dystrophy, is often accompanied by heart problems. The hamsters that Howlett had previously used thus were prone to hereditary cardiomyopathy – a heart disease in which the heart cells spontaneously die. In her previous work, Howlett had been studying muscular dystrophy and was trying to understand how the disease was expressed. Her work started with biochemical studies investigating different proteins, such as the calcium channels in the heart cell membranes. This work led Howlett to believe that she needed to look at the cells themselves to find answers. Her theory was that the voltage-sensitive function, which prevents calcium from entering heart cells, was defective, allowing too much calcium through the cell membrane and causing calcium overload. This interest led her to move from muscular dystrophy to heart disease in order to study the calcium channel in the heart and to see how calcium movement across the cell membrane became deregulated.

At the time of the grant application, Dr Howlett had recently investigated the force-interval relation in the CM hamster heart (Howlett, Bobet and Gordon, 1991) and had used the data within the framework of a recent model of excitation–contraction coupling to predict the relative rates of calcium movement between compartments in the heart. The results of these studies showed that the relative rates of calcium movement between intracellular compartments are generally quite similar in normal and CM hearts, although the rate of calcium re-accumulation (from external pools) following depletion of internal stores by a long rest interval is greatly slowed in cardiomyopathy. The differences between normal and CM hearts were reproduced by experiments in which calcium influx and

loading of internal stores was reduced by the calcium antagonist nifedipine and by a computer simulation in which relative loading of the uptake compartment was reduced (Howlett, Bobet and Gordon, 1991). Howlett had thought that intracellular calcium overload seemed to contribute to myocardial necrosis in this CM model; however, much of the evidence leading up to the grant proposal was indirect and had not been fully explored. Treatment with calcium antagonists had been observed to prevent the development of cardiomyopathy (Ferrier, Moffat and Lukas, 1985). These results had suggested to researchers that the voltage-sensitive calcium channels in the cell membrane of heart muscle cells change in cardiomyopathy.

Dr Howlett concluded that there were two likely explanations for her results: calcium influx may be increased in the CM heart or calcium influx may be unchanged or enhanced in cardiomyopathy but calcium loading of internal stores is impaired. The latter possibility was thought to lead to intracellular calcium overload, although at the time it was unclear whether calcium influx is reduced, enhanced or unchanged in cardiomyopathy. Howlett proposed that this question could be answered by measuring the slow inward current in myocytes isolated from CM hearts.

16.3.2 Desire to fill knowledge gaps concerning age-related heart disease

Scientists' understanding of the cellular mechanisms involved in the development of heart disease was incomplete. For several years the PI had investigated possible causes of cardiomyopathy using the unique strain of genetically CM hamsters that develop a progressive hypertrophic form of cardiomyopathy that results in CHF and premature death. These animals provided a spontaneous reproducible model by which to study basic mechanisms in the development of cardiomyopathy and CHF. As heart disease typically affects older people, Dr Howlett started to look at the animals in three different stages: the young stage, where there is not much damage; the middle stage, where there is damage; and the older stage, where the heart starts to hypertrophy. The animals rarely lived to be two years old, and Howlett wanted to understand the cell mechanisms, properties and changes that were occurring at different disease stages as the animals aged.

In the CM hamster, it was thought that necrosis may have been caused by secondary intracellular calcium overload, although the mechanisms involved had not been identified. Cardiac necrosis can be prevented by treatment with calcium antagonists (Nosek et al., 1986). It was believed that calcium influx via voltage-sensitive calcium channels could be enhanced in cardiomyopathy, although this had never before been demonstrated.

16.3.3 Proximity to breeder

For the two years spanned by this grant, the research team purchased 300 CM hamsters annually from Canadian Hybrid Farms in Nova Scotia – a local breeder. The proximity of the research team to this breeder was definitely an advantage. Buying the hamsters elsewhere – for instance, from the United States, where there were similar breeders – would have greatly elevated the costs of the project.

16.4 Interface A – project specification and selection

The proposal to the HSFC was Dr Howlett's first application for a research grant in electrophysiology. The overall purpose of the proposed research was to determine whether slow inward current is increased in myocytes from CM hamsters and whether the arrhythmogenic transient inward current is more readily induced in these myocytes compared with controls. The team intended to investigate the passive and active membrane properties and the ionic currents in myocytes isolated from CM hearts.

The goals of these studies were to:

1. study the ionic currents and passive and active membrane properties of myocytes isolated from CM hamster hearts
2. study the modulation of slow inward current by the myocardial beta-adrenoceptor and angiotensin II receptor systems in cardiomyopathy
3. determine whether the arrhythmogenic transient inward current is more readily induced in cardiomyopathy.

These studies were conducted in cardiac myocytes isolated from normal and CM hamsters of three different ages: 30 days (pre-necrotic), 90 days (active necrosis) and 200 days (hypertrophic). These ages correspond to three very different stages in the development of hamster cardiomyopathy. Animals at 30 days display little, if any, evidence of myocardial necrosis and are at the pre-necrotic stage, where observations of changes in the cell membrane properties, which are important in the initiation of myocardial necrosis, were expected. At 90 days, the hearts show clear evidence of active necrosis and the animals have developed cardiomyopathy. It was predicted that changes seen at this age will reflect processes involved in active necrosis. At 200 days, the surviving myocytes would have hypertrophied and CHF is imminent. Alterations in cell membrane properties at this age were thought to reflect changes associated with hypertrophy and CHF. The results of these studies were meant to characterise the electrophysiologic properties of normal and CM heart cells at distinct stages in the development of the disease.

Specifically the research team:

- used current and voltage clamp methods to:
  - measure intracellular action potentials and determine at which stages in the development of cardiomyopathy action potentials increased
  - measure the ionic currents of slow inward current and delayed rectifier and determine whether changes in either or both underlie the increased action potential duration in cardiomyopathy
  - determine passive membrane properties
- proposed using voltage clamp in isolated myocytes to:
  - test the effects of isoproterenol on current–voltage curves for the slow inward current to compare activation of the myocardial beta-adrenoceptor system in CM cells

  - study activation and inactivation curves for slow inward current to compare the activation of the myocardial angiotensin II receptor system in normal and diseased cells

- determine whether transient inward current is more readily induced in CM hearts by investigating the transient inward current induced by:
  - the cardiac aglycone acetylstrophanthidin
  - high external calcium.

By using a voltage clamp to control the membrane voltage, the team observed ions moving across the membrane. In doing so, the team witnessed that the cells contract at much higher negative voltages than was commonly understood by, and accepted within, the scientific community. The team assumed that it was a result of the way they were doing their experiments: most people do research at room temperature, which is easier and less expensive, but Howlett and her team were conducting their experiments at body temperature (37°C), which has important implications on cell physiology. The effect of temperature on cellular processes is measured using the Q_{10} temperature coefficient, which compares the rate of a system at one temperature with the rate of a system at a temperature 10°C higher; thus if Q_{10} is 4, the rate of system X is four times faster when the temperature is increased by 10°C.

Many different channels exist in heart cells, which means that the rates slow down when the cells are cooled below body temperature but not necessarily at a uniform rate as some molecules are extremely sensitive to temperature changes. When heart cells are cooled down, they become loaded with calcium. Dr Howlett's study found that heart cells at room temperature were overloaded with calcium. Cells were not overloaded at body temperature, however, thus yielding more room to add more calcium and causing the cells' responses to calcium to get bigger.

At the time of the grant, the team was in the process of setting up the voltage clamp techniques. Patch clamp and voltage clamp are roughly the same; both involve putting an electrode on or in the cell, while keeping the voltage at specified levels. A protocol to control the voltage can be generated through software that creates any sort of desired waveform. The clamp allows an observer to see and measure the currents that are turned on in the cell at given voltages. The current may be carried by calcium, sodium or potassium channels, which move in different directions across the cell membrane. When the channels are turned on, they are called voltage-activated channels. The patch clamp technique is a refinement of the voltage clamp technique, in that it can record the currents of a single ion channel.

The review committee gave the grant proposal a high rating (3.6/4), stating that the application was 'concise, clear and well-written'. The review committee noted that one of the strengths of the proposal was that Dr Howlett seemed to have good collaboration and access to the required expertise and equipment via her colleague Dr Gregory Ferrier, who was well known for his work on cardiac electrophysiology and was also on the staff at Dalhousie University in the Department of Pharmacology. The committee noted some technical issues regarding the current clamp model and use of a membrane time constant, as the input resistance is indirect and more prone to error. It was also thought that pilot

experiments to determine the main currents underlying the action potential might be helpful to optimise the experiments. That said, the review committee thought that, 'the proposal [was] feasible and publishable data should be generated. It was viewed [as] a very good grant from a new investigator and high priority for funding [was] warranted' (Heart and Stroke Foundation of Canada, 1990). Howlett's recollection was that the idea of aging was not supported because the review committee did not think it was interesting to look at the disease in different stages of age.

Dr Howlett told us during her interview that some of the proposed experiments were not completed as suggested and that the project morphed to include additional aspects. This was, in part, because of the time lag between the application submission and receipt of the funding, which Howlett recalled to be about a year. Within that year, new research had emerged and different results were available in the literature. Howlett did not have pilot information, as desired by the review committee, because she was just starting her laboratory at the time (she had received her faculty appointment in 1989 and spent about a year applying for funding). This case study grant was one of the first peer-reviewed funded grants she was awarded. The direction of her project thus also changed, because what was thought to be feasible, in the absence of pilot data, was not. For instance, the hamster cells were found to have a different potassium transient outward current than expected and so the study scope had to be modified to account for this new finding. Howlett explained that she did the spirit of what was written in the proposal but measured the contractions in a different way.

16.5 Stage 1 – inputs to research

16.5.1 Funding

The team had applied for two years of funding, requesting about Can$55,000 per year, as described in Table 16-1.

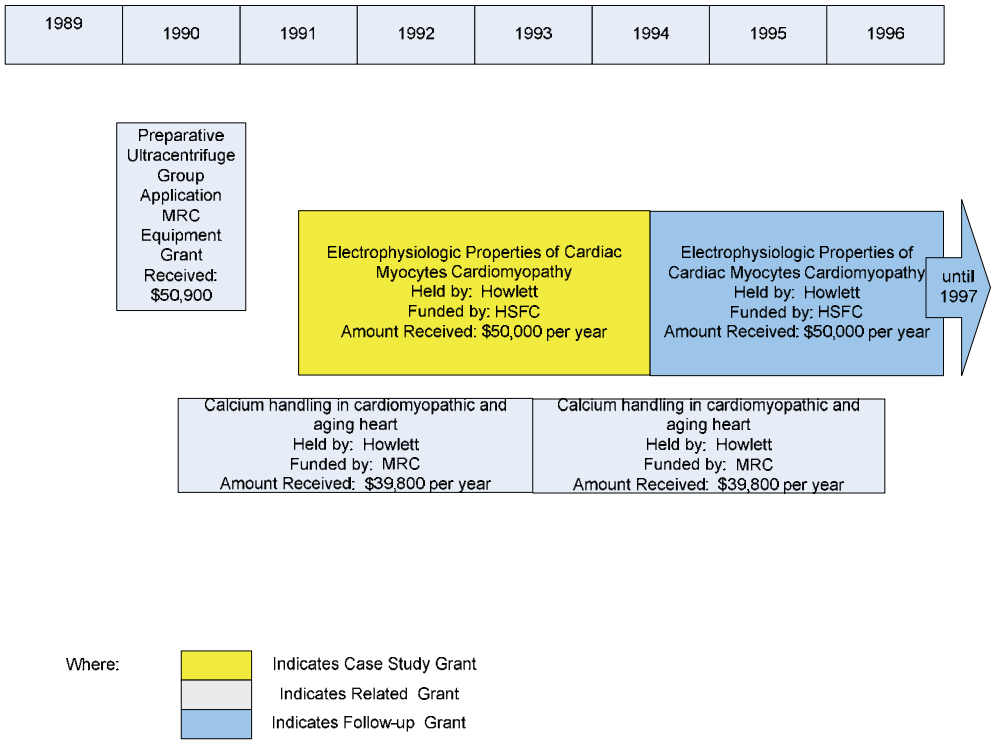
Table 16-1 Proposed budget for case study grant.

Summary	1991–1992 (Can$)	1992–1993 (Can$)
Technician (salary and benefits)	25,300	26,565
Equipment and computer software	2,760	–
Experimental animals (252 hamsters)	10,643	11,707
Materials and supplies	11,146	12,260
Other	5,100	5,100
Total	**54,949**	**55,632**

Howlett and her team were awarded slightly less than the amounts requested, receiving Can$50,000 per year. The team members reflected that money was tight and they had to consider the opportunity cost of buying new animals versus equipment. Computers were being introduced at the time, and although they were expensive, they were necessary for analysis. It was also noted in the application that the PI had participated in a group application to the Medical Research Council (MRC) in 1990, requesting Can$89,515 to purchase a preparative ultracentrifuge for the pharmacology department. The group was awarded Can$50,900. Howlett could not remember if additional funds were acquired

from elsewhere or if a less-expensive unit was purchased. Other money held in the laboratory at the time of this funding is described in Figure 16-1.

Figure 16-1 Peer-reviewed funding held by the PI from 1989 to 1996



Had this grant not been funded by the HSFC, Dr Howlett was not sure if it would have been funded by another organisation. She did think that it would have been difficult to get private funding for this research.

Dr Howlett also elaborated on the difficulties of being a woman doing scientific research in the early 1990s. She said that, because there were not many jobs available in Nova Scotia and she was perhaps not a good negotiator, she accepted a poor salary that was later readjusted after she pointed out that all the women received lower salaries than most of the men (there was one underpaid man at the time). She had her second child during the span of this grant. As a faculty member, Howlett was able to take maternity leave without risk to her position; however, she continued to work throughout her leave in order to keep producing and publishing, thereby maintaining good standing with the funding organisations. She pointed out that, 'there was nowhere on an application form to say that you've had a baby [to explain a reduction in productivity]!'

16.5.2 Collaborations

Dr Howlett had arranged to collaborate with Dr Roberto Orozco, medical doctor and pathologist, who worked in the Department of Pathology at Victoria General Hospital, Halifax. With Orozco's assistance, Howlett proposed to use Orozco's image analysis

system to measure the myocyte area. By the time of funding, however, Howlett had access to an edge detector system and so could measure the cell dimensions on her own. Measuring cell dimensions was important, because some diseases, such as cardiomyopathy, can enlarge cells. By measuring the cells and accounting for the growth that could be attributed to cardiomyopathy, the data could be normalised.

Howlett also mentioned the collaboration she had with Dr Ferrier, who worked with her in the Department of Pharmacology at Dalhousie University. Ferrier reviewed and provided helpful feedback on all of her grant applications. Ferrier had also written a letter that was submitted with the grant application, stating that Howlett could borrow the required equipment in the event that the equipment grant submitted to the MRC was unsuccessful.

16.5.3 Facilities

Dr Howlett remarked that the laboratory she was given was not optimal and was full of old outdated equipment, much of which was not of use. The team salvaged some equipment but had to throw most of it out.

Despite not having their own equipment, a major enabler for the success of this and future research was the ability to borrow what they needed from Dr Ferrier's laboratory, including access to a voltage clamp station. The team was also quite creative in adapting existing equipment to suit new needs.

Nicholl, the technician, recalled that the laboratory space was fairly cramped and that the situation got worse as more students came to work and study in the laboratory.

16.5.4 Research team

The research team included both basic and clinical scientists. Dr Howlett explained that her research team was very new. She had started conducting this research in 1989 without designated trained staff. The grant of interest to this case study was Dr Howlett's first research grant in electrophysiology, and she was just learning how to make the electrophysiology recordings at the time of this funding. Her previous work using the proposed model related to functional responses in intact tissue and binding studies. Howlett had spent several months during her postdoctoral fellowship at the University of Alberta in Edmonton learning how to use the patch clamp from Norman Stockbridge and Joy Steele. From 1986 to 1990, Howlett was involved with eight publications and had several others submitted and in preparation, of which most were related to the case study grant. The review committee wrote that Howlett's previous work suggested experience and competence to carry out the proposed experiments (Heart and Stroke Foundation of Canada, 1990).

In 1990, Peter Nicholl joined the laboratory and worked as Dr Howlett's only technician. Nicholl explained that he had no previous electrophysiology training. His previous experience was in a laboratory where he worked on plants and fish. He was given hands-on training by Howlett. In 1993, Howlett also had a student in her laboratory, named John Sapp, who was working for his bachelor of science in medicine. He said, 'I became very interested in cardiology and I was looking for an opportunity to learn more about cardiac electrophysiology research. I asked around and found out that the very best person to work with was Susan Howlett' (John Sapp interview, 2008).

Sapp recalled that the laboratory consisted of interested, hard-working graduate students. Sapp also recalled that there was great collaboration between Howlett and Ferrier's laboratories. The two laboratories communicated through weekly meetings, during which they would brainstorm and troubleshoot their problems.

16.6 Stage 3 – primary outputs from research

The team was ultimately looking at the size of the current in the hamster myocyte cells, hypothesising that changes in the calcium current might lead to calcium overload. They found that the contractions were suppressed because of an absent voltage-sensitive release mechanism (VSRM). This finding was very controversial at the time and really 'shook up the field' (Howlett interview, 2008). There were two main reasons for the controversy. Firstly, most other researchers do their studies at room temperature, at which this mechanism is inhibited, and so very few had observed the VSRM behave in this way. Howlett was able to obtain her results because she worked at higher temperatures to mimic what was actually happening in the body. Secondly, many other researchers use an electrophysiology recording device in which a solution is injected into the cells; this dilutes the other molecules within the cell, again inhibiting the VSRM. Few people replicated the results Howlett was observing, although she claims few people worked under the same conditions.

These findings were eventually published, after 2–3 years and about 14 iterations, in the *Journal of Physiology* in 1995 (35 citations) (Ferrier and Howlett, 1995). Dr Howlett recalled that the team had to keep adding data to the paper before the journal would publish it. Howlett and Dr Ferrier have been acknowledged as conducting the pioneering studies and making the first observations on the VSRM effect on suppressing contractions (Hobai and Levi, 1999).

The team now believes that the contractions are suppressed because of a defective phosphorylation pathway inside the cells (phosphorylation is the addition of a phosphate (PO_4) group to a protein or other organic molecule). This is explained further in the following section.

16.6.1 Knowledge production

The PI identified 14 papers as being directly related to the case study grant. A selection of them will be discussed in an attempt to give a sense of the knowledge produced via the case study funding. The following papers will be described:

1. Li, G.R., G.R. Ferrier and S.E. Howlett, 'Calcium Currents in Ventricular Myocytes of Prehypertrophic Cardiomyopathic Hamsters', *American Journal of Physiology*, Vol. 268, 1995, pp. H999–H1005.
2. Howlett, S.E., J.Q. Zhu and G.R. Ferrier, 'Contribution of a Voltage-Sensitive Calcium Release Mechanism to Contraction in Cardiac Ventricular Myocytes', *American Journal of Physiology*, Vol. 274, 1998, pp. H155–H170.

3. Howlett, S.E., W. Xiong, C.L. Mapplebeck and G.R. Ferrier, 'Role of Voltage-Sensitive Release Mechanism in Depression of Cardiac Contraction in Myopathic Hamsters', *American Journal of Physiology*, Vol. 277, 1999, pp. H1690-H1700.
4. Ferrier, G.R. and S.E. Howlett, 'Cardiac Excitation–Contraction Howlett Coupling: Role of Membrane Potential in Regulation of Contraction', *American Journal of Physiology*, Vol. 280, 2001, pp. H1928–H1944.
5. Xiong, W., H.M. Moore, S.E. Howlett and G.R. Ferrier, 'In Contrast to Forskolin and 3-Isobutyl-1methyxanthine, Amrinone Stimulates the Cardiac Voltage-Sensitive Release Mechanism Without Increasing Calcium-Induced Calcium Release', *Journal of Pharmacology and Experimental Therapeutics*, Vol. 298, 2001, pp. 954–963.
6. Ferrier, G.R. and S.E. Howlett, 'Differential Effects of Phosphodiesterase Sensitive and Resistant Analogues of cAMP on Initiation of Contraction in Cardiac Ventricular Myocytes', *Journal of Pharmacology and Experimental Therapeutics*, Vol. 306, 2003, pp. 166–178.
7. Xiong, W., G.R. Ferrier and S.E. Howlett, 'Diminished Inotropic Response to Amrinone in Ventricular Myocytes from Myopathic Hamsters is Linked to Depression of High Gain Ca^{2+}-Induced Ca^{2+} Release', *Journal of Pharmacology and Experimental Therapeutics,* Vol. 310, 2004, pp. 761–763.

The first publication above describes the findings when changes in calcium ion currents (ICa), which were believed to contribute to calcium ion overload in young (70- to 100-day-old) CM hamster hearts, were measured in isolated ventricular myocytes with conventional recording and discontinuous single-electrode voltage-clamp techniques (Li, Ferrier and Howlett, 1995). The team found that action potential duration (APD) at 90 percent repolarisation (APD_{90}) was significantly longer in CM myocytes compared with normal cells (APD_{90} 120.3±4.5ms vs. 98.2±5.9ms, $p<0.01$). Input resistance constant and membrane capacitance were observed to be similar in normal and CM myocytes. Current–voltage (I–V) relations for peak ICa were found to be depressed in CM cells compared with normal cells; this difference was statistically significant at the peak of the I–V curve. Activation and inactivation relations for ICa and recovery from inactivation were found to be similar in myocytes from normal and CM hearts, indicating that these changes occurred in myocytes from young CM animals before development of heart failure. Results indicated that increased influx of calcium ions through L-type calcium channels does not account for the longer APD in cardiomyopathy and is not involved in the development of calcium overload in cardiomyopathy.

The second paper mentioned above describes the results of a study with the following three objectives: 1) to determine whether the VSRM exhibits steady-state inactivation and the voltage range over which inactivation occurs, 2) to determine and compare the effects of protocols designed to alter sarcoplasmic reticulum (SR) calcium load on components of contraction initiated by the VSRM or by L-type calcium ion currents and 3) to evaluate the role of the VSRM in restitution (Howlett, Zhu and Ferrier, 1998). The team's observations demonstrated that a component of contraction, which they have called a VSRM, plays an important part in excitation–contraction coupling in the heart. They

concluded that when the VSRM is inactivated, the maximum magnitude of contraction is reduced and contraction is weak at membrane potentials corresponding to the peak and plateau phases of the ventricular action potential. However, when the VSRM is available for activation, the magnitude of contraction is greater and becomes independent of voltage at potentials positive to approximately 20mV. The VSRM then operates as a trigger mechanism, which initiates maximal contractions over the entire range of potentials corresponding to the overshoot, initial repolarisation and plateau of the action potential. The VSRM also plays an important role in determining restitution of contraction following a previous activation and is very sensitive to manipulations that alter SR calcium loading. L-type calcium ion currents activate a component of contraction that is less sensitive than the VSRM to loading of SR stores and also plays an important role in loading SR calcium stores. The team's observations thus supported their hypothesis that the VSRM is a major link that couples cardiac contraction to membrane potential, activation interval and SR calcium loading.

The next paper, published in 1999, outlines the findings of a study in which the team investigated excitation–contraction (EC) coupling in isolated ventricular myocytes from prehypertrophic CM hamster hearts (Howlett et al., 1999). Conventional and voltage-clamp recordings were made with high-resistance microelectrodes, and cell shortening was measured with a video-edge detector at 37°C. Contractions were depressed in myocytes from CM hearts, whether they were initiated by action potentials or voltage-clamp steps. As the team had previously indicated in guinea pig and rat studies (Ferrier and Howlett, 1995), contraction in hamster myocytes could be triggered by a VSRM or by calcium ion-induced calcium release (CICR). Selective activation of these mechanisms demonstrated that the defect in EC coupling was primarily caused by a defect in the VSRM. However, activation and inactivation properties of the VSRM were not altered. When the VSRM was inhibited, the remaining contractions induced by CICR exhibited identical bell-shaped contraction voltage relations in normal and CM myocytes. Inward calcium ion current was unchanged. The team thus concluded that a defect in the VSRM component of EC coupling precedes the development of hypertrophy and failure in CM hamster hearts. This conclusion raised the possibility that defects in the VSRM might contribute to the development of cardiac hypertrophy and/or heart failure.

By 2001, there was increasing evidence that the VSRM and CICR represent two different mechanisms for EC coupling in the heart, both of which were believed to contribute to initiation of cardiac contraction. These two mechanisms have distinct electrophysiological and pharmacological characteristics that allow them to be distinguished from each other in the same cell. Due to the mutually exclusive nature of many of these characteristics, the VSRM and CICR cannot be explained by a single mechanism. In the fourth paper listed above, the team reported that VSRM and CICR are two independent mechanisms for release of SR calcium ions, yet concluded that it is unclear whether the VSRM operates a separate population of ryanodine receptors or opens the same population of release channels as CICR (Ferrier and Howlett, 2001). The functional characteristics of the VSRM resemble characteristics associated with calcium ion release coupled with gating charge in skeletal muscle. The team therefore suggested a physical link between a channel moiety, functioning as a voltage sensor in the sarcolemma, and the calcium ion release channel in the SR. The molecular identity of the VSRM was not yet known, and the team

believed that other mechanisms should be considered. Phosphorylation was known to play a major role in activation of the VSRM and was thought to allow the VSRM to serve as a major regulatory mechanism for cardiac contraction in normal heart. Phosphorylation sites for protein kinase A and calmodulin-dependent kinase were thought to be located on one or more components of the VSRM, although it was speculative to suggest what sites would regulate the VSRM. It was thought that if the VSRM and CICR are separate parallel mechanisms, this would allow for separate alterations of either mechanism in disease states. The VSRM plays a major role in contractile dysfunction in several models of heart failure and may contribute to impaired contractile performance in heart disease. As the team's information about the VSRM accumulated, it was becoming clear that this important trigger for cardiac contraction plays a central role in regulation of cardiac contraction in normal and diseased myocardium.

The fifth paper outlines the results of a study to determine whether the VSRM can be stimulated independently from the CICR by drugs that elevate intracellular cAMP (Xiong et al., 2001). Contractions were measured in voltage-clamped guinea pig ventricular myocytes at 37°C. The team compared the effects of agents that elevate levels of cAMP through activation of adenylyl cyclase (AC; 1 μM forskolin), non-specific inhibition of phosphodiesterases (PDEs) (100μM 3-isobutyl-1-methylxanthine (IBMX)) and selective inhibition of PDE3 (100–500μM amrinone) on contractions initiated by the VSRM and CICR. The team found that forskolin and IBMX significantly increased peak calcium ion current and CICR. In addition, these agents also markedly increased contractions elicited by test steps from –65mV to –40mV, which activate the VSRM. However, because these steps also induced inward current in the presence of forskolin or IBMX, CICR could not be excluded. In contrast, amrinone caused a large, concentration-dependent increase in VSRM contractions but had no effect on CICR contractions or calcium ion current. Sarcolemma calcium ion load, as assessed by rapid application of caffeine (10mM), was increased only modestly by all three drugs. Normalisation of contractions to caffeine contractures indicated that amrinone increased fractional release by the VSRM but not CICR. Forskolin and IBMX increased fractional release elicited by steps to –40mV. Increases in CICR induced by forskolin and IBMX were proportional to caffeine contractures. Thus, positive inotropic effects of cAMP on VSRM contractions may be compartmentalised separately from effects on calcium ion current and CICR.

These results suggested that the VSRM may serve as a major regulatory site for cardiac contraction by the AC–protein kinase A (PKA) pathway. Furthermore, the VSRM can be regulated independently of CICR and independently of effects on L-type calcium ion current. Thus, this study provided evidence that the AC–PKA cascade has divergent paths that regulate the CICR and VSRM separately. These results also indicated that stimulation of the VSRM by the AC–PKA pathway results in release of a greater fraction of SR calcium ions. Furthermore, the phosphorylation site(s) for the VSRM are regulated by PDE3, and local changes in cAMP caused by inhibition of PDE3 may affect the VSRM without affecting CICR or L-type calcium ion current. In contrast, most of the inotropic effect of forskolin and IBMX on CICR could be accounted for on the basis of increased SR load under the conditions of this study. The team concluded that pharmacological agents that affect the AC–PKA cascade at different points may have very different effects on cardiac EC coupling.

The team next studied the effects of different phosphodiesterase (PDE)-resistant and PDE-sensitive analogues of cAMP on CICR and investigated whether cAMP sensitises CICR so that small currents induce large contractions (Ferrier and Howlett, 2003). Experiments were conducted in voltage-clamped guinea pig ventricular myocytes at 37°C, with different analogues of cAMP added to patch pipette solutions. This study demonstrated that intracellular dialysis with cAMP can result in large contractions and calcium ion transients occurring with little inward current. This occurs only if degradation of cAMP is restricted by use of PDE-resistant analogues or by treating the cells with PDE inhibitors. The team's results indicated that PDE could serve an important regulatory role, modulating cardiac contraction by altering the relationship between membrane potential and calcium ion release.

In 2004, the team investigated whether 100–1000 μM amrinone, a PDE3 inhibitor, can alleviate depression of contractions in ventricular myocytes from prefailure CM hamsters (80–100 days) (Xiong, Ferrier and Howlett, 2004). Cell shortening and ion currents were measured in voltage-clamped cells at 37°C. The team's results demonstrated that amrinone selectively enhances high-gain CICR in normal hamster myocytes, but this agent is not able to restore high-gain CICR, which is depressed in myocytes from CM hamsters, except in the presence of forskolin, which stimulates AC. Furthermore, the positive inotropic effect of amrinone in normal myocytes cannot be attributed to effects on L-type calcium ion current, which is inhibited in hamster myocytes by this drug. However, it was thought that the positive inotropic effect could reflect increased SR calcium ion stores because increased SR stores accompanied the positive inotropic effect in normal myocytes but were absent in CM myocytes. Interestingly, loss in sensitivity to PDE3 inhibitors had been observed in end-stage heart failure in humans (Feldman et al., 1987, and Bohm et al., 1988). Because the loss of sensitivity to PDE3 inhibitors in CM hamster myocytes occurred prior to the onset of heart failure, the results suggest that this defect may contribute to the development of heart failure in these animals.

Bibliometric analysis was conducted on all 14 publications identified by the PI as directly related to the case study grant. Of the 14 articles, 13 were included in the citation analysis. The one excluded article was not indexed in the Web of Science and thus could not be included in the citation analysis.

Table 16-2 Publication output and impact[1]

Number of journal articles:	14
Number of articles included in citation analysis:	13
Total number of citations (all papers):	314
Aggregate relative citation impact:	1.06 (Class III)
Self-citations:	29%

Distribution of publications across relative citation impact classes:

	Class I (uncited)	Class II (>0 and < 0.8 citations)	Class III (0.8 to 1.2 citations)	Class IV (1.2 to 2.0 citations)	Class V (> 2.0 citations)
Number of publications	2	6	1	1	3
Proportion of total output	15%	46%	8%	8%	23%

Most highly cited publication[2]:	Hobai, I.A., F.C. Howarth, V.K. Pabbathi, G.R. Dalton, J.C. Hancox, J.Q. Zhu, S.E. Howlett, G.R. Ferrier and A.J. Levi, 'Voltage-Activated Ca Release in Rabbit, Rat and Guinea Pig Cardiac Myocytes and Modulation by Internal cAMP', *Pflügers Archiv*, Vol. 435, 1997, pp. 164–173
Times cited:	63

16.6.2 Dissemination

Dr Howlett explained that her primary mode of dissemination was via published papers, commonly in the *American Journal of Physiology*. She claimed that of her work, her controversial findings seem to receive the most citations. She still tells her students that her best papers were rejected by at least two publishers before eventually being accepted.

Dr Howett claimed that she would have had to attend various meetings to have had a larger impact. However, in the early 1990s, during the case study grant funding, she was the mother of two small children and thus did not travel to many conferences. Howlett did present findings related to the case study grant at an invited seminar at the annual meeting of the British Pharmacology Society in Bath, England, in July 1996 and in a keynote address called 'A New Trigger for Contraction in Heart' to the University of Alberta, University of Calgary Joint Symposium on Ion Channels in May 1998. She and members of the team also presented many poster presentations locally, nationally and internationally. These conferences would have been attended by both researchers and clinicians.

As another mode of dissemination, Dr Howlett started conducting high-school tours through her laboratory in 1994. She has presented to younger students, bringing her microscope and samples to the classroom and talking about careers in science. She also

[1] In addition, two publications were indirectly linked to this grant. These publications were all indexed in Web of Science and received 21 citations in total, giving a relative citation impact of 0.33. Both were in relative citation impact Class II and the self citation rate was 19 percent.

[2] Citation count extracted April 2009.

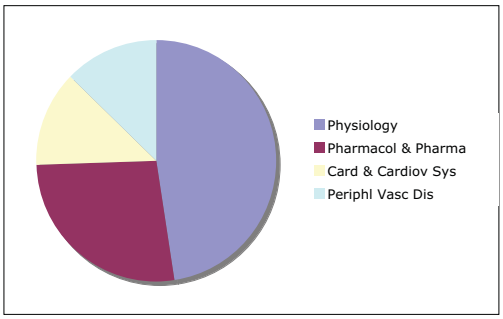
works with local high schools to allow students to spend a day job shadowing her. Fundraisers from Dalhousie Medical Research Foundation also recently toured Howlett's laboratory.

Dr Howlett began interacting with the media in 2002 and continues to use the media as a tool to communicate her research findings. This work also contributed to a book chapter, as seen in the *Proceedings of the World Heart Congress* (Howlett and Ferrier, 2003). In addition, she co-authored a book chapter focused on the technique in 1997 with Dr Ferrier (Ferrier and Howlett, 1997).

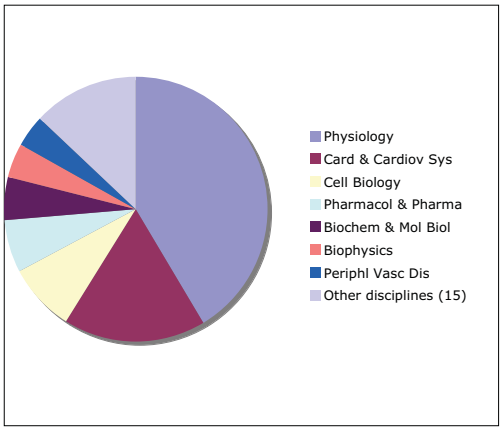
The bibliometric analysis also investigated knowledge diffusion. It was found that Howlett and her team most commonly publish in the area of physiology, followed by pharmacology and pharmaceuticals. Their work is most commonly cited by those working in cardiology and cardiovascular systems in the United States and United Kingdom.

Figure 16-2 Knowledge diffusion: fields of research of output (a), fields of citing papers (b) and countries of citing papers (c)

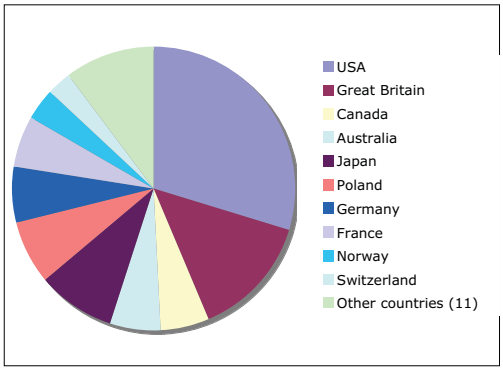
(a)



(b)



(c)



16.6.3 Training and capacity building

Howlett said that the research from this grant still influences everything she currently does. Prior to this funding from the HSFC, she had not led any studies in electrophysiology. However, since receiving the initial grant, she has maintained continuous funding to

pursue this line of inquiry. Her current work, funded by both the Canadian Institutes of Health Research (CIHR) and the HSFC, is directed at three major themes:

1. investigation of changes in EC coupling that occur with age in intact cardiac ventricular myocytes
2. exploration of the mechanisms whereby aging may exacerbate deleterious effects of ischaemia and reperfusion
3. evaluation of the impact of ischaemic preconditioning on calcium cycling in a cellular model of ischaemia and reperfusion.

Moving from CM hamsters, Howlett's studies are now conducted in isolated ventricular myocytes from guinea pig, mouse and rat hearts. Transmembrane currents are measured with voltage clamp and patch clamp techniques (skills she developed through the case study grant); cardiac contraction is measured as cell shortening through a video-edge detector. Howlett continues to measure intracellular calcium levels, although she now measures them as calcium transients in cells loaded with calcium-sensitive dyes (eg Fura-2).

Dr Howlett's work is now aimed at contributing new information about the mechanisms of contractile dysfunction in the aged heart and will help explain why elderly people are more susceptible to the deleterious effects of myocardial ischaemia, which is closely related to her work in the early 1990s. In addition, the results of these studies are expected to enhance overall understanding of the cellular mechanisms that underlie ischaemic preconditioning in the heart and, in the long term, should help develop treatment strategies that mimic the beneficial effects of ischaemic preconditioning in ischaemic heart. Howlett is a recent recipient (2004–2005) of the CIHR Mid-Career Salary Award for Aging Research and continues to organise laboratories and teach within both pharmacology and physiology studies.

The papers Dr Howlett published with John Sapp were quite instrumental in his career (Sapp interview, 2008). He is now an electrophysiological cardiologist and associate professor of Cardiology at the Queen Elizabeth II Health Sciences Centre, and continues to do research. He said that his time and training in Howlett's laboratory gave him a foundation in basic research and animal research that he had not previously been exposed to. When doing a fellowship in cardiac electrophysiology in Boston, he set up his own animal laboratory using the methods and animal research skills that came from his experiences with Howlett.

In working with Dr Howlett, Sapp described how he was taught the value of true collaboration. This, he said, was learned by observing the synergy between Howlett and Ferrier, who had the laboratory next door. He added, 'they had a very dynamic and excellent collaboration and a lot of ideas went back and forth. The lab meetings were very interesting…That collaboration was inspirational. That example is why I now have a similar collaboration with a colleague. There's no ego in the way, it's simply about finding new knowledge and getting good work done'. He referred to Howlett as an amazing mentor who always had or made time for her students. He reflected that she was approachable, bright and very insightful.

He finished by stating, 'I can't give you a meaningful assessment about the impact of the research itself. I can give you a personal account about how influential it was for me. My

time in Howlett's lab gave me a research foundation…It was foundational and inspirational. It's part of how I got into a high-level fellowship that I did and has allowed me to undertake novel research work as a fellow and now applies to my clinical practice. My work truly has been bench to bedside. The methodological and scientific curiosity approach that I learned from Susan is a huge part of that' (Sapp interview, 2009).

Dr Howlett claimed that the case study grant contributed to her ability to recruit numerous graduate students, of which she proudly reports that all of her doctor of philosophy students still work in science and continue to do research. She claimed that this research did feed into the training of her students. The HSFC grant allowed Howlett to hire a technician and expand her research group. Howlett believes the HSFC funding helped create a positive reputation for her laboratory and helped her to grow her operation and to obtain subsequent funds. Peter Nicholl continues to work as Howlett's full-time technician.

16.6.4 Benefits to future research and research use

The results of this study enhanced understanding of the events that occur at the cellular level and evaluated the calcium overload hypothesis. This body of work over time has helped people to think differently about how cells connect electrical excitation with their physical contraction, how it is modulated and what connections the cell uses to translate an electrical signal into a mechanical action.

The published findings that stated that contractions were suppressed because of an absent VSRM were very controversial. Although some members of the scientific community came on side, most disputed the team's findings. Howlett continues to work in the field of excitation contraction coupling. In 2001, the team published a paper stating that the VSRM 'is strongly modulated by phosphorylation and provides a new regulatory mechanism for cardiac contraction. The VSRM is depressed in heart failure and may play an important role in contractile dysfunction' (Ferrier and Howlett, 2001). These experiments were initially about the currents in the membrane. Now, with advances in technology, Howlett is able to measure contractions and calcium levels. She can also measure individual calcium release events called 'calcium sparks'[3], which were discovered in the late 1990s.

The work conducted via the HSFC funding has also led to international collaborations involving a British group (Hobai et al., 1997) and researchers from Norway (Louch et al., 2005). Howlett also mentioned that a group in Poland, led by Bohdan Lewartowski, replicated their work on the VSRM.

With respect to the aging component of these studies, Dr Howlett says she abandoned the idea for years because she did not gain support and says she 'didn't want to commit career suicide' (Howlett interview, 2008). However, aging and heart disease has remained an

[3] In full contraction in a beating heart cell, there is a coordinated release of calcium from stores within the cell. Anything that interrupts this coordinated release is problematic, because it can lead to extra beats or poor relaxation of the heart, among other things. If a calcium current is fully activated, it will cause many smaller releases to fire off simultaneously, resulting in a big release. The big releases of calcium are made up of smaller releases or 'sparks'. Changes in these smaller events, the sparks, such as increases or decreases in frequency, are indicative of an area in a cell that might change with, for example, age or disease.

interest of hers. In her view, 'looking at heart disease in young and adolescent animals is akin to looking for your keys over where the light is good even though you dropped them elsewhere' (Howlett interview, 2008). She has recently become very active in the field of aging and heart disease; having received funding from the CIHR Institute of Aging and the HSFC, along with other grant applications that have been submitted. She is also broadening her research to include the affect of age and gender. Very little research has been done in this area, as investigators typically work with male animals because it removes any menstrual cycle fluctuations, a variable which can complicate things.

Dr Howlett finished her interview by commenting on the difficulty of drawing a line between what she was doing in 1990 and now because it is all interwoven and data obtained in one project cannot help but influence your thoughts on all of your projects.

16.7 Stage 4 – secondary outputs

This research supported by the HSFC grant has not to date informed policies, device or drug development.

16.8 Stage 5 – adoption by practice and the public

The basic research supported by the HSFC grant has not been taken up by the health service, doctors or public health officials.

16.9 Stage 6 – broad health and economic outcomes

This research has not led to a broad effect on society through improved health in the population, spin-off companies or employment opportunities, other than those who were working in the laboratory at the time.

16.10 Payback

The payback framework describes the process from research to several categories of impact. The categories allow us to compare across different case studies. Table 16-3 shows, in point form and by impact category, some of the impacts, described more fully above, that have emerged from this grant.

Table 16-3 Payback

Payback category	Impacts from case study
Knowledge production	• 14 related-peer reviewed articles • Keynote and invited speaker, poster presentations at various meetings
Research targeting and capacity building	• Knowledge transfer within laboratory to students and postdoctoral researchers • Techniques taught to students and new technician • Positive influence on PI, as a new researcher and her ability to recruit, obtain subsequent funding and generate new collaborations
Informing policy and product development	• Not applicable
Health and health sector benefits	• Not applicable
Broader social and economic benefits	• Not applicable

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CHAPTER 17 To study the effects of angiotensin-converting enzyme inhibitors on tissue and cardiac angiotensin-converting enzyme

17.1 Overview of case study

This case study examines the evolution and impacts of a National Heart Foundation of Australia (NHFA)-funded basic research grant 'Effects of angiotensin converting enzyme inhibitors on tissue and cardiac angiotensin converting enzyme', awarded to Dr Bruce Jackson – the principal investigator (PI) – for the period 1988 to 1989 (grant reference 2317).

With the prevention of chronic heart failure in mind, this project sought to better understand the characteristics of an enzyme called angiotensin-converting enzyme (ACE) in relation to blood pressure control. Following this, the study sought to observe the effects of a pharmaceutical that inhibited this enzyme. This project provided, for the first time, a direct assessment of the cardiac tissue effects of ACE inhibitors. Factors contributing to differences in tissue effect were explored and led to a rationale for development of specific therapeutic approaches to blood pressure control.

The project had three key aims: to examine the in-vivo effects of ACE inhibitors on ACE in different tissues to examine the factors contributing to differential tissue effects of ACE inhibitors in vivo and to examine the characteristics of ACE in the heart tissue specifically and how it related to other molecular signalling systems. The project built on work previously undertaken by Dr Jackson's laboratory.

The research study found that concentrations of ACE were not identical in all tissues and that ACE in individual tissues is under tissue-specific controls. In particular, it showed that tissue ACE and its involvement with the renin–angiotensin–aldosterone system (RAAS) in the heart could be manipulated independently of effects on circulating substances of the RAAS.

17.2 Introduction to case study grant

17.2.1 Scientific background

The RAAS is a hormone system responsible for regulating blood pressure and water–fluid balance via a series of reactions that occur in the bloodstream. It is a complex system that has clinical associations with hypertension, atherosclerosis, heart failure, stroke and kidney disease, thus affording wide-ranging options and opportunities for interested researchers. When blood pressure falls, the kidneys release the enzyme renin into the bloodstream. Renin splits angiotensinogen (a large protein circulating in the blood) into two pieces. One of these pieces is called angiotensin I. Angiotensin I, which is relatively inactive, is split into angiotensin II by ACE. Angiotensin II is very active and acts as a hormone, causing blood vessel constriction and increasing blood pressure.

Angiotensin II also triggers the release of aldosterone from the adrenal cortex. This hormone increases reabsorption of sodium and water by the kidneys, which increases blood volume and therefore further increases blood pressure. Proper regulation of the RAAS has important implications for blood pressure – if the system is too active, blood pressure will be too high and hypertension results.

The enzyme ACE, which converts angiotensin I into angiotensin II, is found in many body tissues, including the kidneys, blood vessel walls, brain and lungs. ACE is also involved in the inactivation of a second signalling molecule bradykinin (a potent vasodilator).

Restricting the action of ACE is, therefore, an ideal treatment target for a number of conditions, including high blood pressure and heart failure. ACE inhibitors are just one family of pharmaceuticals that are used in the treatment of hypertension and congestive heart failure and, in some cases, are the drug of choice. ACE inhibitors are often combined with diuretics when an ACE inhibitor alone proves insufficient to control hypertension.

At the time of the grant application, ACE inhibition was already being used in the clinical treatment of hypertension, heart failure and renal failure (a further consequence of high blood pressure), with Dr Jackson himself publishing in these areas.

Studies had been conducted, using various techniques, to identify where in the body ACE was located. These techniques included antibody labelling, labelling with radioactive substrates or inhibitors, and testing for ACE activity in tissue extracts. Different research groups had shown ACE to be present in high concentrations in several parts of the body specifically: the walls of blood vessels (Caldwell et al., 1976, and Soffer, 1976), the kidneys (Ward et al., 1976), the wall of the gut (Ward et al., 1980), discrete nuclei in the brain (Strittmatter et al., 1984) and the reproductive tract (Strittmatter and Snyder, 1984). According to Dr Jackson, these groups tended to measure ACE activity using a synthetic substrate (based on a amino-acid trimer of sequence Hip-His-Leu) to demonstrate enzyme activity and to measure the distribution of ACE by labelling it with antibodies. This process, together with immunological characterisation, Jackson believed, led to the general conclusion that ACE is identical in all tissues.

However, evidence from a number of studies using several approaches was emerging to suggest otherwise. This growing body of evidence included work from Dr Jackson's laboratory, and Dr Jackson referenced particular work conducted by Sakharov et al.

(1987), which had shown that ACE from the heart exists in several different forms. The sum of this work and observations made suggested to Dr Jackson that ACE existed in tissue-specific forms and was under tissue-specific controls.

At the time it was, according to the grant application, well recognised that the tissue RAAS was important to cardiovascular regulation. Work conducted by the group had shown that the relationship between changes in the circulating RAAS and the cardiovascular response was inconstant following acute or chronic ACE inhibition therapy (Johnston et al., 1984; Jackson, Cubela and Johnston, 1984; and Jackson and Johnston, 1984). The grant sought to examine the possibility that the tissue RAAS, in particular ACE in the heart, could be manipulated independently of effects on the circulating RAAS.

17.2.2 PI's background

Dr Jackson was both a biomedical researcher and a clinician, and although initially interested in nephrology, his experience with the RAAS saw a natural diversification to include the cardiovascular field. Jackson notes that he was able to get in on the early research work on the RAAS in the mid 1970s, moving on to the bench work and further through to clinical application.

Dr Jackson started his academic career as an engineering student but switched in his first year to science before finally settling on medicine. While at the Royal Melbourne Hospital he worked under Professor Kincaid-Smith, a leading figure in the renal world who was perhaps most well known for highlighting the misuse of analgesics and demonstrating a link with kidney damage,[1] where his interest in pathology started. After completing his medical training, he became a nephrologist and went to the United States on a scholarship, where he gained a strong introduction to the RAAS. On his return to Australia, he went into research on a National Health and Medical Research Council (NHMRC) research fellowship in the early 1980s, and his career progressed to the role of Associate Professor and most recently Clinical Dean at Casey Hospital, Victoria.

Initially based at the Baker Institute and Prince Henry's Hospital in Victoria, Dr Jackson and his team moved to the Austin Hospital, which is affiliated with the University of Melbourne. Austin Hospital is, and was, a major public teaching hospital in Victoria, Australia. It was while based here as an NHMRC-funded senior research fellow that he submitted this grant application. During this period, Jackson was primarily a biomedical researcher with a clinical interest, supporting himself and a research group with various grants including from the NHF. In his 10-year period as an NHMRC research fellow and senior research fellow, Jackson's competitive research funding grants totalled Aus$1,284,223; this was in addition to industry funding.

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[1] Professor Priscilla Kincaid-Smith demonstrated the link between kidney damage and the overuse of analgesics in the form of the aspirin–phenacetin–caffeine headache powders that were very popular in Australia at the time. She was also very involved in setting up the renal transplant programme at the Royal Melbourne Hospital and has numerous recognitions, including Commander of the Order of the British Empire (1975) and President of the Royal Australasian College of Physicians (1986–1988); in 1989, she received the David Hume Award from the National Kidney Foundation (USA) and became a Companion of the Order of Australia. Dr Jackson's thesis for his bachelor of medical science degree was on analgesic nephropathy, with Kincaid-Smith as supervisor.

At the time of this particular grant, Dr Jackson's emphasis was more that of biomedical researcher with clinical interests (he was running other clinical research projects at the same time as undertaking this basic research project). Over time, this has shifted to clinician with biomedical research interests and involvement in a number of clinical research trials. He was able to collaborate and experience cross-pollination with basic scientists.

17.2.3 The case study approach

The case study based on this research grant involved a combination of: a review of documentation for the grant; one face-to-face interview with the PI on the project; a review of the PI's curriculum vitae; and documentary analysis of key citing papers, publications and conference abstracts arising from it.

17.3 Stage 0 – topic/issue identification

This research built directly on Dr Jackson's previous research experience and, in his own words, 'It seemed like a good idea at the time!' (Jackson interview, 2008). Jackson could see a potential knowledge yield, and potential clinical applications were also an important driver to the identification of this research issue. Finally, the institutional environment provided intellectual stimulation for Jackson. These points are discussed further below.

17.3.1 Previous research experience and proven track record in the research topic

At the time of the grant application, Dr Jackson was well established in his field of choice; the application referenced 24 publications he had authored in refereed journals that were directly relevant to the proposed research and a further 17 other major references in the five years prior to application. Indeed, this background was commented on by the assessor: 'this…application…represents an extension of previous work in which the applicant has achieved significant experience and significant results' (Grant-in-Aid Assessor Report, 1987) and, according to the Committee Chairman's report, Jackson presented the case very well at interview.

Dr Jackson's proven track record in securing funding was also a key factor. At the time of the grant application he was the recipient of four research grants, including two from the NHMRC, one from the National Heart Foundation of Australia and one from the Australian Kidney Foundation (AKF), as shown in Table 17-1. The grant application also makes it very clear that this project would examine 'areas separate to those covered by the currently held NHMRC project grant titled "Angiotensin-Converting Enzyme: Studies by Radioinhibitor Binding"'.

Table 17-1 Grants held by Dr Jackson at time of application

Grant title	Period	Funder
Angiotensin-Converting Enzyme Studies by Radioinhibitor Binding	1987–1989	NHMRC
Antihypertensive Drug Modulation of Renal Injury in Renal Failure Models	1987 –1989	NHMRC (included Senior Research Fellowship for Dr Jackson)
Atrial Natriuretic Peptide: Studies in and Effect on Progression of Renal Failure	1987–1988	NHF
Renin–Angiotensin System and Human Diabetic Renal Disease	1987	AKF

Dr Jackson himself attributes his background to the success of the application: 'I think it's probably a reasonable observation that very few of the National Health and Medical Research Council or [National] Heart Foundation [of Australia]-funded projects are first applications, first success funding. People applying for these things usually come with a heritage of baggage, whatever you like to call it, which is judged. And the prospect of productivity, bang for your buck, is one of the key things for the very small number of dollars available here' (Jackson interview, 2008).

17.3.2 Curiosity and potential for knowledge yield

Dr Jackson had a strong background in the RAAS, and although he had 'no burning ambition to become a guru' in this field, the topic of the cardiac RAAS was, in his eyes at least, 'a significant area of burgeoning interest' at this time. Furthermore, new tools and technologies were available to support further research.

Dr Jackson's laboratory was already active in research concerning ACE and had shown that pulmonary, vascular and renal ACE were induced to different degrees during chronic ACE inhibitor therapy, with the suggestion of differences in control mechanisms.

The laboratory had also shown there to be measurable differences in how tightly ACE from different rat tissues bound to angiotensin, further supporting the suggestion that there may be variations at the active site of ACE between tissues. At this stage, two papers had been published and a further paper was in press at the time the application was made (Johnston, Matthews and Jackson, 1983; Johnston and Jackson, 1983; and Johnston et al., 1983). According to Dr Jackson, 'We could see a yield [of new knowledge, further research and potential clinical applications] that was going to come at the end of it' (Jackson interview, 2008). This appealed to his curiosity. His anticipation was shared by the assessor, who commented that: 'The research is likely to contribute significant new knowledge' (Grant-in-Aid Assessor Report, 1987).

17.3.3 Clinical interest and potential clinical applications

Dr Jackson's clinical interest was also a driver behind the research topic. According to Jackson in the grant application, ACE in the heart was 'a topic of growing clinical importance'. He said, 'I am not a basic science-driven person, driven by the knowledge of basic science, I am driven by an application at the end of the day that has a bottom line in terms of clinical practice' (Jackson interview, 2008).

17.3.4 Institutional environment

The topic selection was also influenced by those around him at Austin Hospital, who Dr Jackson respected, including biomedical researchers and clinicians, and with whom he was able to 'workshop', both formally (for example in 'think tanks') and informally prior to submitting his application. It also meant that Jackson had access to a diverse skill set for ongoing research projects.

Another influence was the experience Dr Jackson gained through participation in a consortium led by Norman Sharp from New Zealand; as a local team leader, Jackson was involved in a larger clinical project that introduced the clinical concept of beta blockers being used in the management of heart failure and provided him with a good

understanding of the nature of heart failure. An interesting aside of this project was the evolution from its initial impetus around the use of beta blockers to looking at peptides.

17.4 Interface A – project specification and selection

The grant application was drafted by Dr Jackson with input from both his team and those who worked at Austin Hospital at the time and had an interest in the research study.

As noted above, the proposal built on the track record of Dr Jackson and his laboratory and was designed to provide, for the first time, a direct assessment of the tissue effects of ACE inhibitors. The research explored factors contributing to differences in tissue effect, and it was postulated that this might lead to a rationale for the development of specific therapeutic approaches.

The project had three key aims: to examine the in-vivo effects of ACE inhibitors on ACE in tissue, to examine the factors contributing to differential tissue effects of ACE inhibitors in vivo and to examine the characteristics of ACE in the heart and how it related to other molecular signalling systems. The research used a radioligand binding assay system modified for use on tissue sections to measure ACE and describe its anatomic distribution and in vivo inhibition.

The assessor[2] and committee were very positive about the proposed research, noting it to be 'very professionally written' and praising the presentation at interview. No comments were made against the proposed research and, indeed, the assessor commented: 'The hypotheses are well set out, adequately substantiated from existing knowledge and capable of testing by the methodology proposed'. In his report of the interview, the spokesman notes the preliminary photograph (a digital densitomitary image) to be 'very impressive'.

The only revisions made to the grant were its duration and the funding for staff. The project was reduced from three years to two, as it was felt that part of the project had already been done. The request for a research technician (Grade II) was reduced to a technical officer. The amount awarded for maintenance was also reduced; it is not clear why this was so.

It seems that a reduction in budget was not unexpected for Dr Jackson: 'I have always worked with the basis that you submit a fully costed project…then negotiation takes place as a slash-and-burn negotiation, which is really not negotiation, because if you costed your grant at [Aus]$100,000 and you are given [Aus]$60,000, the grant giver often expects [Aus]$100,000 worth of work. The grant recipient tries to slash and burn the [integrity] of the original project to get [Aus]$50,000 or [Aus]$60,000, whatever' (Jackson interview, 2008).

The panel recommended the grant be funded, with a score of 4.5. Had this funding not been awarded, Dr Jackson believes the grant would have been reworked and resubmitted to the National Heart Foundation of Australia, as well as other potential external funders, according to who was funding what at the time.

[2] Only one assessor report was provided for this grant.

17.5 Stage 1 – inputs to research

According to Dr Jackson, the key to the success of the project was the availability, at that time, of newly developed techniques that staff at Austin Hospital had been developing. However, several other key ingredients should be factored in, including: funding availability, knowledge and expertise, available facilities and the reputation and personal drive of Jackson, as well as strong collaboration and support from Jackson's institution. These are discussed below.

17.5.1 Financial

Funding was important to the project – ie 'if the money is there, you'd do the work' – and part of Dr Jackson's remit was to secure funding to support his laboratory, which he believes assisted his proficiency in preparing research applications.

The grant from the National Heart Foundation of Australia provided funding for a technical officer and maintenance, which included rats, radiopharmaceuticals, photographic consumables and x-ray cassettes for autoradiography.

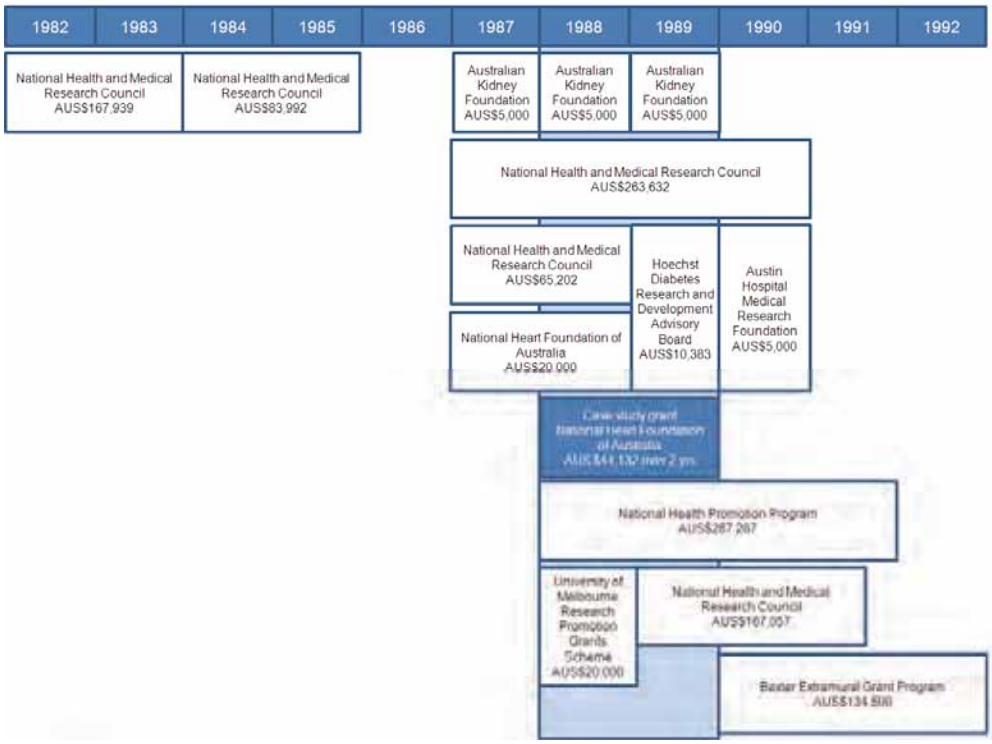
As Figure 17-1 shows, this particular grant overlapped with several others, including another from the National Heart Foundation of Australia. These other grants funded research projects in related fields:

- ACE Studies by Radioinhibitor Binding (NHMRC)
- Antihypertensive Drug Modulation of Renal Injury in Renal Failure Models (NHMRC)
- Atrial Natriuretic Peptide: Studies in and Effect of Progression of Renal Failure (NHF)
- Renin–Angiotensin System and Human Diabetic Renal Disease (AKF).

By Dr Jackson's admission, it is hard to attribute outputs to individual grant funding in this kind of situation.

Resources for the project also came from other sources outside the grant amount, including space, equipment and researchers from the University of Melbourne, where Dr Jackson was an NHMRC-funded senior research fellow.

Figure 17-1 Dr Jackson's grant history by year, awarding body and amount



17.5.2 Knowledge and expertise

Research experience

As noted above, Dr Jackson had a strong background in the RAAS, which was valuable to the project inception, funding application and conduct of the research.

In addition to Dr Jackson's knowledge and expertise, the research also benefited from a wealth of knowledge and expertise, as well as access to staff and clinicians at Austin Hospital who were interested in the research project. In particular, this included John McNeil, from the Department of Clinical Pharmacology; Professor Colin Johnston and his technical staff, with whom Dr Jackson moved across from his previous institution to Austin Hospital, and Dr Fred Mendelsohn, who was named on the grant application as another investigator associated with the project and who was very interested in blood pressure and cardiovascular issues. Jackson noted at interview that formal and informal interactions with colleagues active in their own research projects were very important to all of the research he conducted: 'To work in isolation is extraordinarily difficult. You just cannot underestimate the value of those casual conversations in the coffee room, the formal weekly meetings, where you justify your position and the progress you are making, and the opportunity for informal collaboration' (Jackson interview, 2008).

Clinical experience

Dr Jackson himself brought both basic and clinical research expertise to the project. He believes his clinical experience gave him a different perspective to basic research in two key ways. Firstly, it helped him to anticipate the impact it could have, including on the quality of life of patients. Jackson said, 'I have been working in dialysis units and you realise that

the quality of life on dialysis is not perfect. You are pretty keen to try and prevent kidney failure' (Jackson interview, 2008).

Secondly, having 'a foot in each camp', as Dr Jackson put it, had a further advantage of the ability to see things that may be otherwise overlooked. According to Jackson, 'When you go to clinical trials, there is always a very strong need for the basic scientist to be involved', and he points to outcomes of trials that are far from the original impetus. The reverse is also true – that an event may be observed clinically with a patient who seems to be out of left field, which triggers thinking as to why this occurred, with basic research principles being used to develop a hypothesis about what is happening that can be tested through research.

It should be noted too that this 'foot in each camp', from Dr Jackson's perspective, has potential benefits for outcomes, enabling the PI to take the outcomes from this basic research through to clinical trials and policy adoption. Jackson said, 'To me, the excitement was being able to take what you'd seen at the bench, in the test tube, and to stick it into an animal model, show that it worked and then to jump around the corner and say that I have a patient that looks just like the rat, why don't we do this?' (Jackson interview, 2008).

17.5.3 Techniques

New techniques developed as part of previous grants were crucial to the success of the research project, particularly techniques that had been developed in conjunction with Mendelsohn's group.

The new techniques were important to the study, as studying tissue ACE had previously posed problems for investigators. Two of these key difficulties were that other enzymes in the tissues broke down the synthetic substrates, leading to overestimates for the activity of ACE, and that the chemicals that the synthetic substrate was broken down into could inhibit ACE, leading to underestimates for activity. General enzyme inhibitors went some way to addressing these problems but also affected ACE. Although purification of ACE from tissues helped address some of the methodological issues, the purification process itself caused structural alterations in ACE, making comparative studies of ACE difficult to interpret.

To counter these difficulties, the group developed a technique for measuring plasma and tissue ACE that involved a binding assay with a new radioinhibitor derived from an ACE inhibitor. The ACE inhibitor was radiolabelled to become a probe for autoradiographic and radioinhibitor binding studies of ACE. In its radioactive state, the ACE inhibitor bound tightly to ACE, blocking its active site and hence inhibiting the enzyme. By measuring the amount of bound inhibitor through radiography, it was possible to directly estimate the amount of ACE present. This technique could be used both in vitro and in vivo; this was a relatively recent development, with publications appearing from 1983 (Johnston, Matthews and Jackson, 1983; Johnston et al., 1983; Larmour et al., 1985; McGrath et al., 1985; Johnston et al., 1986; and Johnston and Jackson, 1986). The technique was then being used at Austin Hospital; another group based at the Minerva Institute at the University of Helsinki, Finland, was also publishing work using inhibitor

binding assays for ACE (Fyhrquist et al., 1984, and Forslund, Kouvonen and Fyhrquist, 1984).

In this project, the technique was applied to measure characteristics of ACE in the heart and to study in-vivo interactions of ACE inhibitors with the active site of the enzyme in a variety of tissues. To provide data on the anatomic distribution of ACE within an organ, in this case the heart, tissue homogenates could not be used. Instead, frozen sections of tissue were incubated with the radioligand, washed to remove any non-specific binding and then exposed to x-ray film. The resulting images (autoradiographs) were digitised (a digital sensor was used initially) to quantify the distribution of ACE, and this was represented with different colours for different levels of ACE; the images were calibrated against standard measures from the same process.

The grant application noted that for a ligand-binding study to be valid it was important firstly that the ligand and ligand–enzyme complex were stable under assay conditions and secondly that there was only a low level of non-specific binding. The application stated that the team had established optimal binding conditions and demonstrated the required criteria for validity.

Colour-coded, computer-generated, digital-densitometry images were the visual output from this technique.

17.5.4 Reputation

Apart from the hospital's contribution to facilitators such as space, equipment and personnel, it also importantly contributed its intellectual capability, resources and reputation.

At the time, Austin Hospital was 'home' to several key research groups and was seen as a highly stimulating environment to work in – both as a teaching hospital and an 'intellectual hub'. Dr Jackson himself moved to Austin Hospital when the Chairman at his previous institution moved to take up a position of Head of Medicine at Austin Hospital.

Being at Austin Hospital gave Dr Jackson 'access to the whole spectrum of people', clinical and non-clinical, who could be involved in any piece of research, either formally or informally. This included access to pure biomedical researchers, mathematicians and statistical advisors, if needed for clinical trials, and cardiologists to discuss current practices and thoughts.

17.5.5 Equipment, infrastructure and space

Austin Hospital provided the major equipment and facilities required for the project, including full animal-house services and facilities, a small-animal surgery suite, radioisotope-handling facilities (including iodination laboratory and gamma counters), a cryostat, a computerised image and analysis system, a darkroom, an x-ray film developer and a general laboratory.

The department at Austin Hospital provided laboratory space; however, this was dependent on Dr Jackson. Given this was onsite at the hospital, Jackson noted it was difficult at times, 'making the division between who owned what and paid for what.'

The institution also provided animal-house staff (four days a month) and the services of a research assistant and a research officer (one day each month).

17.5.6 Consumables

Key consumables for this basic research project were the rats used for tissue sampling.

17.5.7 Collaborators

As noted above, in-house collaboration was seen by Dr Jackson as critical to this and other projects. The collaboration was through networks, brainstorming and conversations, which allowed ideas to be pooled.

A key collaboration was with Dr Mendelsohn and his team. This was important to advance the autoradiography techniques employed in this research: while Mendelsohn's team applied this technique to neuroreceptors and adrenaline, Dr Jackson's team applied it to measuring ACE enzyme levels in the heart.

17.6 Stage 2 – research process

As noted above in the discussion on research inputs, the project used a method that had been developed at Austin Hospital to examine the distribution of ACE in tissues; this was the first time this particular method had been used for research in the heart. The technique countered key difficulties of studying ACE in tissues. The assessor commented of the technique: 'If the radioinhibitor binding technique is sufficiently specific to separate out the effects of a specific ACE from those of all non-specific peptidases which act on angiotensin I, then this is a powerful methodological tool' (Grant-in-Aid Assessor Report, 1987).

The technique involved radiolabelling of known ACE inhibitors, which would bind to ACE, enabling the distribution of ACE to be observed and quantified through autoradiography. Autoradiography is a technique that enables molecules, or fragments of molecules, to be visualised by labelling them with radioactive material; in autoradiography, the specimen itself is a source of radiation; this is in contrast to radiography, where a source of radiation is external to the specimen. The result is an image captured by placing the sample on x-ray film or a digital sensor. At the time, the technique of autoradiography was being perfected by Dr Mendelsohn at Austin Hospital. Mendelsohn had gone one step further by doing digital densitometric analysis to give a clear and visual distribution of the target in question. Mendelsohn was using the technique in the neural arena, and Dr Jackson's team was able to adopt this approach for the renal arena, creating a new probe for a component of the RAAS. This probe could then be used for studies of the heart, and, according to Jackson, while the technique is important, the key to success is having a probe: 'I started looking at kidneys, Fred [Mendelsohn] was looking in the brain, and together we looked in the heart' (Jackson interview, 2008).

In the research process, several different ACE inhibitors were used, including lisinopril, enalapril, cilazapril, fosenopril and CGS14831. A variety of tissues were also used.

Autoradiograph analysis and ligand-displacement studies of tissue homogenates were used to show the variable effects of ACE inhibitors in different tissues.

17.7 Stage 3 – primary outputs from research

17.7.1 Knowledge

One of the drivers when applying for the initial grant was that Dr Jackson was fairly certain of a knowledge yield and potential clinical applications from this research, and, as noted above, his anticipation was shared by the grant assessor. The number of publications, and their well-above level of citation for their age and field (see Table 17-2), shows that these expectations about the project were justified.

Table 17-2 Publication output and impact[3]

Number of journal articles:	12
Number of articles included in citation analysis:	8
Total number of citations (all papers):	498
Aggregate relative citation impact:	2.63 (Class V)
Self-citations:	10%

Distribution of publications across relative citation impact classes:

	Class I (uncited)	Class II (>0 and <0.8 citations)	Class III (0.8 to 1.2 citations)	Class IV (1.2 to 2.0 citations)	Class V (>2.0 citations)
Number of publications		2	1	1	4
Proportion of total output		25%	13%	13%	50%

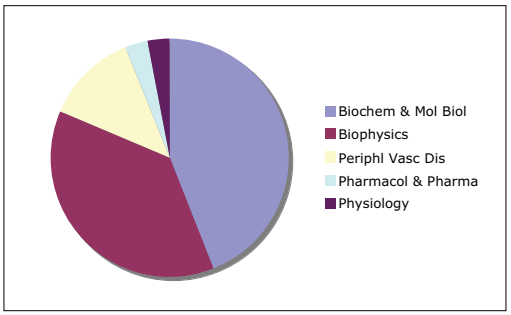
Most highly cited publication[4]:	Yamada, H., B. Fabris, A.M. Allen, B. Jackson, C.I. Johnston and F.A. Mendelsohn, 'Localization of Angiotensin Converting Enzyme in Rat Heart', *Circulation Research,* 1991, Vol. 68, No. 1, p. 141–149
Times cited:	182

[3] In addition, four publications were indirectly linked to this grant. These publications were all indexed in Web of Science and received 340 citations in total, giving a relative citation impact of 2.54. Half were in citation impact Class IV and half in Class V; their self-citation rate was 26%.

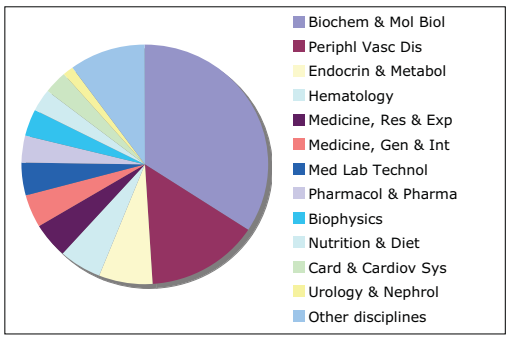
[4] Citation count extracted April 2009.

Figure 17-2 Knowledge diffusion: fields of research of output (a), fields of citing papers (b) and countries of citing papers (c)

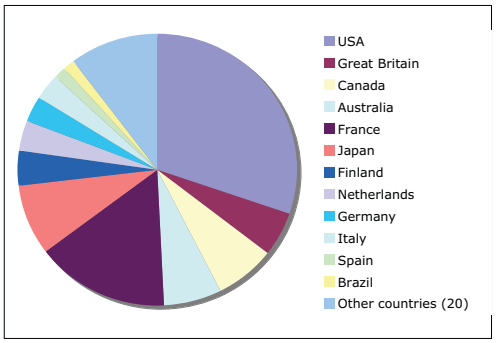
(a)



(b



(c



The main elements of the research proposed in the grant application were achieved and successful. This included examining the in-vivo effects of ACE inhibitors on ACE tissue in the heart, the factors contributing to the differential tissue effects of ACE inhibitors in vivo and the characteristics of ACE in the heart and how it relates to other molecular signalling systems.

The research produced 11 publications that can be directly attributed to the work funded by the grant and a further four publications that are indirectly attributable. The 11 directly attributable publications are listed below, with the number of citations to April 2009 also noted:

- Sakaguchi, K., S.Y. Chai, B. Jackson, C.I. Johnston and F.A.O. Mendelsohn, 'Differential Angiotensin Converting Enzyme in Brain After Oral Administration of Perindopril Demonstrated by Quantitative In Vitro Autoradiography', *Neuroendocrinology*, Vol. 48, No. 3, 1988, p223–228 (cited 29 times).
- Sakaguchi, K., B. Jackson, S.Y. Chai, F.A.O. Mendelsohn and C.I. Johnston, 'Effects of Perindopril on Tissue Angiotensin Converting Enzyme Activity Demonstrated by Quantitative In Vitro Autoradiography', *Journal of Cardiovascular Pharmacology*, Vol. 12, No. 6, 1988, pp. 710–717 (cited 38 times).
- Jackson, B. and C.I. Johnston 'Angiotensin-Converting Enzyme Inhibition in Renal Disease: Contrasting Effects on Renal Function in Renal Artery Stenosis and Progressive Renal Injury', *Journal of Human Hypertension*, Vol. 3, Suppl. 1, 1989, pp. 107–115 (cited nine times).
- Jackson, B., R.B. Cubela and C.I. Johnston, 'Inhibition of Tissue Angiotensin Converting Enzyme by Perindopril – In Vivo Assessment in the Rat Using Radioinhibitor Binding Displacement', *Journal of Pharmacology and Experimental Therapeutics*, Vol. 245, No. 3, 1988, pp. 950–955 (cited 34 times).
- Jackson, B., R. Cubela and C.I. Johnston, 'Effects of Perindopril on Angiotensin Converting Enzyme in Tissues of Rat Heart', *Journal of Hypertension*, Vol. 6, Suppl. 3, pp. s51-s54, 1989 (cited 11 times).
- Johnston, C.I., B. Fabris, H. Yamada, F.A.O. Mendelsohn, R. Cubela, D. Sivell and B. Jackson, 'Comparative Studies of Tissue Inhibition by Angiotensin Converting Enzyme Inhibitors', *Journal of Hypertension, Supplements*, Vol. 7, Suppl. 5, 1989, pp. S11–16 (cited 80 times).
- Fabris, B., B. Jackson, R. Cubela, F.A.O. Mendelsohn and C.I. Johnston, 'Angiotensin Converting Enzyme in the Rat Heart: Studies of its Inhibition In Vitro and Ex Vivo', *Clinical and Experimental Pharmacology and Physiology*, Vol. 16, No. 4, 1989, pp. 309–313 (cited 52 times).
- Fabris, B., B. Jackson, M. Kohzuki, R. Perich and C.I. Johnston, 'Increased Cardiac Angiotensin-Converting Enzyme in Rats with Chronic Heart Failure', *Clinical and Experimental Pharmacology and Physiology*, Vol. 17, No. 4, 1990, pp. 309–314 (cited 87 times).
- Kohzuki, M., C.I. Johnston, S.Y. Chai, B. Jackson, R. Perich, D. Paxon and F.A. Mendelsohn, 'Measurement of Angiotensin Converting Enzyme Induction and Inhibition Using Quantitative In Vitro Autoradiography – Tissue Selective Induction After Chronic Lisinopril Treatment', *Hypertension*, Vol. 9, No. 7, 1991, pp. 579–587 (cited 39 times).
- Yamada, H., B. Fabris, A.M. Allen, B. Jackson, C.I. Johnston and F.A. Mendelsohn, 'Localization of Angiotensin Converting Enzyme in Rat Heart', *Circulation Research,* 1991, Vol. 68, No. 1, p. 141–149 (cited 182 times).

- Perich, R.B., B. Jackson and C.I. Johnston, 'Structural Constraints of Inhibitors for Binding at Two Active Sites on Somatic Angiotensin Converting Enzyme', *European Journal of Hypertension*, Vol. 266, No. 3, 1994, p 201 (cited 25 times).

The first papers were produced in 1988, showing a very quick delivery of results and knowledge from the grant. The 1988 papers were referenced in the first progress report submitted by Dr Jackson to the funder (along with three papers due for publication in 1989); they both acknowledged the funding from the National Heart Foundation of Australia, however funding from NHMRC was also acknowledged, showing the difficulty in attributing knowledge production to the grant in question. The publications continued through to 1994; no papers were published in 1992 and 1993, which perhaps reflects Jackson's sabbatical in the United States. A brief account of each paper follows, outlining the key findings and the knowledge produced by the study.

The first paper looked at the study of the pattern of tissue ACE in rats after being treated orally by a then new ACE inhibitor drug called perindopril (Sakaguchi et al., *Neuroendocrinology*, 1988). In this study, plasma levels of perindopril were undetectable by 24 hours after its administration. Inhibition of ACE within tissues relevant to blood pressure control had always been suggested to be independent of effects on plasma ACE. This study, using quantitative in-vitro radiography, showed that acute oral administration of perindopril inhibited ACE in the kidney, lung, aorta and smaller blood vessels of tissues. The major difference in this study, in contrast with previous studies, was that it demonstrated the anatomic localisation of ACE inhibition within each organ and distinguished ACE inhibition in different ACE tissues.

The second publication examined ACE inhibition in the brain after oral administration of perindopril (Sakaguchi et al., *Journal of Cardiovascular Pharmacology*, 1988). It found that following small doses of perindopril, two areas of the brain (the subfornical organ and organum vasculosum) were markedly inhibited and only partially recovered after 24 hours. No or little change in ACE was observed in other areas of the brain that are rich in the enzyme. However, after large doses of perindopril, all areas of the brain progressively inhibited ACE. These findings fitted with the deficient blood–brain barrier known to occur in circumventricular organs. In combination with other, then-current observations, the paper concluded that the circumventricular organs were potential targets for the centrally mediated actions of ACE inhibitors.

The third publication studied ACE inhibition in renal disease (Jackson and Johnston, 1989). Acute renal failure is a common consequence of ACE inhibitor therapy in patients with renal artery stenosis. This paper looked at the suggestion that these effects are reversible – ie that function returns after withdrawal of ACE inhibitor therapy. After examining the long-term (12-month) effects of ACE inhibitors on rats, ACE inhibitors seemed to have a paradoxical effect on renal function. Whether the observations obtained from animal models were applicable to man was felt to require clinical confirmation.

The fourth paper again looked at the inhibition of ACE in rats using perindopril, this time using radioinhibitor binding displacement (Jackson, Cubela and Johnston, 1988). The study showed that the degree of ACE inhibition by perindopril varied between different tissues. Plasma ACE was almost completely inhibited two hours after oral treatment, as was ACE in the kidney.

The fifth paper looked at the effects of perindopril on ACE in rat tissues (Jackson, Cubela and Johnston, 1989). The results in this study were very similar to those of previous studies but highlighted the profound inhibition of ACE in kidney tissue following all doses of perindopril, with maximum inhibition 1–2 hours after gavage. There was also a good correlation between plasma ACE activity and kidney ACE estimated by radioligand displacement.

The sixth publication examined and compared the advent of the ligand-binding methods, together with the array of ACE inhibitors of different potencies (Johnston et al., 1989). This allowed more-detailed studies of the active site of enzyme in various tissues (heart, brain, lungs and testes), accurate anatomic mapping of the enzyme and analysis of the effects of ACE inhibitors on tissue. The publication looked at the physiological and clinical implications of inhibition of ACE tissue. It concluded that until ACE is purified from the various tissues and studied in more rigidly defined conditions, it will not be possible to determine whether the active site is the same in all tissues. It suggested that it may be feasible to design drugs that will inhibit ACE in specific areas – for example, in cardiovascular disease, it may be particularly important to inhibit ACE in the heart, vasculature and kidneys.

The seventh publication studied ACE in the myocardium (heart tissue) of male Sprague-Dawley rats both in vivo and ex vivo (Fabris et al., 1989). The study evaluated the binding characteristics of the active site of ACE from different regions of the heart. It provided evidence to show that ACE derived from different tissues is not identical. The existence of ACE in the myocardium supports the possibility of a local RAAS with the ability to generate angiotensin II. The study showed that small differences in the ligand-binding characteristics of ACE from the atria and ventricles do not seem to affect the in-vivo inhibition of rat myocardial ACE.

The eighth publication reported a study of ACE in heart membrane preparations and sections in a model of chronic cardiac failure in rats with left ventricular myocardial infarction (Fabris et al., 1990). The study demonstrated significant increase in myocardial ACE content in all heart chambers and a marked increase of ACE in the myocardial area: 'Over the 4 week period of the study 15 out of the 26 rats tested were found to have evidence of myocardial infarction on inspection of the left ventricle wall'. Following myocardial infarction (heart attack) and the loss of functional myocardium, there is a systolic and diastolic ventricular dysfunction resulting in hypertrophic remodelling of the heart (an increased cardiac size and volume). The precise stimuli triggering the hypertrophic remodelling were not discovered. The study demonstrated the increase of ACE in hypertrophic myocardium and supports the notion that the RAAS may be dysregulated, allowing the potential of local tissue production of angiotensin II.

The ninth publication examines ACE inhibition by lisinopril treatment using quantitative in-vitro autoradiography (Kohzuki et al., 1991). The results and pattern of ACE inhibition shown were similar to those seen in previous acute studies. However, induction of ACE was found to be organ specific. During chronic treatment with lisinopril, ACE activity in all organs was inhibited with low levels of free ACE. There was neither induction nor inhibition in the testis. The conclusion suggested that ACE may be under tissue-specific controls.

The tenth publication reported on the localisation of ACE in the rat heart by in-vitro quantitative autoradiography (Yamada et al., 1991). It also examined the binding properties of cardiac ACE. The study showed that the highest density of ACE in the heart was found on valve leaflets (aortic, pulmonary, mitral and tricuspid), which contrasted with very low ACE labelling in the endocardium (the innermost layer of tissue that lines the chambers of the heart). The coronary arteries also showed a high density of ACE, and the right atrium had a moderate density, which was higher than that in the left atrium and ventricles. The results of the study revealed a markedly non-uniform localisation of ACE in the rat heart and suggested possible sites for local generation of angiotensin II. Dr Jackson noted: 'This is a most important paper. Subsequent to that work I went to America and did a sabbatical study leave. So this one here is now being fairly widely cited and is one of the first benchmark studies that doctors of the heart and pre-heart failures refer to' (Jackson interview, 2008).

The eleventh publication looked at the structural constraints of inhibitors (Perich, Jackson and Johnston, 1994). ACE active sites from rat plasma, lung, kidney and testis were assessed by comparative radioligand-binding studies. The resulting data demonstrated that the two binding sites on native plasma and somatic ACE are potentially of different and structural natures, suggesting they may have different substrate specificities.

As noted above, a further four publications can be indirectly attributed to this grant. One in particular has attracted 156 citations to date, and it is this paper that Dr Jackson believes is the most important to be associated with this grant:

- Schunkert, H., B. Jackson, S.S. Tang, F.J. Schoen, J.F.M. Smits, C.S. Apstein and B.H. Lorell, 'Distribution and Functional Significance of Cardiac Angiotensin-Converting Enzyme in Hypertrophied Rat Hearts', *Circulation*, Vol. 87, No. 4, 1993, pp. 1328–1339.

This publication reported on the study in which ACE inhibitors were used as a tool to study the distribution of cardiac ACE and provided evidence that inhibition of cardiac ACE is sufficient to prevent hypertrophy in the rat model of this study (Schunkert et al., 1993). It also looked at the role of the enzyme inside the rat heart in terms of the conversion of angiotensin I to angiotensin II. The major findings of this study were that the fractional conversion from angiotensin I to angiotensin II is amplified in isolated beating hearts with left ventricular hypertrophy (LVH), ACE inhibition reduces the conversion rate and functional related changes in rat hearts with LVH, and ACE density is increased throughout the myocardium of rat hearts with LVH; in fact, the findings revealed that ACE density is two-fold higher in the myocardium of rats with LVH.

The evidence presented above demonstrates that the project did, as anticipated, make a significant contribution to knowledge in this field.

17.7.2 Benefits to future research and research use

Capacity building and career development

The PI

It is extremely difficult to ascertain the impact of this specific grant on Dr Jackson's career. He continued in research after the grant and remains an active researcher today, as well as

being involved in clinical practice and having a consultative capacity in a number of special interest areas.

Today Dr Jackson oversees a programme that practices much of what has been developed over the last 20 years. His clinical research interests remain with cardiovascular risks and in particular hypertension and the kidney, heart and brain. Jackson is still an active clinician, undertaking some private practice and involvement with clinical research. He works part-time and is also involved with teaching. He holds a number of appointments in general medicine and is involved with a number of special interests groups. In addition to being Deputy Head of General Medicine at Casey Hospital, he is also Unit Head of General Medicine at Dandenong; a consultant for the vascular medicine group of Southern Health in Victoria; and Medical Head for a chronic disease programme. He has specific interests in chronic disease management and is involved in a number of related activities with organisations, including Senior Clinician to a chronic heart failure programme and involvement with diabetes intervention groups through his kidney and cardiovascular risk knowledge. His research projects today include service delivery, newer modes of treatment, newer pharmacological agents and quality assurance. Jackson said, 'I still do have a foot in each camp…It's a big clinical foot now, because I can't read fast enough to keep up with all that biomedical research, and I don't have an academic home base to compete with the likes of the Baker [Institute] and so on' (Jackson interview, 2008).

From the perspective of career development, Dr Jackson felt the projects and research that he was doing in the period of this specific grant had been of importance with regard to the evolution of his career. He said, 'The product that you see is not just a clinician, it's a clinician with a strong background in the laboratory' (Jackson interview, 2008).

Quite how much this particular grant versus Dr Jackson's other work impacted on his career development is very difficult to ascertain, but it is clear that this was part of an important body of work for him.

Other staff

The technical assistant funded by the grant, Rose Cubela, went on to do a doctoral thesis on the topic, with Dr Jackson as a supervisor. From there she moved to the pharmaceutical industry and then to a regulatory authority, where it is believed she is currently involved with new product development and regulatory authority administration. We were unable to speak with her at this time. In terms of the impact of this grant on her career, Jackson believes that: 'She wouldn't have had that role if it hadn't been for the involvement in the trials' (Jackson interview, 2008).

Targeting of future research

There were two key outputs from the research with regard to the targeting of future research.

The research was part of a body of work that lead to an evolutionary paradigm shift in thinking about the use of ACE inhibitors. Jackson said, 'It was an evolutionary [shift] and this happened over a period of years, at multiple centres around the world, we were just one contributing strongly to that…and Colin Johnston was certainly extraordinarily adept at drawing together the strands from our different work groups to bring those together and make the presentations' (Jackson interview, 2008).

This particular research was proof of principle and the start of a chain that stepped next to functional proof and then to clinical proof, with different teams being involved along the way.

According to Dr Jackson, the research 'opened a whole can of worms' for the tissue angiotensin system. It showed that ACE was different in different tissues, meaning that the ACE situation was much more complicated than had been thought. It also drew attention to the idea of measuring drug/enzyme activity directly in tissues. However, his group was just one of several involved in prising off this lid. The implications of this body of work were broad and the story continues today.

A further key output of this research was the establishment of a method for the direct assessment of tissue ACE and its inhibitors. The project helped to establish the diverse potential of tissue autoradiography techniques: although tissue autoradiography itself was not new at the time, this was the first time it had been used to measure ACE in the department, university and Australia. This approach was widely adopted within the department following this research and was then taken up by endocrinologists looking at diabetic kidney disease. Today it has a generalised nature and is a laboratory standard probe technique. According to Dr Jackson, commenting upon the major impact of his research, key to the technique is having a probe: 'It is part of the justification of the use of ACE treatment of hypertension, treatment of cardiac disease, in particular the treatment of heart failure and kidney disease – not just hypertension associated with kidney disease but in the preservation of kidney function' (Jackson interview, 2008). This comment is backed up by the number of times his publications have been cited.

Furthermore, Dr Jackson also noted that he introduced the idea of tissue autoradiography to the team in the United States during his sabbatical to Boston. A decision was taken at the time by the team to send tissues back to the laboratory at Austin Hospital rather than establish a parallel laboratory in Boston at the time.

17.8 Interface B – dissemination

Publications are an obvious form of dissemination and, as is seen above, Dr Jackson produced a number of highly cited papers from this research. It is perhaps worth noting here Jackson's approach to work he presented: he believed that anything he presented should be in a publishable form, and he believes this has contributed to his list of publications – 172 publications between the years 1970 and 2005.

Dissemination also involved both in-house and out-of-house educational activities for undergraduates and postgraduates and influenced clinical practice. At the time, Dr Jackson was involved in the undergraduate teaching programme at Austin Hospital, University of Melbourne, as a clinical tutor and examiner at fourth and final year levels, continuing from a role at Monash University, Victoria. Reflecting his involvement with postgraduate teaching, he was appointed Director of Physician Training for both basic and advanced training at the Northern Hospital in 1992.

He continues to be involved today in the undergraduate teaching programme at Monash University, where he lectures and is involved with clinical tutorials and the problem-based

learning curriculum. In the interim, he has had responsibility for the undergraduate medicine teaching programme at Northern Hospital, where he coordinated across all six years of the curriculum, as well as delivering small-group clinical tutorials, clinical pathological case presentation discussions and combined medical and surgical presentations personally. Again, it is uncertain to what extent the knowledge from this research grant was disseminated through these practices, but there was certainly an avenue available.

Dr Jackson was not the only person involved in dissemination of the research findings. According to Jackson, Professor Johnston, as department head, would also have spoken out about Jackson's work as part of the bigger picture of what was happening at Austin Hospital, giving greater publicity to it.

Professor Johnston was invited to speak at the Cardiovascular Centre, College of Medicine, University of Iowa, Iowa City, on 23 November 1992. He was the principal discussant on a clinical conference titled 'Tissue Angiotensin Converting Enzyme in Cardiac and Vascular Hypertrophy, Repair and Remodelling'. It is likely that his talk drew on the work by Dr Jackson's team.

17.8.1 Collaboration

Involvement in collaborations, for example in the United States shortly after this research project, saw Dr Jackson spreading the word informally, formally, nationally and internationally. Jackson said, 'In-house collaboration is terribly important, in the sense that one has a collegiate group and there is a critical mass, conversations that occur in the coffee room and corridors are probably just as important as ones that occur in the presentation room. Internationally…it is not just a collaboration in the sense of a brainstorming…it is more akin to networking, "we are doing this, they are doing that, why don't we pool our ideas and see then if something comes out of it?"' (Jackson interview, 2008).

With reference to this specific grant, Dr Jackson points in particular to his experience during a sabbatical study leave to Boston. During his time in Boston, Jackson was able to take the next step in the process to show a functional consequence. He said, 'They had a project over there that needed a worker with insight into an area…and for the person doing that work there must be some additional benefit, so for me it was to go into the whole adventure of molecular biology, which we sort of studiously skirted around here. So I spent six months with my nose in a molecular biology book but at the same time took the autoradiography techniques to the Boston group, said this is what we can do and this is how we do it…They ended up saying, "Well that is probably better that we send you the tissues and you do it in Melbourne rather than we set up a parallel competing group in Boston"' (Jackson interview, 2008).

17.9 Stage 4 – secondary outputs

This particular piece of research was effectively proof-of-principle research. When combined with the work of others, it generated key findings that were used to inform clinical trials and were later used in the development of clinical guidelines that are now used in policy and practice – for example, treatment of diabetic patients with early kidney

disease. These areas may not seem to be related to cardiovascular areas, but, as Dr Jackson notes: 'It depends if you see the kidneys as an appendage of the heart…or the heart as an appendage of the kidney' (Jackson interview, 2008).

Dr Jackson felt that his multidisciplinary background helped to progress these ideas, in a way that would have been far less possible for a biomedical researcher. The research was 'a small cog in the big work' influencing the use of ACE inhibitors for cardiovascular disease and kidney disease. The pharmaceutical industry has since invested significant money into subsequent clinical trials that have proved the clinical benefits of the agents explored (perindopril and lisinopril).

One relevant clinical trial paper makes references to several of Dr Jackson's publications that are directly appropriated to this specific grant:

- Mancini, G.B., G.C. Henry, C. Macaya, B.J. O'Neill, A.L. Pucillo, R.G. Carere, T.J. Wargovich, H. Mudra, T.F. Lüscher, M.I. Klibaner, H.E. Haber, A.C. Uprichard, C.J. Pepine and B. Pitt, 'Angiotensin-Converting Enzyme Inhibition with Quinapril Improves Endothelial Vasomotor Dysfunction in Patients with Coronary Artery Disease', *Circulation*, Vol. 94, 1996, pp. 258–265 (cited 885 times).

17.10 Stage 5 – adoption by practice and the public

Being a basic science project, this project itself did not lead to clinical guidelines for the treatment of hypertension. However, the findings from the research supported the use of ACE inhibitors in the treatment of hypertension, cardiac disease and kidney disease and in preservation of kidney function, and it led on to further work that eventually led to major clinical trials and the basis of policies for the treatment for diabetic patients with early kidney disease.

Dr Jackson himself has, through other research, been involved in work that has led to the development of clinical guidelines. Jackson noted that clinical trials are often funded by the pharmaceutical industry – perhaps then we should not expect to see a huge contribution to clinical guidelines from NHF-funded research.

It is difficult to determine whether the guidelines to which this work indirectly contributed in fact had an impact on clinical practice. Dr Jackson notes that current use of ACE inhibitors in heart failure management is now enshrined in clinical guidelines. He currently supervises heart failure programmes in patient management where the origins of the management have come from the basic research in which he participated.

17.11 Stage 6 – final outcomes

The impact of the use of ACE inhibitors has improved mortality and has been significant to the evolution of management and intervention; however, this has brought its own set of new clinical challenges and a need for clinical practice to change. As Dr Jackson explained: 'We are winning on the cardiovascular front at the moment. The epidemic of heart attack, strokes, kidney disease and peripheral vascular disease is much different to when I started

back in the 1960s. No longer is an acute myocardial infarct killing you. You are now having the problem of surviving the first infarct and the second and the third and the fourth, having your interventions, living and having the end-stage damaged heart failure issue. So that is an evolution of our management interventions…out of that comes the fact that cardiovascular disease is still around but it's now changed its spots…what is really happening is that we are now all living longer, perhaps to die of other diseases, not acute illness but chronic organ failure' (Jackson interview, 2008).

In Dr Jackson's opinion, both quality of life and life expectancy have increased with the use of ACE inhibitors, while utilisation of the public sector/hospital facilities and the cost of keeping the person alive and health system costs have increased.

As mentioned earlier, the research has also benefited the pharmaceutical industry. By showing that ACE inhibitors work, the pharmaceutical industry has subsequently invested 'a large amount of money' (Jackson interview, 2008) into clinical trials. Jackson has himself been involved in these clinical trials, which have clearly demonstrated clinical benefits of ACE inhibitors. This has resulted in pharmaceutical products such as perindopril and lisinopril being sold by the pharmaceutical industry, leading to employment and likely profits.

17.12 Additional observations

It is worth considering the impact of funding and funding probabilities; at the end of the day, researchers have to survive personally as well as professionally. Dr Jackson notes that generally clinical funding is more readily available than in biomedical areas, and this contributed to his move away from basic research and towards clinical research, while retaining his clinical practice. Jackson said, 'Is it better to play with the As or captain the Bs?' (Jackson interview, 2008). He also noted that in hospital systems today, no allowance is made for clinical research – timesheets are taken up with everything else.

17.13 Summary of case study impacts

The payback framework describes the process from research to several categories of impact. The categories allow us to compare across different case studies. Table 17-3 shows, in point form and by impact category, some of the impacts, described more fully above, that have emerged from this grant.

Table 17-3 Payback

Payback category	Impacts from case study
Knowledge production	• 11 peer-reviewed publications from the year the grant commenced • Proved that ACE is not identical in all tissues
Research targeting and capacity building	• Technical assistant went on to do her doctoral thesis on the same topic but then left basic research for clinical trials in the pharmaceutical industry and thereafter regulatory authorities • Dr Jackson is still involved in cardiovascular disease but more so on the clinical side; he feels his basic research background and experience helps him in his many roles today • Part of a body of work that led to an evolutionary paradigm shift in thinking about the use of ACE inhibitors • Establishment of a method for the direct assessment of tissue ACE and its inhibitors; the diverse potential of tissue autoradiography techniques; laboratory work at Austin Hospital on behalf of parallel laboratory in Boston, United States • New area of investigation, looking at what happens in situ in tissue
Informing policy and product development	• Provided some justification for the use of ACE inhibitors in the treatment of LVH, hypertension, cardiac disease, heart failure and kidney disease • Provided proof of principle, which then led into clinical trials • Led into clinical work that has informed policy for treatment of patients with diabetes
Health and health sector benefits	• ACE inhibitors have improved quality of life and life expectancy
Broader social and economic benefits	• Fed into clinical trials and pharmaceutical industry

17.14 References

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CHAPTER 18 Vasoactive substances and pathogenesis of essential hypertension

18.1 Overview of case study grant

The grant titled 'Vasoactive Substances and Pathogenesis of Essential Hypertension' was funded by the Medical Research Council of Canada (MRC) from 1989 to 1991. This research was conducted at Laval University in Quebec City. Led by Dr Lebel, a professor at the university and an associate in nephrology at the university's hospital and research centre, the team examined the role of the kidney in the pathogenesis of hypertension. They approached the problem by identifying and studying various vasoactive compounds found within the kidney. The goal of this research was to understand the natural mechanisms within the kidney that protect against hypertension.

The research undertaken through this MRC grant suggested that prostaglandins (PGs) may have a role in modulating the renal haemodynamic effect of vasopressor substances. The team's research showed that people with pre-hypertension or borderline hypertension had lower total blood volume and lower extracellular fluid volume; there was an inverse relationship between blood pressure and volume. The team found that young people who have borderline hypertension have a defence mechanism in their kidney that fights to delay the hypertension as long as possible.

18.2 Introduction to case study

Hypertension is a medical condition in which blood pressure is chronically elevated. Hypertension can be classified as either essential (primary) or secondary. Essential hypertension is a genetic disease that individuals are born with and therefore no specific underlying medical cause can be found to explain the condition. Secondary hypertension occurs when high blood pressure is a result of (ie, secondary to) another condition, such as renal disease or tumours. Persistent hypertension of either form is a known risk factor for stroke, heart attack, heart failure and arterial aneurysm and is a leading cause of chronic renal failure. Hypertension is usually treated first with lifestyle changes (weight loss, smoking cessation, etc). If non-pharmacological treatments fail to reduce blood pressure, drug treatment is initiated. Multiple drug therapies are available.

The kidney is known to occupy a central role in the pathogenesis of both essential and secondary hypertension. Experiments and system analysis conducted by Arthur C. Guyton (1989) led the research community to commonly accept that the kidney determines the 'pressure–natriuresis' relationship, because in a healthy individual, when blood pressure increases, the kidney will excrete more sodium to compensate for the increasing blood pressure. When the kidney is able to do its job correctly and consistently, hypertension will not occur. Guyton suggested that every type of high blood pressure begins as volume overload arising from an inability of a dysfunctional kidney to excrete salt. Thus the kidney was of overriding importance for ongoing control of blood pressure in any form of hypertension. Over time, however, the kidney can tolerate higher blood pressures and will stop working to decrease the blood volume and thus hypertension progressively appears. In other words, the 'pressure–natriuresis' mechanism is shifted to the right and less sodium is excreted by the kidney despite higher blood pressures.

Around the same time, researchers in France had studied men with sustained or well-established hypertension and found the opposite to be true (Safar and London, 1987). They found that as soon as the blood pressure was established or started to become severe, the blood volume increased. These findings are in keeping with the hypothesis that when the 'pressure–natriuresis' mechanism fails, the pre-hypertension condition may evolve to sustained hypertension with a concomitant increase in blood volume. The vasoactive compounds abundantly produced within the kidney and regulating the renal haemodynamics may contribute to this evolutionary process.

Three other sources of evidence that emerged in the mid-seventies and early eighties strongly supported the importance of the kidneys in determining the arterial pressure in both normotension and hypertension. Firstly, when kidneys were transplanted from hypertensive animals to normotensive animals, the hypertension 'followed' the kidneys (Dahl and Heine, 1975, and Bianchi et al., 1974). Similarly, when kidneys were transplanted from normotensive animals to hypertensive animals, the normotension again 'followed' the kidneys. Also important were studies published by Curtis et al. (1983), in which normal kidneys were transplanted into six patients with essential hypertension in whom end-stage hypertensive renal disease had occurred; in each instance the arterial pressure reverted to normal. Evidence of this type had given increasing credence to the concept that the function of the kidney was a key factor in all or virtually all types of long-term arterial pressure regulation.

Another important area of science relevant to this project was the discovery of new vasoactive substances – compounds that either dilate or restrict blood vessels – and their effects on renal haemodynamics. Acute infusions of noradrenaline and angiotensin II (AII) were observed to induce profound changes in blood movement within the kidney and in secretion of water and electrolytes, concurrently with the elevation of blood pressure (Brown and Peart, 1962, and Smythe, Nickel and Bradley, 1952). Results obtained in animal models suggested that eicosanoids synthesised and released by the kidney possibly smoothed the renal vasoconstrictor activity of pressor substances and were therefore protecting renal circulation and function from ischaemic results (Aiken and Vane, 1973, and McGiff et al., 1972). On the contrary, the administration of PG synthetase inhibitors under the same experimental conditions was seen to enhance renal vasoconstriction (Aiken and Vane, 1973).

Animal and *in-vitro* studies indicated that noradrenaline and AII can induce the release of eicosanoids by the intact kidney (reviewed in Schlondorff and Ardaillou, 1986). Clinical studies had also shown that AII and noradrenaline may increase the urinary excretion of some eicosanoids (Nadler, et al., 1983, and Loup, Favre and Vallotton, 1986). However the relationships between the effects of these vasopressors and PG release, renal haemodynamics and electrolyte excretion in man had not been simultaneously evaluated.

This case study examines the grant entitled 'Vasoactive Substances and Pathogenesis of Essential Hypertension', which was funded by the MRC from 1989 to 1991, with Can$99,019 per year. Through this project, the research team was trying to elucidate the mechanisms leading to the development of essential hypertension. Individuals are born with essential hypertension but often do not display signs or symptoms of the disease until their 40s. This delay suggests that the body will fight against genetics, trying to slow the start of hypertension. With this knowledge, the research team studied people who had pre-hypertension, young people with borderline hypertension and older people with established hypertension, focusing on the kidney's mechanisms to fight against essential hypertension. Specifically, the research team was interested in knowing how vasoactive substances affect the onset of essential hypertension, its progress and evolution as related to the kidney, as the kidney is responsible for the control of all body fluid volumes. The team aimed to identify hormonal components involved in controlling sodium metabolism and body fluid volumes produced by the kidney or elsewhere in the body.

This research was conducted at Laval University Research Center by a team led by Dr Marcel Lebel, who was the principal investigator (PI). Lebel was an associate professor at Laval University from 1986 to 1990, became a full professor in 1990 and was Director of the Clinical Research Unit of the Hôtel-Dieu Hospital of Québec, which is associated with Laval University, from 1987 to 1999.

18.2.1 The case study approach

The findings presented in this case study are based on a combination of three face-to-face interviews, with Dr Marcel Lebel (the PI), Dr Paul Isenring (a member of the research team) and Dr Richard Larivière (who was researching vasoactive substances at the time of the grant and joined Lebel's team a few years after the grant); a review of the PI's curriculum vitae; documentary analysis of the scientific literature; and bibliometric analysis. A review of the original grant application and supporting documentation was not possible as the grant application for this project was unfortunately no longer available.

18.3 Stage 0 – topic/issue identification

The idea for this research was based on the PI's background and training, his previous work and the lack of concordance in evidence available in the scientific literature at the time. These points are elaborated on below.

18.3.1 Training

Marcel Lebel was trained in nephrology and internal medicine at Laval University. He completed a doctoral degree in 1970. He then did a fellowship in experimental hypertension at the Clinical Research Institute of Montreal (CRIM) for the following two

years under the supervision of Jacques Genest, W. Nowaczynski and O. Kuchel. The CRIM was known as the 'Mecca' of hypertension research at the time. During his fellowship, Lebel's time was spent almost entirely on research. Upon its completion, Dr Genest encouraged Lebel to do a second post-doctoral fellowship in nephrology and hypertension at the University of Glasgow's Medical Research Council Blood Pressure Unit. Lebel describes that fellowship as very productive, in that it resulted in two publications in the *Lancet* as well as a book chapter. Lebel claimed that these publications were a great start for a career in hypertensive research.

When Dr Lebel returned to Canada from the United Kingdom in 1973, he was recruited to work at the University of Laval. He applied for and received a five-year Scholarship (Junior I and II) Research Award with a recognised speciality in nephrology from the Fonds de la Recherche en Santé du Québec (FRSQ) – a non-profit-funding organisation in Quebec that supports health research and researchers in Quebec. His funding was renewed in 1982, and Lebel received another Scholarship (Senior I and II) Research Award for the following four years, again with a recognised speciality in nephrology. In 1986, Lebel was hired by the University of Laval as an associate professor.

18.3.2 Previous research

In the early 1980s, Lebel's research focused on the mechanisms of essential and secondary hypertension. Due to his nephrology training, his research focused on the kidney, which was known to play a key role in maintenance of blood pressure. He started studying body fluid volumes because the literature was beginning to indicate that hypertension was highly related to sodium metabolism and fluid volumes. To advance the research knowledge, Lebel and his team decided to study the role of vasoactive compounds within the kidney.

Lebel claimed his research interests have always been orientated towards pathogenetic mechanisms in the areas of pharmacology and physiology as a result of his clinical training.

18.3.3 Scientific literature

At the time of this project, Dr Lebel was interested in primary and secondary hypertension. As discussed earlier, his views were influenced by Arthur C. Guyton, a famous physiologist of the 1960s, who proposed that the kidney was the organ from which hypertension originates. This idea was central to the team's hypothesis.

18.4 Interface A – project specification and selection

Dr Lebel wrote the grant application with input from John Grose, a co-applicant. The focus of this grant was to look at the effect of vasoactive substances on hypertension, which was part of the broader research programme ongoing in the PI's laboratory at the time. This funding enabled the research team to investigate the following vasoactive agents:

- angiotensin II
- noradrenaline (which has a dual role as a hormone and neurotransmitter)
- eicosanoids
  - prostaglandin F2 (PGF2)

- prostaglandin E_2 (PGE_2)
- prostacyclin I_2 (PGI_2)
- thromboxane A_2 (TXA_2).

Specifically, the team proposed to investigate:

- renal eicosanoid production during basal conditions and during infusion of pressor doses of noradrenaline
- whether renal eicosanoid production attenuated the noradrenaline-induced renal vasoconstriction similarly in two groups (patients with borderline hypertension and normal controls)
- whether the induced release of vasodilatory PGs (PGE_2 and $PGF_{2\alpha}$) can attenuate the degree of renal vasoconstriction
- the interaction of haemodynamic and hormonal factors with water, electrolyte and metabolite excretion during systematic infusion of pressor doses of noradrenaline
- possible variations in the phosphorylation patterns of specific intracellular proteins during modulation of PGI_2 secretion in cultured bovine aortic endothelial cells.

The funding from the MRC supported *in-vitro* studies using cultured bovine aortic endothelial cells and human studies. For the human studies, subjects were hospitalised in the Metabolic Unit of the Hôtel-Dieu de Québec. To measure hormone levels, blood samples were drawn after overnight recumbency. Urine samples to determine urinary electrolytes were collected at the same time daily. Renal function studies and infusion of noradrenaline were performed on the fourth day. The team monitored effective renal plasma flow; renal vascular resistance; glomerular filtration rate; filtration fraction; fractional sodium excretion; systolic, diastolic and mean blood pressure; pulse rate and plasma concentrations of noradrenaline and adrenaline.

Approximately two months after the first protocol, a sample of the initial group of subjects were re-hospitalised and submitted to the same renal function studies but were infused with AII. The team monitored inulin (glomerular filtation rate), p-aminohippurate (renal blood flow), sodium, potassium, chloride and osmolality through urine samples and plasma concentrations, as well as sodium excretion and arterial pressure. Plasma and urine samples were then assayed for inulin and p-aminohippurate levels. Radioimmunoassays were used to determine plasma renin activity, plasma AII levels, urinary prostaglandin levels and plasma aldosterone levels. A radioenzymatic assay determined plasma catecholamines.

This project occurred during an era of radioimmunoassays. The team developed all of their own assays, although nowadays researchers typically buy commercial kits. The team also developed a purification step and the antiserum required to develop the assays. In doing so, the team took measurements that involved a long and very tedious process of extraction, recovery, purification and, finally, the radioimmunoassay.

As the project was rather fundamental in nature, regarding the mechanisms of high blood pressure, patients were not provided with direct feedback. Study subjects may have read

the resulting publications, although Lebel thought this was unlikely. Lebel stated that the subjects would have benefitted from the extra care they received.

The PI reflected that the grant review system is generally good and reviewers can propose new helpful approaches. He recalled having both positive and negative feedback, although he did not recollect the specifics and the Canadian Institutes of Health Research (CIHR) formerly the MRC, did not retain these. Dr Isenring recalled that the proposed methods were appropriate, although he was not involved in drafting the grant application. Isenring stated that 'Lebel always has very clear-cut objectives and he sticks to the primary objectives' (Isenring interview, 2008).

18.5 Stage 1 – inputs to research

18.5.1 Funding

The grant entitled 'Vasoactive Substances and Pathogenesis of Essential Hypertension' was funded by the MRC from 1989 to 1991 with an amount of Can$99,019; Can$78,474 was initially requested. The PI had applied for three years of funding, but he received only two years of funding in his first attempt as this was a continuation of a long-term research programme.

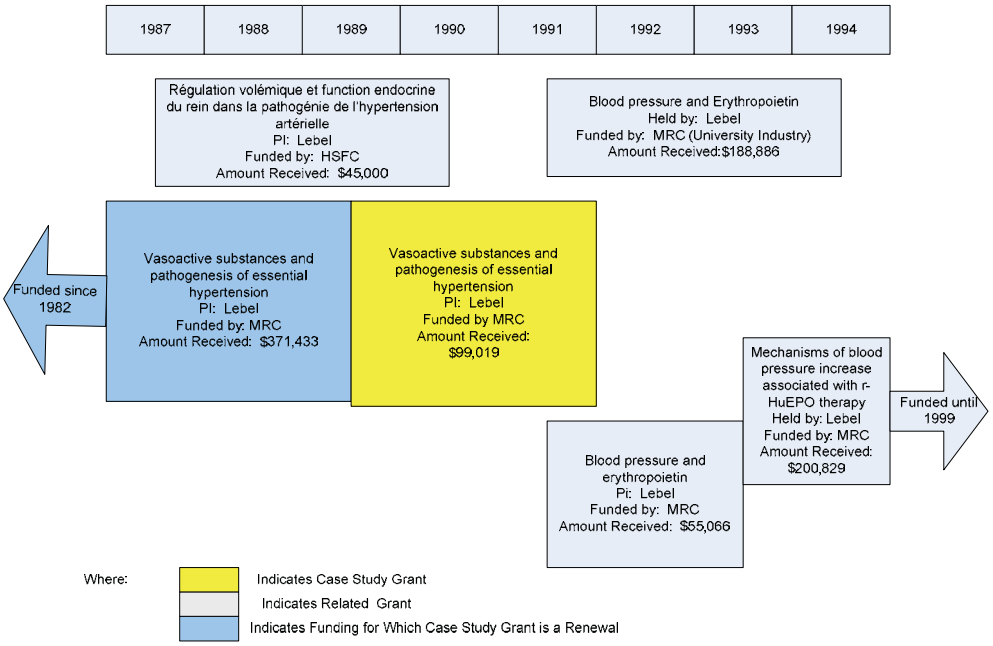
Dr Lebel acknowledged that there was a small overlap between the MRC grant and another grant from the Heart and Stroke Foundation of Quebec (HSFQ), which is described below. The amounts of HSFQ grants are nominal, typically Can$15,000–20,000, which was approximately how much it costs to hire one student. More valuable than the grant dollars is the recognition from the foundation, which is considered prestigious and can help bring in additional funding (Lebel interview, 2008)

Dr Lebel also claimed that some of this money was used to advance other areas of research and interest within his group to expand their research programme. Lebel said, 'if you only investigate what you've written in the grant you cannot move ahead or develop other things' (Lebel interview, 2008). For example, ET-1 was discovered in 1988 and was believed to be a very important compound for their research at the time. Although it was not included in the grant proposal, the money from the MRC supported the development of an assay to be used with ET-1.

Dr Lebel said he thought he had enough funding to complete his proposed project. Lebel said that without the MRC and HSFQ grants it would have been impossible to complete this project. Dr Isenring echoed this comment (Isenring interview, 2008). Other sources of funding potentially available to the research group were the Kidney Foundation and industry. Lebel also said that there was, and still is, local money available in competitive funding from the university, the hospital and the department.

Figure 18-1 shows other funds held by the PI plus and minus a two-year window spanning the case study grant.

Figure 18-1 Related funds held by the PI from 1987 to 1994



18.5.2 Facilities

This research was conducted at Laval University and its associated hospital, L'Hôtel-Dieu de Québec. Le Centre de Recherche de L'Hôtel-Dieu de Québec was founded in the late 1970s and early 1980s, with a vision of orienting its research around the clinical vocations of the hospital. The general aim of Laval's health research is the development of clinically relevant knowledge and the training of young researchers and students. L'Hôtel-Dieu de Québec is a tertiary medical centre that occupies a strong role in oncology, nephrology and cardiology. L'Hôtel-Dieu de Québec was and remains the only centre for dialysis or transplantation in eastern Quebec. Dr Larivière referred to the clinical unit at L'Hôtel-Dieu as 'one of the most interesting in Canada and particularly so in Quebec.' He also said that sharing and communication among the researchers at L'Hôtel-Dieu was very good, involving researchers with 'very good academic backgrounds' (Larivière interview, 2008).

This research required a laboratory to measure compounds within the kidney. Dr Lebel recalled that he had a very good laboratory and an experienced rigorous biochemist working with him. Dr Isenring recalled, however, that the facilities were suboptimal, as space was cramped, refrigerators were in the halls and the security was not stringent. Lebel's team moved into the modern facilities of the expanded research centre in 1992.

Dr Lebel said that his research team, which included research nurses, was very well organised within the hospital. Called the Metabolic Unit, his team was able to study patients under strict, controlled conditions. Lebel's patients were referred to him by colleagues in the surrounding area who were unable to measure the same compounds within the kidney themselves. Lebel's team was 'at the forefront of the science' (Lebel interview, 2008), being able to conduct clinical studies and fundamental experiments. Lebel explained that the research programme meant that he and his team had access to these techniques and equipment that were unavailable at the hospital and other clinics.

18.5.3 Research team

The PI said that the team consisted of strong researchers and the team functioned well. Dr Lebel, a clinical scientist, together with his colleague and co-applicant John Grose, a biochemist, 'made a good team' (Lebel interview, 2008). At the time, John Grose, PhD, was a professor at Laval University; he is now retired. Lebel also said that there is great collaboration between basic and clinical researchers at Laval, remarking that this was one of the team's strengths.

The research team formally consisted of Dr Lebel and the co-applicant John Grose, who worked with Lebel to develop the technique to measure renal haemodynamics and vasoactive substances and to conduct fundamental experiments using cell culture systems. Dr Isenring claimed that this research could not have been done if these techniques had not been developed. Many students and collaborators were also involved in the project and are briefly described below. Input was received from clinicians and basic scientists:

- Dr Paul Isenring was a resident in internal medicine with an interest in nephrology. Dr Lebel approached him to ask if he was interested in participating in this study. Isenring recalled that his role was to evaluate patients, collect data and enter the data for computer analysis. He shared his work with a 'meticulous' research nurse who was also heavily involved in this project. He was supervised by the nurse and Lebel, who said that he had approached Isenring because he thought he had the potential to be a good researcher.
- Alain Milot was a resident at the time. Dr Milot is now a researcher in the department of medicine at University of Laval.
- Monique Richer was coached by Lebel at the University of Laval and now holds a doctorate in pharmacology. In the early 1990s, she was working on a masters degree.
- Pierre Falardeau was a collaborator from CRIM who had access to mass-spectrometry equipment and was able to measure a metabolite for the team.
- Luc Caron was a professional assistant and masters of arts student.
- Guy Drapeau was a collaborator within the team's research centre. Lebel referred to him as a peptide specialist who was able to develop specific molecules.
- Iris Kingma was recruited to the University of Laval at the time of this grant after completing her PhD at Calgary. She became very involved in this project, officially joining the team in 1994.
- Serge Langlois is a nephrologist who contributed by recruiting and referring patients. He was not involved in the laboratory aspects of the project.

Dr Lebel mentioned the difficulties he faces in recruiting and retaining people. The competition has been fierce from the University of Toronto, an institution with a lot of money and thus opportunities. Lebel claims he has been lucky in his retention of bachelor of science students who continue on for a PhD. He says he has spent a lot of time over the years training and mentoring students.

18.5.4 Other facilitators

Dr Lebel said that other researchers at L'Hôtel-Dieu de Québec were always willing to collaborate with him because of his clinical skills and access to patients and clinical samples. Dr Larivière commented that Lebel was well known and connected to other nephrologists, meeting them regularly at seminars and meetings and sharing information.

18.6 Stage 3 – primary outputs from research

The studies supported by the MRC grant suggested that PGs may have a role in modulating the renal haemodynamic effect of vasopressor substances. The team's research showed that people with pre-hypertension or borderline hypertension had lower total blood volume and lower extra-cellular fluid volume; there was an inverse relationship between blood pressure and volume. Specifically, the team found for the first time that young people who have borderline hypertension have a defence mechanism in their kidney that fights to delay the hypertension as long as possible. Lebel, who is known to say to his colleagues that the kidney is the heart of hypertension, explained that the kidney is a very active organ, as it is responsible for volume control and excretion of sodium, among other substances (Lebel interview, 2008).

In addition, the team found that noradrenaline and catecholamines usually induce renal vasorestriction, but all of these responses were found in patients that were treated with non-steroidal anti-inflammatory drugs (NSAIDs), which were used by the team as an antagonistic tool to test the role of the hormones and other compounds on renal haemodynamics. Lebel and his team were one of the first groups to show how the kidney can defend itself when patients are taking NSAIDs, which essentially block PGs.

18.6.1 Knowledge production

The PI identified the following papers as directly related to the funding received by the MRC from 1989 to 1991, although it must be noted that all of these papers also relate to a wider research programme ongoing within the laboratory at the time:

1. Lebel, M., J,H. Grose and P. Falardeau, 'Modulation of Renal Hemodynamics by Renal Eicosanoids During Vasopressor Infusions in Man', *Prostaglandins, Leukotrienes and Essential Fatty Acids*, Vol. 35, 1989, pp. 41–49.
2. Grose, J.H., L. Caron, M. Lebel and J. Landry, 'Prostacyclin Secretion and Specific Intracellular Protein Phosphorylation', *Advances in Prostaglandin, Thromboxane, and Leukotriene Research*, Vol. 21A, 1990, pp. 145–148.
3. Lebel, M. and J.H. Grose, 'Renal Eicosanoids and Renal Hemodynamics in Early Borderline Hypertension', *Clinical and Investigative Medicine*, Vol. 14, No. 6, 1991, pp. 525–534.
4. Grose, J.H., L. Caron, G. Drapeau and M. Lebel, 'The Regulation of Prostacyclin Secretion in Endothelial Cells', In: Bailey, J.M., ed., *Prostaglandins, Leukotrienes, Lipoxins and PAF*, New York: Plenum Press, 1991.

5. Isenring, P., M. Lebel and J.H. Grose, 'Endocrine Sodium and Volume Regulation in Familial Hyperkalemia with Hypertension', *Hypertension*, Vol. 19, No. 4, 1992, pp. 371–377.
6. Isenring, P., M. Lebel, P. Falardeau and J.H. Grose, 'Eiconsanoid Modulation of the Norepinephrine Effect on Blood Pressure and Renal Hemodynamics in Humans', *Prostaglandins, Leukotrienes and Essential Fatty Acids*, Vol. 54, 1996, pp. 59–64.

The first paper acknowledges funding from the MRC and the Canadian Heart Foundation (Lebel, Grose and Falardeau, 1989). This paper presents the first attempt, nationally, to directly relate renal haemodynamic changes to renal PGs under acute pressor challenges (AII and noradrenaline) in normal human subjects. An interesting and original finding of the study, which involved eight healthy men, was the constant elevation in urinary PGF2α observed in all subjects during the administration of both vasopressors, which suggests that PGF2α is the most sensitive index of noradrenaline- and AII-induced release of renal PGs and that these vasoactive hormones induced profound changes in the intrarenal synthesis and/or catabolism of these PGs. The data obtained from this study also demonstrated the importance of measuring PGF2α and PGE_2 when studying the influences of a given stimulus on the renal production of PGs. The team concluded that their data showed that renal eicosanoids may modulate the effect of acute vasopressor administration on renal haemodynamics and therefore on electrolyte excretion. Noradrenaline and AII were thought to exert distinctive regional effects on the production of PGs by various compartments within the kidney. PGI_2, levels of which were observed to increase, was believed to be compensatory and proportional to the plasma levels of the vasopressors infused and to the increase in renal vascular resistance. The capacity of the kidney to produce primary PGs was thought to be involved in attenuating the renal haemodynamic alterations induced by the administration of vasopressors.

The second paper listed above is an example of how results in one field can affect another field (Grose et al., 1990). Landry was a cancer researcher, while Lebel's team focused on cardiovascular disease; however, both were interested in the mechanisms of abnormal cells and were focusing on PGI_2. The team investigated possible variations in the phosphorylation patterns of specific intracellular proteins during modulation of PGI_2 secretion in cultured bovine aortic endothelial cells and their associated relationships. This paper was the first to report globally the implications of the prostacyclin family in the modulation of PGI_2 secretion, possibly though the dynamic equilibrium of the phosphorylation states of the pre-existing proteins within the cell. The authors found that the secretion of PGI_2 is modulated by different protein kinase C (PKC)-sensitive mechanisms.

In 1991, Lebel and Grose took the results obtained in the first paper further by studying ten young patients with borderline hypertension to investigate the role of renal eicosanoids on renal haemodynamics and electrolyte excretion by infusing patients with pressor doses of noradrenaline (Lebel and Grose, 1991). In this study they found that renal haemodynamic changes tended to be more pronounced in those with borderline hypertension compared with the control group of 13 subjects. The results demonstrated that, in both groups, pressor infusion of noradrenaline induced significant modifications in

renal haemodynamics and urinary excretion of electrolytes and eicosanoids. The vasodilatory component of the renal eicosanoid system seemed hyper-responsive in borderline hypertension, which may represent an early antihypertensive defence mechanism. The team thought that this increased response to renal vascular resistance was likely an early mechanism that would fail progressively in patients who eventually develop established hypertension.

In the 1991 paper by Grose et al., the team reported an investigation of the relationship between intracellular protein phosphorylation and the principal signalling pathways during modulation of PGI_2 secretion in cultured bovine aortic endothelial cells (Grose et al., 1991). Through this study the team found that agonist-induced PGI_2 in endothelial cells occurs in the presence of increased phosphorylation of intracellular proteins and concluded that PGI_2 secretion in endothelial cells is controlled by complex mechanisms.

The paper authored by Isenring, Lebel and Grose (1992) again investigated the role of eicosanoids in modulating the effect of noradrenaline on blood pressure and renal haemodynamics during administration of noradrenaline. Experiments involved eight healthy volunteers, and the team observed that pressor doses of noradrenaline induced marked alterations in renal haemodynamics and concomitant increases in eicosanoid excretion rates. The production of vasodilatory PGI_2 was observed to be 2.7 times higher than that of the constrictor thromboxane. This investigation demonstrated the important role of the eicosanoid system in easing the systemic and renal haemodynamic vasoconstrictor effects of noradrenaline in normotensive control subjects. During administration of noradrenaline, PGs are predominately produced relative to the combined constrictor effects of noradrenaline and thromboxane.

The final paper listed above was not part of the research funded by the case study grant, but was a spin-off study (facilitated by the MRC funding) that allowed the team to pursue this research (Isenring et al., 1996). In this project the research team studied three patients, two of whom were related, with familial hyperkalaemic acidosis and hypertension (FHH) syndrome, also known as Gordon's syndrome. Using previously developed tools and techniques, the team examined the patients' endocrine factors, which control body fluid homeostasis and blood pressure under controlled conditions, in an effort to characterise these very rare cases. This paper concluded that FHH syndrome seemed to be related to an inherited tubular defect in potassium handling, with relatively normal endocrine sodium regulatory mechanisms. The body fluid compartment dynamics resembled subgroups of mineralocorticoid excess and low renin essential hypertension. This study is an example of how grant funding can open additional avenues that would otherwise have been impossible.

Bibliometric analysis was conducted on three of six publications identified by the PI as directly related to the case study grant. Publications have been excluded from the analysis when the publication type is not a 'primary research publication' (ie an article, review or note). One paper (Lebel, Grose and Falardeau, 1989) was excluded due to misinformation concerning the start date of the case study grant. This publication will be included in an update of the bibliometric analysis. Three of the four additional articles identified by the PI as indirectly related to the grant of interest have been included in the citation analysis.

Table 18-1 Publication output and impact of directly related publications[1]

Number of journal articles:	3
Number of articles included in citation analysis:	3[1]
Total number of citations (all papers):	16
Aggregate relative citation impact:	0.17 (Class II)
Self-citations:	19%

Distribution of publications across relative citation impact classes:

	Class I (uncited)	Class II (>0 and <0.8 citations)	Class III (0.8 to 1.2 citations)	Class IV (1.2 to 2.0 citations)	Class V (>2.0 citations)
Number of publications		3			
Proportion of total output		100%			

Most highly cited publication[2]:	Isenring, P., M. Lebel, P. Falardeau and J.H. Grose, 'Eiconsanoid Modulation of the Norepinephrine Effect on Blood Pressure and Renal Hemodynamics in Humans', *Prostaglandins, Leukotrienes and Essential Fatty Acids*, Vol. 54, 1996, pp. 59–64
Times cited:	6

18.6.2 Dissemination

The main methods of dissemination were through publications and presentations. The PI said that he and/or his team members always presented in the province of Quebec, throughout Canada and abroad. Dr Lebel presented the findings at the Canadian meeting of the Royal College and Clinical Investigations Society, which at the time included all subspecialties of the royal college. Specialty groups have since split from the college to form the Canadian Hypertension Society and the Canadian Cardiovascular Society. Approximately 4000 people attend the Canadian Cardiovascular Society meetings annually. Dr Isenring recalled presenting their work at various meetings across Quebec and Canada, including the Canadian Hypertension Society meeting. Lebel said that the students presented various poster presentations, nationally and internationally, as part of their training.

In 1990, the International Society of Hypertension met in Montreal. It was the first meeting of the society in Canada, and the team was able to present their findings to an international audience within Canada. Dr Lebel also organised a satellite symposium entitled 'The Kidney in Hypertension', which took place in Quebec City after the Montreal meeting. It was well attended, involving 250 people from 18 countries. Lebel was a keynote speaker at this symposium. The proceedings were published in a regular issue of *Clinical and Investigative Medicine* (Symposium, 1991). Lebel was also a speaker in

[1] In addition, four publications were indirectly linked to this grant. Three of these publications were indexed in Web of Science and received 43 citations in total, giving a relative citation impact of 0.47. The publications were in relative citation classes I, II and IV, and the self-citation rate was 33 %.

[2] Citation count extracted April 2009.

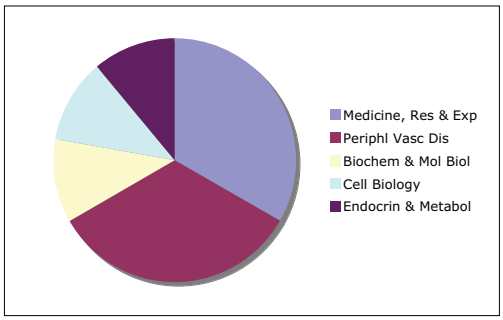
Australia in 1999 at the first international meeting on primary aldosteronism. This presentation was later published in 2001 in the journal *Hypertension* (Agharazii, 2001).

Dr Lebel has also disseminated his findings through continuing medical education (CME) seminars and workshops, which reach general practitioners, specialists and occasionally even lay people. His work has been covered locally in the media via the University of Laval's journal.

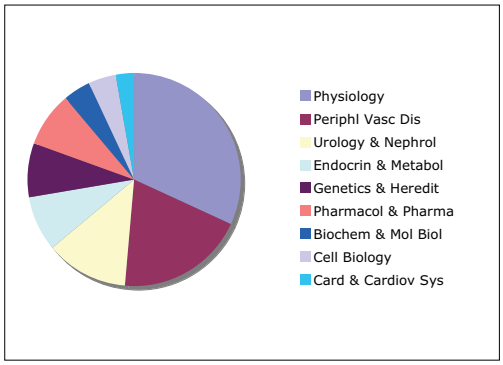
The bibliometric analysis also investigated knowledge diffusion, as shown in Figure 18-2. Again this analysis includes three of six publications identified as directly relevant to this grant by the PI. This analysis shows that Dr Lebel and his team most commonly publish in the areas of 'medicine, research and experiments' and peripheral vascular disease. Their work is most commonly cited by those working in physiology or peripheral vascular disease in the United States and United Kingdom.

Figure 18-2 Knowledge diffusion: fields of research of output (a), fields of citing papers (b) and countries of citing papers (c)

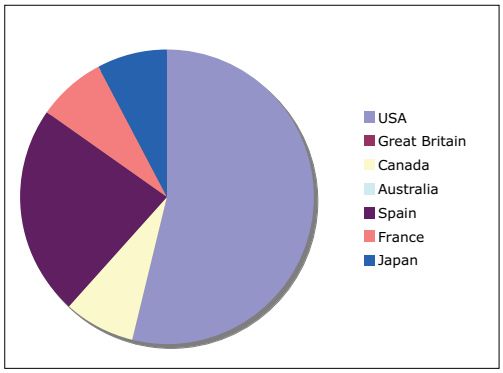
(a)



(b)



(c)



18.7 Training and capacity development

Dr Lebel claimed that this grant, as a part of the larger research programme, helped recruit researchers to his laboratory, had a positive influence on the reputation of his group and the university and expanded the research group. For instance, Dr Isenring, who had little research experience in the early 1990s, now has his own independent laboratory and works at the University of Laval as a clinician scientist. Iris Kingma was recruited at the same

time through the previously mentioned symposium in Quebec City and became a professor at the University of Laval.

Dr Lebel did not feel that the case study grant led to improved physical infrastructure. However, he did believe that this grant assisted the team in acquiring an equipment grant at the same time. Lebel explained that one is more likely to get an equipment grant to assist an already funded project. Lebel also felt that this grant helped the team in acquiring subsequent grants, such as the MRC University:Industry Grant, which was awarded from 1991 to 1994.

Overall, Lebel claimed that, although this was a small grant, there were great returns. For instance, their assays, which were based on blood and tissue samples, were very helpful to other researchers. It was a very original technique at the time, and the team taught their methods to various collaborators and hospital biochemists from Montreal, other parts of Quebec and Toronto (Lebel interview, 2008)

The PI also spoke of the members of the research team, saying that his collaboration with John Grose was mutually beneficial to both of their careers. Dr Lebel said that Dr Isenring, who was at the heart of the project, carried on to complete a PhD at Yale. He was later recruited back to the University of Laval, where he is now a well-established clinician scientist. He has many grants and was awarded the Canada Research Chair in Molecular Physiology for work on cation-chloride co-transporters two years ago. Lebel said he 'is now king' (Lebel interview, 2008). Isenring said that Lebel had realised his interest in research and helped him to participate in this research project. This involvement reassured him that his decision to pursue a career in research was worth it. He continued by saying, 'I don't think I would have pursued research if I hadn't participated in this grant, even though I was interested in research. I had a lot of coaching from Lebel' (Isenring interview, 2008). Although Isenring no longer investigates the pathogenesis of vasoactive substances, he is still interested in renal haemodynamics. Isenring continues to collaborate with Lebel on research projects and maintains a clinical practice in nephrology.

Dr Isenring thought that the most important thing that came from this grant for him and other people involved in this research project was that it generated other types of research, creating new careers. Dr Lebel claimed that his research programme has developed over the last 15 years and now involves a very interesting group of researchers. Lebel, as the team leader, received a new emerging team (NET) grant from CIHR in 2002, which involved an amount of Can$1,250,000 over five years to study cardiovascular health and renal failure. Lebel claimed that this team grant has stabilised his group and facilitated further recruitment. He also claimed that receiving the NET funding is a real example of how the MRC grant was useful in building his programme, saying 'we are recognised by the CIHR and this helps with further funding' (Lebel interview, 2008).

18.8 Benefits to future research and research use

After the MRC grant, the team changed their research focus to secondary hypertension, focusing on patients with kidney failure who develop hypertension related to erythropoietin (EPO) replacement therapy for renal anaemia. As previously discussed, when the kidney is deficient, hypertension can develop. In addition, a deficient kidney

results in a lack of EPO, which is produced in the kidney, causing individuals to become severely anaemic so that they require transfusions every three weeks and therefore are susceptible to viruses. In 1989, EPO became available to treat anaemia. However, an unpredicted side-effect of this therapy was that patients developed hypertension. Researchers did not understand why this happened, as this form of secondary hypertension is seen only in patients with renal failure. The team thus moved their research focus to try to elucidate the mechanism of EPO in hypertension with chronic renal failure. The team made slight modifications to the assays and methods previously developed with the MRC grant and applied them to examine the mechanisms of secondary hypertension related to EPO treatment, as described in a paper by Lebel et al. (1998).

Richard Larivière, who was hired by the University of Laval 2–3 years after the case study grant, claimed he has benefitted from the techniques. Larivière was recruited in 1994 from Montreal, where he had worked with the vasoactive compound ET-1. When he arrived at Laval, he did not bring any antibodies or assays with him. Lebel's team provided him the assay for ET-1, which they had been using to measure ET-1 in plasma and urine, and so began Larivière's first collaboration with Lebel's team. Using the assay, Larivière was able to measure the concentration of ET-I in vascular tissues. He has since used the methods to measure thromboxane and prostacyclin and has published papers with Lebel describing this. These techniques have since been translated to research transgenic animal models and histology.

The team's research on secondary hypertension progressed to animal studies. The team developed a rat model of renal failure and was able to confirm their earlier findings that the kidney has a defence mechanism that was observed to be deficient in rats with renal failure.

As mentioned earlier, a spin-off project made possible by the MRC funding from 1989 to 1991 allowed the team to pursue their interest in genetics and describe individuals with a monogenetic form of hypertension that is extremely rare and specific using the same tools and techniques. About five years after the publication of the paper by Isenring, Lebel and Grose (1992), an international study led by Richard Lifton of Yale University used all published cases around the world to study the genetics of this population. Lifton asked Lebel's team to share serum samples from their patients with FHH. Lebel's team participated in this project, which resulted in a publication in *Nature Genetics* (Mansfield et al., 1997). Lebel said this is an example of how financial support can open new opportunities that otherwise would have been impossible; this study was very significant and would not otherwise have been done.

With the knowledge that essential hypertension is controlled through diet and lifestyle, Dr Lebel and his team made various efforts through the Quebec Hypertension Society to teach patients about the importance of diet, as such information was not readily known at the time.

18.9 Stage 4 – secondary outputs

Dr Lebel thought that this research may have influenced assay kits but recalls that he did not have any time to patent things, referring to the patent process as very difficult and time

consuming. He spoke of some colleagues who were so involved in companies and patents that they did not publish and subsequently lost their grants.

Through his work, Dr Lebel has been on multiple advisory boards. Dr Isenring said that 'in doing his research [Lebel] has acquired a reputation and was often asked to be on hypertension advisory board committees'. Lebel remains well respected in the field of hypertension as evident by his current position as Vice Chair of the Canadian Education Program on Hypertension – a Canadian Hypertension Institution programme. At the time of writing, he was working to prepare the next meeting in Edmonton, which was scheduled for October 2009. Every year this group updates the recommendations for the treatment of hypertension; Lebel is the co-chair of the task force on treatment recommendations (the recommendations were published in the *Canadian Journal of Cardiology* in 2008 (Canadian Hypertension Education Program, 2008)). Although not directly related to this grant, Lebel has also been involved in development of the Kidney and Hypertension Society's guidelines for hypertension therapy due to his expertise and knowledge of hypertension related to renal disease.

18.10 Stage 5 – adoption by practice and the public

The techniques developed by Dr Lebel and his team for measuring vasoactive substances in biological fluids were clinically relevant and are now used at many hospitals in Quebec. Some patients produce too much catecholamines or aldosterone, which cause hypertension. In the 1990s, whenever a patient was suspected to have such a disorder, hospitals across Quebec would send samples to Lebel's laboratory for analysis.

18.11 Stage 6 – broad health and economic outcomes

It is difficult to quantify the health gains obtained by identification of hormonal disorders or the effect of Dr Lebel's work on anaemic patients with kidney disease. No spin-off companies or employment opportunities outside of the laboratory have been created. Lebel has not marketed any products as a result of the MRC funding.

18.12 Payback

The payback framework describes the process from research to several categories of impact. The categories allow us to compare across different case studies. Table 18-2 shows, in point form and by impact category, some of the impacts, described more fully above, that have emerged from this grant.

Table 18-2 Payback

Payback category	Impacts from case study
Knowledge production	• Six directly related peer-reviewed articles • Assay development
Research targeting and capacity building	• Knowledge transfer within laboratory to colleagues, students and postdoctoral researchers • Valuable spin-off projects studying secondary hypertension and monogenic forms of hypertension • PI's laboratory hosted biochemists from Quebec and Ontario to teach them their techniques • Grant and overall programme has assisted in recruitment and attaining subsequent funding • All work has led to NET grant that has facilitated greater recruitment and retention
Informing policy and product development	• PI involved in annual Canadian recommendations for treatment of hypertension
Health and health sector benefits	• PI's laboratory responsible for identifying hormonal overproduction for hypertensive patients in Eastern Quebec using their assay technique • May have influenced outcomes for patients with renal anaemia receiving therapy with EPO
Broader social and economic benefits	• Not applicable

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CHAPTER 19 Immunoelectron microscopy of amine and peptide synapses on sympathetic preganglionic neurons

19.1 Overview of case study grant

In 1988, Dr Ida Llewellyn-Smith (the principal investigator (PI)) received a grant-in-aid of Aus$137,819 for two years from the National Heart Foundation of Australia (NHFA) to study 'Immunoelectron Microscopy of Amine and Peptide Synapses on Sympathetic Preganglionic Neurons' (grant reference: 2356). The research project was conducted at the Centre for Neuroscience at the Flinders Medical Centre in South Australia, where the PI had access to high-quality facilities and expertise, including the renowned Professor John Chalmers (Chair of Medicine at Flinders University). The project used advanced electron microscope techniques to identify the brain and spinal cord pathways responsible for control of blood pressure; this represented a key breakthrough in cardiovascular research, as it established these linkages at the synaptic level.

The project added precision and certainty about the function and role of these pathways, helped to lay the building blocks of our current understanding of sympathetic nervous control of the cardiovascular system and developed a series of techniques that have been used to facilitate research in this and other areas. This helped give the Flinders Medical Centre greater confidence to pursue new avenues of research in relation to the sympathetic nervous system and how it controls the heart, blood vessels and other organs. The project was also very important in establishing the PI as an independent investigator in her own right, opening up avenues for further funding, particularly from the National Health and Medical Research Council (NHMRC).

19.2 Introduction to case study

19.2.1 Overview

The research for this grant focused on understanding the role of the central nervous system (brain and spinal cord) in blood pressure regulation. It explored the role of nerve pathways in autonomic functions, including blood pressure control. Autonomic functions are those over which we have little, if any, conscious control.

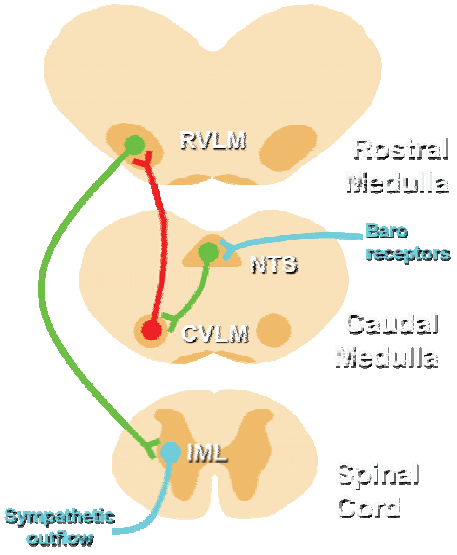
The research from this case study established that the nerves in the brain are principally responsible for running the nerves in the spinal cord that control blood pressure. The project found that spinal nerve cells that regulate blood pressure and other autonomic functions in fact receive some two thirds of their input from the brain.

Changes in the activity of sympathetic neurons (which facilitate communication between the peripheral and central nervous systems via chemical synapses in the ganglia) elicit profound effects on a variety of target organs, including the heart and blood vessels, as well as the release of hormones such as adrenaline in the sympatho-adrenal response of the body commonly known as the fight-or-flight response. Two groups of neurons were understood to be involved in these responses:

- sympathetic postganglionic (or postsynaptic) neurons, whose cell bodies are situated in the peripheral ganglia (knot-like nerve-cell groupings)
- sympathetic preganglionic (or presynaptic) neurons, whose cell bodies lie in the intermediolateral cell column of the spinal cord (the grey matter that runs through the thoracic and lumbar sections of the spine).

The activity of these neurons is modulated by bulbospinal pathways, which connect the medulla (brain stem) with the spinal cord, as shown in Figure 19-1.

Figure 19-1 Nerve pathways in the brain and spinal cord that are involved in controlling blood pressure

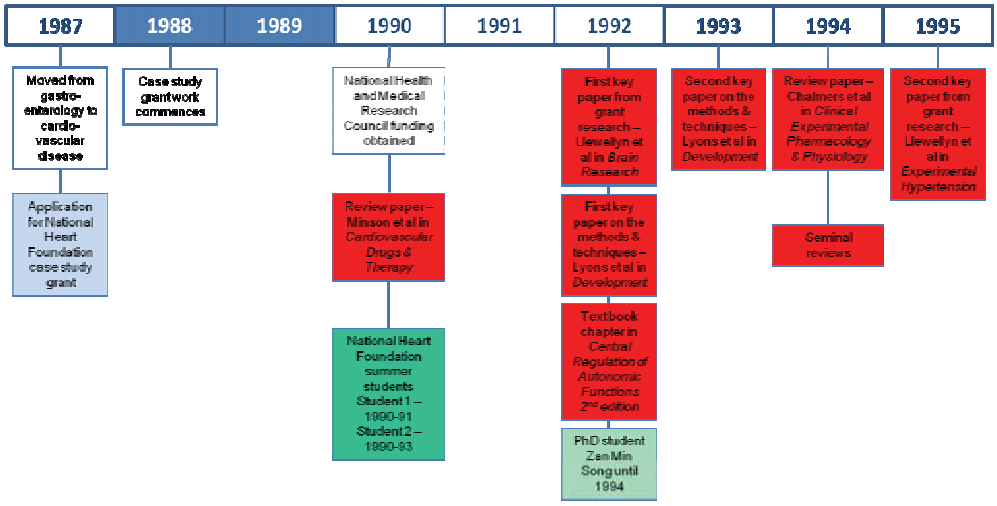


Of particular importance in the control of blood pressure are the vasopressor neurons in the rostral ventrolateral medulla. Increases in blood pressure and sympathetic nerve activity occur when the rostral ventrolateral medulla is stimulated, either electrically or chemically, whereas inhibition of the area results in decreases in blood pressure and activity.

The goal of the research for this case study was to establish the existence of anatomical circuits from the rostral ventrolateral medulla to sympathetic preganglionic neurons in the intermediolateral cell column in the spinal cord.

Figure 19-2 illustrates the timeline of key events relating to the grant. Clear knowledge outputs from the grant are shown in red and clear training outputs in turquoise. Where the impact of the grant on particular outputs is debated, a lighter shade has been used. The years coloured blue correspond to those for which money from the grant was supplied.

Figure 19-2 Timeline illustrating key events relating to the grant



19.2.2 Understanding the broader research field

Investigation into the spinal nerve pathways responsible for regulating the activity of blood pressure had been limited up until this research. Dr Llewellyn-Smith said, 'They were something of a black box' (Llewellyn-Smith interview, 2008).

Findings from previous immunohistochemical studies had suggested that different functional groups of sympathetic preganglionic neurons may have anatomically distinct inputs from the medulla. In addition, functional studies in rats had demonstrated that the neurotransmitters serotonin, neuropeptide Y (an amino acid that is important in regulating food intake and that also occurs in the adrenaline cells in the medulla) and substance P (a neuropeptide that is present in some serotonin nerve cells in the medulla and that influences pain and inflammation responses) are involved in vasopressor (blood vessel constriction) responses involving adrenaline, noradrenaline and phenylephrine.

It was felt essential to provide electron microscopic evidence for the presence of adrenaline/neuropeptide Y and serotonin/substance P synapses on sympathetic preganglionic neurons in order to prove that neurons in the ventrolateral medulla connect with these spinal neurons.

19.2.3 The case study approach

The case study based on this research grant involved a combination of: a review of documentation for the grant; one face-to-face interview with the PI on the project; interviews with a senior investigator and other associated investigators; a review of the PI's curriculum vitae; and documentary analysis of key citing papers, publications and conference abstracts arising from it.

19.3 Stage 0 – topic/issue identification

In short, the project was an opportunity for Dr Llewellyn-Smith to apply specialised techniques in a new field to create a niche and to assist in development of her independent research career. The project also brought important new skills and techniques to the field of cardiovascular disease research and to the research team. Llewellyn-Smith summarised it as 'people, place and opportunity', combined with her confidence to be able to apply the skills and techniques she had developed in gastroenterology.

The idea for the project arose from discussions with key people in the Department of Medicine at Flinders University, where the PI was based. The move to cardiovascular disease research and to undertake the project was prompted by four key factors:

1. timing – the PI had a desire to obtain her own funding and run her own laboratory so that she could research her 'own ideas'
2. knowledge gaps and valuable skills of the PI developed in another area of research – the PI's skills developed in another area of research were seen as being important in the identification of the brain and spinal cord pathways responsible for the control of blood pressure
3. mentor and initial grant providing track record in new field of research – the research group leader provided mentoring to obtain the initial research funding, access to facilities and a research team the PI knew and could work with well
4. challenging disease-focussed research and solving big clinical problems.

19.3.1 Timing

Dr Llewellyn-Smith had been a muscle biochemist and then spent up to nine years becoming an increasingly senior postdoctoral researcher working in neurogastroenterology (the study of the interactions of the central nervous system and the digestive system and its disorders) – a period she describes as 'the beginning of a golden period. Neuroscientists were just starting to use antibodies to trace nerve pathways and the lab I was in was one of the very first to do that. So there was an explosion of knowledge and it was incredibly interesting time. In five years I went from a knowledge base of zero to people seeking me out to talk to me' (Llewellyn-Smith interview, 2008).

After nine years, Dr Llewellyn-Smith felt that the golden age was declining in regard to breakthrough research in neurogastroenterology and a phase of 'dotting 'i's and crossing 't's' was beginning. Finding a new research area would have meant leaving the hospital-based institution, Flinders Medical Centre at Flinders University, where she was located. While working in the neurogastroenterology laboratory, Llewellyn-Smith had established expertise in electron microscopic immunocytochemistry (the use of marked antibodies to identify the locale of particular antigens or proteins within a cell). This technique was seen as important for a new area of research in relation to control circuitry for blood pressure. Llewellyn-Smith was also at a stage in her career where she wanted the opportunity to establish her own laboratory and her own research. She said, 'I had this body of expertise which I could just plunk into the completely open area of the spinal cord blood pressure control circuitry. By then I had decided I really did want to be responsible for myself and

get my own money and run my own laboratory and investigate my own ideas rather than somebody else's ideas' (Llewellyn-Smith interview, 2008).

19.3.2 Knowledge gaps and valuable skills of the PI developed in another area of research

In the late 1980s, there was an open niche in relation to understanding of the role of the central nervous system (brain and spinal cord) in blood pressure regulation. Existing histological methods could show that there were nerve endings near other nerve endings but could not show that there was communication between the nerves through chemical synapsis. The techniques applied in this project demonstrated the communication links between neurons, which then allowed the pathways between the brain and the cardiovascular system to be identified. Professor Chalmers said, 'So, what Ida [Llewellyn-Smith] was able to do was to add the dimension of showing that one of the nerves that you traced from the brain stem down to the spinal cord actually made a synapse with a nerve which was going out to a blood vessel. So it got us down to very precise capacity to establish links between brain pathways and outflows (functional outflows), and the ones which interested us were the ones to blood vessels and/or the heart, because we were interested in blood pressure and the circulation. So that's what she got us down to – really got us down to the single cell and the link between structure and function, because we had been able to demonstrate structural juxtaposition but she was able to say there is actually a functional link: they actually talk' (Chalmers interview, 2009).

As noted earlier, Dr Llewellyn-Smith had expertise in electron microscopic immunocytochemistry that was seen as important to this new area of research and could fill a key gap in the research team's skills base. It was therefore proposed that Llewellyn-Smith move from the Department of Anatomy and Histology to the cardiovascular neuroscience research team in the Department of Medicine at Flinders, where she could work on blood pressure control circuits in the central nervous system. Professor Chalmers said, 'What we were looking at was trying to identify and trace the specific pathways through the central nervous system that control blood pressure and how they did it, and in order to advance along that we had to get more precision into our techniques, and so Ida [Llewellyn-Smith] added a huge level of precision and capacity with the electron microscopic approach and its combination with immunohistochem[istry] and neuropharmacological techniques' (Chalmers interview, 2009).

19.3.3 Mentor and initial grant providing track record in new field of research

Although the space, most of the equipment required and the facilities were provided by Flinders Medical Centre, there was no funding for Dr Llewellyn-Smith's salary and associated research costs. She was guided by Professor John Chalmers, the Chair of Medicine at the Flinders University ('who was very savvy about funding'), to apply for a National Heart Foundation of Australia fellowship in order to establish a track record of gaining her own funding, which would then lead to the opportunity to successfully apply for NHMRC funding. Chalmers said, 'She hadn't been independent enough. She had been, if you like, a bit of a 'supertech'…rather than an independent investigator. And when she joined us she became a key independent investigator and her career flourished and she went on to get an NHMRC fellowship. So it was important for her career, important for the group as a whole and important for her contributions to knowledge' (Chalmers interview, 2009).

Dr Llewellyn-Smith obtained the National Heart Foundation of Australia fellowship for this case study in 1988 and 1989 and then moved to the NHMRC fellowship in 1990. She said, 'So really…if I hadn't have gotten the NHF [National Heart Foundation of Australia] funding I don't know where I'd be now!' (Llewellyn-Smith interview, 2008).

19.3.4 Challenging disease-focussed research and solving big clinical problems

Another important factor raised by Dr Llewellyn-Smith in moving to cardiovascular disease and working on the particular research topic was her desire to do clinically relevant basic research. She wanted a disease focus for her basic research and to link it to solving a well-defined important clinical problem. She said, 'Because [the work in neurogastroenterology] was all fairly esoteric…not particularly disease focused, I have always been interested in tying what I do, no matter how far in the future, [to solving a big clinical problem], because all of the research questions that I investigate are really very basic…the information that [I generated] in my very early period in central cardiovascular control is now in the textbooks. It's taken about 20 years to get there' (Llewellyn-Smith interview, 2008).

As indicated earlier, Dr Llewellyn-Smith also wanted to work in a challenging new research niche, where there was little existing knowledge and the potential to have a significant impact. She said, 'When I got into the area of central cardiovascular control, there was so little basic knowledge [and so] I really have spent almost 20 years generating very basic information. It's only in the last three, four or five years that I have [focussed] more on cardiovascular conditions in rat models, like heart failure' (Llewellyn-Smith interview, 2008).

19.4 Interface A – project specification and selection

As already identified, the career stage, skills and techniques of the PI were key motivating factors underlying the grant application.

Professor Chalmers had been a cardiologist who had also moved into basic research. By the late 1980s, he had been working in the area for approximately 20 years and had a very high profile. He provided a clinician and a basic researcher perspective. Professor Llewellyn-Smith said, 'His research was always very basic…it was very much looking at how drugs affected blood pressure when you gave them to the brain' (Llewellyn-Smith interview, 2008).

Up until the case study research project, the cardiovascular research had been mainly focused on the physiological and structural aspects, and understanding of the role of brain chemistry in the control of blood pressure was still very basic. Professor Chalmers was looking for different approaches to investigate the same problem and recognised the power of physiology combined with anatomy to obtain the whole picture. Dr Llewellyn-Smith was identified by staff working in Chalmers' laboratory as a person with a lot of skills and, importantly, a person who had skills to fill a gap in the research group. Llewellyn-Smith said, 'He was very much a "big picture" person. He had an impressive grasp of where the gaps were. And I think he saw that by getting me into the group there was the potential to fill a big gap in knowledge' (Llewellyn-Smith interview, 2008).

The knowledge and experience of Professor Chalmers and the particular skills and techniques that Dr Llewellyn-Smith brought to the project in understanding what was happening at the spinal cord level were specifically indicated in the grant application. The review process had very little impact on the project specification. Llewellyn-Smith observed that this was because it was such a new area and already had the support of Chalmers. Llewellyn-Smith said, 'I mean I was proposing to do experiments that absolutely nobody else in the world was doing, and it was sufficiently sophisticated…and I had [Chalmers'] support' (Llewellyn-Smith interview, 2008).

The committee was overwhelmingly supportive of the application and very enthusiastic about the planned research (Grant-in-Aid Assessment Forms, 1987):

- 'This excellent project is likely to yield significant results.'
- 'The planned studies have a real possibility of increasing the level of understanding of control of sympathetic activity from higher centres'.

There were therefore no major conceptual changes, only some clarifying and fine tuning of the project. For example, queries were raised in relation to:

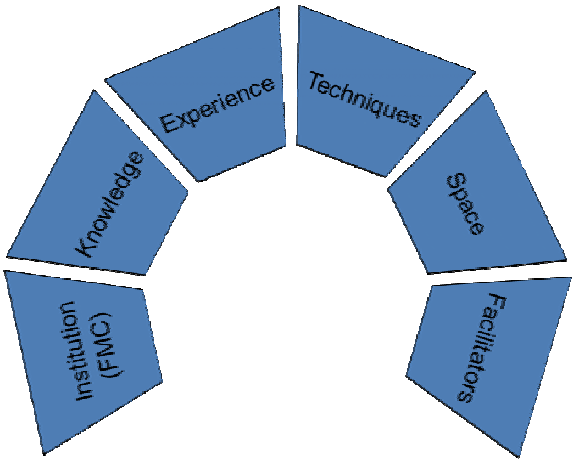
- how the proposed comparison of the inputs to the functionally different types of neurons would be done and whether 'morphometric or other quantitative techniques' could be applied if qualitative techniques failed to yield results
- how the functionally different neurons would be distinguished in the proposed retrograde tracing experiments.

In relation to funding, only two variations were apparent between the application and the grant. The grant was made for two years rather than the requested three years, and the funding for equipment was reduced by Aus$8,735 as the committee concluded that (apart from a diamond knife) the equipment required should already be available at Flinders Medical Centre.

19.5 Stage 1 – inputs to research

In this section we outline the key inputs to the research. These are illustrated in Figure 19-3.

Figure 19-3 Key inputs

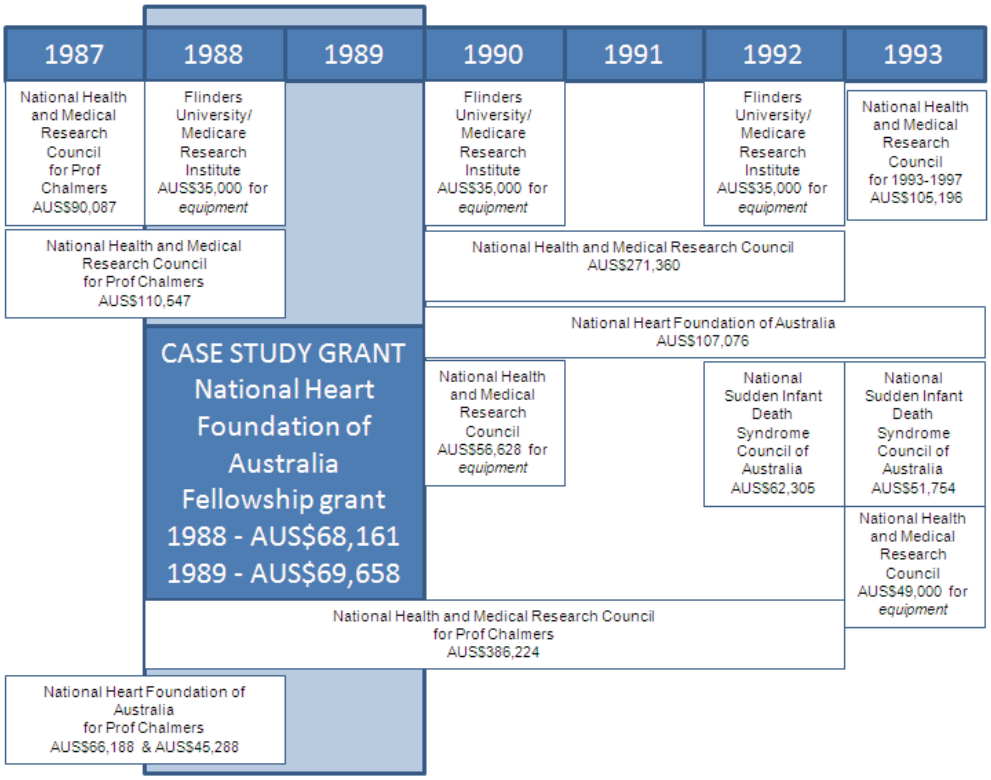


Facilitators were seen as critical and led to providing the time and space for the project. Knowledge, expertise and techniques were equally important, followed by space.

19.5.1 Facilitators

The key facilitator for the research was the grant from the National Heart Foundation of Australia. This totalled Aus$137,819.00 over the two years of 1988 and 1989 and covered Dr Llewellyn-Smith's salary and the basic costs of the project.

Figure 19-4 Funding map for the grant, showing major inputs to the PI's laboratory and sources identified in the grant application over the period 1987 to 1993



Professor Chalmers said, 'I think it [the National Heart Foundation of Australia grant] was critical in enabling Ida [Llewellyn-Smith] to join us, and that helped kick us into a completely new dimension. So it was a very important step in expanding the horizon of our group by going to a new methodology' (Chalmers interview, 2009).

Another key facilitator was that a lot of the equipment, infrastructure, laboratory space and facilities were made available to Dr Llewellyn-Smith in the Department of Medicine at Flinders University. She said, 'So in terms of starting off, I was actually really well off as opposed to moving somewhere else and starting from scratch' (Llewellyn-Smith interview, 2008).

In reputational terms, Professor Chalmers brought significant capital to the project, while Dr Llewellyn-Smith brought important skills, techniques and a good track record in those skills and techniques. Llewellyn-Smith said, 'Within Australia, if you publish with [Chalmers] that meant your name registered in people's heads (Llewellyn-Smith interview, 2008).

19.5.2 Knowledge, expertise and techniques

As noted earlier, Professor Chalmers (Chair of Medicine at Flinders Medical Centre) had pre-eminent expertise and experience in researching blood pressure control, for which he was awarded the Wellcome Medal in 1981, the R.T. Hall Prize of the Cardiac Society of Australia and New Zealand, and the Volhard Medal of the International Society of Hypertension. It was recognised that Dr Llewellyn-Smith would bring particular skills and techniques in understanding how the spinal cord is involved in blood pressure control.

In particular, it was noted in the grant application that Dr Llewellyn-Smith had shown in previous ultrasound studies of the enteric nervous system that a variety of chemically identified types of nerve fibres form synapses on some enteric neurons, indicating the presence of specific connections and the existence of specific pathways. Llewellyn-Smith had also developed a technique for correlating light and electron microscopy to allow the synaptic input of neurons identified using the light microscope to be studied electron microscopically. The correlated light and electron microscopic immunocytochemistry had proved very successful for studying connectivity in the brain. The method that Llewellyn-Smith had previously developed was to be used in the spinal cord to compare the synaptic inputs from different types of immunocytochemically defined nerve fibres to specific subpopulations of sympathetic preganglionic neurons.

As part of the project, there was a need for perfusion (injection of fluid into a blood vessel in order to reach an organ or tissues) of the spinal cord. At the time, Dr Llewellyn-Smith did not have experience in perfusion, but the Flinders Medical Centre, where she was based, was doing perfusion.

Another benefit of the institution being hospital based was that it helped provide a clinical focus to the research and easy access to clinicians who were interested in doing research. This sometimes enabled research that others were not in a position to do so easily. Dr Llewellyn-Smith said, 'One of the great things about being [at Flinders Medical Centre] has always been that if you wanted to do something clinical, the people are right here…so they are willing to talk to you…and they are interested in doing research and in collaborating' (Llewellyn-Smith interview, 2008).

19.5.3 Space, equipment and personnel

The research was undertaken within the hospital-based institution, where there were basic research laboratories, and Dr Llewellyn-Smith had access to a laboratory and other facilities, such as an animal house for the rats being used in her study.

The grant application indicated that there was a transmission electron microscope and ultramicrotomes dedicated to research use at the Flinders Medical Centre, where the project was carried out, and a senior technician who maintained the microscope and supervised the running of the unit. In addition, it was indicated that common service fluorescence microscopes were available, including a light microscope that could be adopted for interference contrast microscopy and a vibratome. Professor Chalmers said, '[Dr Llewellyn-Smith] came into a lab which was, you know, a multidisciplinary lab with physiological, pharmacological, biochemical, immunohistochemical capacity in relation to blood pressure and so on. And so she brought this new technique, but she fitted into a

group which was well established with grants from many other bodies' (Chalmers interview, 2009).

Two other investigators associated with the project were listed in the grant application. One had experience with retrograde tracing methods (the tracing of neural connections from their termination to their source) and light microscopic immunohistochemical techniques (the use of antibodies to target specific peptides or antigens) and would assist with these aspects of the project (four days per month). The other was to be involved in the retrograde tracing experiments and studies on plastic sections, mainly in a consultative role, because of his experience with lectin-gold and semi-thin section immunocytochemistry (one day per month). The grant application also advised that existing technical staff were fully committed to other projects and that there were no staff in the research group laboratory with experience in the preparation of tissue for electron microscopy. However, Dr Llewellyn-Smith recalled in her interview that in the end she had the support of a technical assistant but not a research assistant.

There were weekly meetings of the Hypertension Basic Research Group, which Dr Llewellyn-Smith joined when the case study application was successful. Professor Chalmers said, 'There was an active centre for neuroscience and young people from across different labs. We had a weekly seminar – cross disciplinary, cross group – and people chatted and knew who was doing what in what lab. There [were] about 40–50 people [who] would go to the seminars each week' (Chalmers interview, 2009). As each of the members of the research group were doing different sorts of work and had their own area of expertise, the discussions at the meetings were in relation to general directions, what each member of the group had discovered, any problems that others in the group may be able to help with and tracking spending of funds.

There were few collaborations in relation to this research project beyond the research group due to the highly specialised nature and newness of the research. According to Professor Chalmers, there were no other laboratories in Australia with the same capabilities and only two or three other laboratories around the world had similar technical capabilities but were focused on different areas of research.

Professor Chalmers indicated, however, that two important collaborations strongly influenced the direction and success of Dr Llewellyn-Smith's research. Dr Peter Somogyi (Deputy Director of the Medical Research Council's Anatomical Neuropharmacology Unit at Oxford University, who had previously collaborated with Chalmers) visited Flinders Medical Centre for three months in 1988 and brought his skills in autoradiography to the project. This opened up a new line of investigation for the project in relation to tracing amino acid pathways in the medulla and spinal cord.

There was also an ongoing collaboration with Dr Janusz Lipski at Auckland University in New Zealand to investigate chemically identified synaptic inputs to electrophysiologically characterised sympathetic preganglionic neurons. In 1988, Dr Llewellyn-Smith visited Lipksi's laboratory in New Zealand to further this collaboration, and Dr Paul Pilowsky from Lipski's laboratory would later come to the Hypertension Research Group at Flinders Medical Centre to further his research in this area.

19.6 Stage 2 – research process

The techniques proposed for the research developed by Dr Llewellyn-Smith in gastroenterology were found to be appropriate and effective in this project; however, it also involved developing new skills and techniques. For example, perfusion was critical to the ultimate outcome and the spinal cord was the most difficult part of the central nervous system to perfuse. Llewellyn-Smith said, 'I figured out a few tricks to make sure a lot of fixative got to that critical bit [of the central nervous system], I figured out you needed to be really fast, and once I figured those things out, I was fine' (Llewellyn-Smith interview, 2008).

To develop a method for quantitative electron microscopic immunocytochemistry of the central nervous tissue, Professor Llewellyn-Smith tested the extent to which ethanol treatment would improve the penetration of immunoreagents (antibodies and proteins to test for particular reactions) through vibratome sections (rapidly frozen tissue) preserved in high concentrations of glutaraldehyde without compromising the cellular structure (ultrastructure). The technique made possible quantitative electron microscopic immunocytochemical studies and proved a useful tool for defining synaptic connections in the central nervous system.

The techniques developed and used for the project are now well established, commonly used around the world and described in textbooks (for example, *Central Regulation of Autonomic Functions* (1990) included a chapter on the key methods).

19.7 Stage 3 – primary outputs from research

19.7.1 Knowledge production

The main elements of the research and techniques proposed in the grant application were achieved and successful. This included starting to look at neurochemically identified inputs to sympathetic preganglionic neurons, how to retrogradely label these and other neurons and how to perform immunocytochemistry on the spinal cord at the light and electron microscope levels.

The required techniques were developed successfully, and the evidence generated by the project established the presence of adrenaline/neuropeptide Y and serotonin/substance P synapses on sympathetic preganglionic neurons, proving that neurons in the ventrolateral medulla connect with these neurons.

Dr Llewellyn-Smith indicated that other than publications relating to her earlier work in gastroenterology, virtually all of her published work of approximately 90 articles and notes since then have resulted from the methods pioneered in this grant project – her first on nerve pathways involved in controlling the cardiovascular system.

Eight articles and notes were identified through the bibliometrics analysis as directly attributable to the grant project. Dr Llewellyn-Smith identified Llewellyn-Smith et al. (1992) and Llewellyn-Smith et al. (1995), which outline the research purpose, approach and findings, as the main articles from the grant research. The articles have been cited 83 and 38 times, respectively. Llewellyn-Smith identified Llewellyn-Smith and Minson

(1992) and Llewellyn-Smith et al. (1993) as the main articles providing details on the key methods and techniques used in and developed from the grant research project. The articles have been cited 114 and 139 times, respectively. Other articles attributed directly to the grant project included: Somogyi et al (1989), Pilowsky et al (1990), Llewellyn-Smith et al (1990) and Llewellyn-Smith et al (1990).

As mentioned earlier, the textbook *Central Regulation of Autonomic Functions* (1990) also included a chapter on the key methods.

Dr Llewellyn-Smith identified ten related review articles, including Minson et al. (*Cardiovascular Drugs and Therapy*, 1990), Arnolda et al. (1992), Chalmers et al. (*European Heart Journal* and *Journal of Hypertension*, 1992), Chalmers et al. (1994), Arnolda et al. (1997), Minson et al. (1997), Pilowsky et al. (1997), Arnolda et al. (1999) and Llewellyn-Smith et al. (2006).

A couple of seminal reviews in 1994 cited Dr Llewellyn-Smith's work on the grant project. She said, 'In terms of influencing the way that people thought about the field, my data from this project got out there pretty rapidly actually and started to affect the thought processes of [basic] researchers' (Llewellyn-Smith interview, 2008).

Table 19-1 and Figure 19-5 illustrate the publication output attributed to the case study grant application, its impact and the extent of the knowledge diffusion.

Table 19-1 Publication output and impact[1]

Number of journal articles:	18
Number of articles included in citation analysis:	18
Total number of citations (all papers):	780
Aggregate relative citation impact:	1.61 (Class IV)
Self-citations:	18%

Distribution of publications across relative citation impact classes:

	Class I (uncited)	Class II (>0 and <0.8 citations)	Class III (0.8 to 1.2 citations)	Class IV (1.2 to 2.0 citations)	Class V (>2.0 citations)
Number of publications	1	8	1	3	5
Proportion of total output	6%	44%	6%	17%	28%

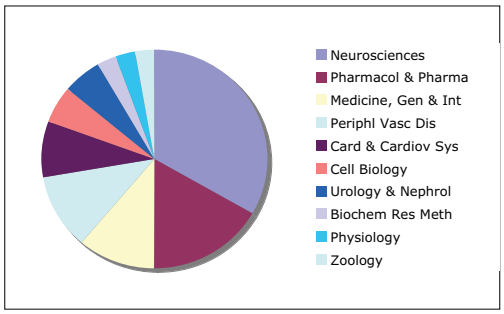
Most highly cited publication[2]:	Llewellyn-Smith, I.J., P. Pilowsky and J.B. Minson, 'The Tungstate-Stabilized Tetramethylbenzidine Reaction for Light and Electron-Microscopic Immunocytochemistry and for Revealing Biocytin-Filled Neurons', *Journal of Neuroscience Methods*, Vol. 46, No. 1, 1993, pp. 27–40
Times cited:	139

[1] In addition, four publications were indirectly linked to this grant. These publications were all indexed in Web of Science and received 202 citations in total, giving a relative citation impact of 1.60. Two were in relative citation impact Class V and one each in Classes II and IV; their self-citation rate was 22%.

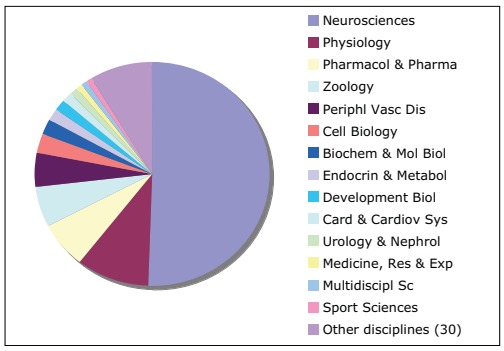
[2] Citation count extracted April 2009.

Figure 19-5 Knowledge diffusion: fields of research of output (a), fields of citing papers (b) and countries of citing papers (c)

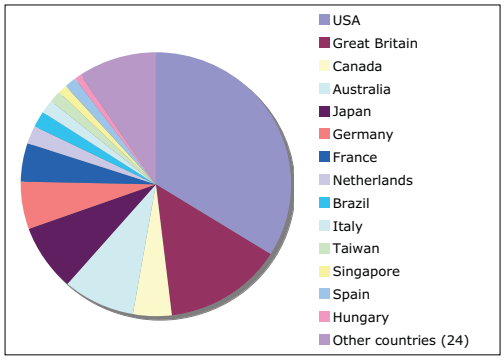
(a)



(b)



(c)



19.7.2 Benefits to future research and research use

Capacity building and career development

A key outcome from the project was that it enabled the PI, Dr Llewellyn-Smith, to establish a track record in research on the central nervous system in relation to cardiovascular disease. It also enabled her to establish a track record of being self-funded in this new area of research. This was important in terms of obtaining future funding from sources such as the NHMRC. Llewellyn-Smith said, 'You need to be able to show that, after you have moved, you can successfully do research in that area…that you can be self-funding' (Llewellyn-Smith interview, 2008).

The project established for Dr Llewellyn-Smith recognition and credentials that have enabled her not only to obtain funding for future work but also to build on the project with high-quality research during her career and establish important research collaborations around the world driven by her specific expertise. Llewellyn-Smith said, 'The collaborations now are because I do something very sophisticated and very well, which nobody else in the world does' (Llewellyn-Smith interview, 2008).

In addition, since the grant project Dr Llewellyn-Smith has trained two PhD students in her laboratory and five postdoctoral fellows in the techniques.

Targeting future research

The research project established a new area of neuroscience (identification of the brain and spinal cord pathways responsible for the control of blood pressure), which created an understanding of central nervous system involvement in cardiovascular disease.

In addition, the specialised neuroscience techniques that Dr Llewellyn-Smith developed as part of the project are commonly used around the world and cited in textbooks and other reference sources. Llewellyn-Smith said, 'Now many of the people who do [electron microscopy] immunocytochemistry on [the] central nervous system use my method without quoting me' (Llewellyn-Smith interview, 2008).

Dr Llewellyn-Smith's subsequent work with spinal cord injuries has also shown they wreak havoc in terms of the body's ability to control blood pressure and can lead to life-threatening aberrations. People with spinal cord injuries suffer from autonomic dysreflexia, which means they experience episodes of very high blood pressure in response to stimuli that would not produce any effect in someone with an intact cord. For someone with spinal cord injury, being patted on the leg, for instance, can produce a spike in blood pressure high enough to cause a stroke. For the same reasons, childbirth among women with spinal cord injuries can have potentially fatal consequences.

Using the same techniques applied to spinal nerves controlling blood pressure, Dr Llewellyn-Smith discovered that the neurons in the spine that control the pelvic organs are run in a completely different way and mainly from within the spinal cord. Whereas spinal nerves controlling blood pressure and most other autonomic functions derive some two thirds of their input from the brain, she found that disconnection from the brain affects only about ten percent of the nerves directing the pelvic viscera. Llewellyn-Smith said, 'That has huge implications for treating bladder, bowel and reproductive organ problems due to spinal cord injury. If most of the control is within the spine, it will probably be much easier to figure out combinations of drugs to treat them' (Llewellyn-Smith interview, 2005). It is understood that the key thing about the work Dr Llewellyn-Smith has done on the circuits that control the pelvic viscera is that it shows that you do not have to get a large amount to regrow, because most of the control comes from within the spine.

Dr Llewellyn-Smith has more recently started work on a heart failure project due to the interest of an honours student. They have established a heart failure model in rats, and preliminary results are very suggestive that the brain changes in rats with heart failure. Llewellyn-Smith said, 'So then the question is if you ever come up with a treatment for heart failure, are there some changes in the brain that are permanent and some changes

that are not? And if you have a heart failure patient whose brain has permanently changed and you treat them...there may be big problems' (Llewellyn-Smith interview, 2008).

19.8 Interface B – dissemination

The key dissemination vehicles for the study's findings were peer-reviewed publications and conferences. In addition to the publications identified in Section 19.7.1, the PI, Dr Llewellyn Smith, made it a point to go to conferences, talk to people and visit people within Australia and overseas. Llewellyn-Smith has a naturally outgoing personality and learnt early in her career that it was important to provide a face to the work being done and to promote the work herself. She said, 'People attribute work to faces not names. Every time I go overseas I try to give a lecture somewhere…several lectures [wherever possible]' (Llewellyn-Smith interview, 2008).

19.9 Stage 4 – secondary outputs

19.9.1 Policy/product impacts

A patent for one of the techniques was issued in the United States on 23 April 2002 (US Patent no. 6,376,460, 'A Method of Modulating Cellular Activity'). Unfortunately this has no commercial outcomes as it has limited application in a therapeutic setting. This patent has, however, been referenced as prior art in two subsequent American patents for clinical methods (Patent no. 6,776,991 in 2004 and 7,041,296 in 2006). Llewellyn-Smith said, 'The problem with the method is it kills nerve cells, so it's a one-hit cure…pharmaceutical companies are not interested in that' (Llewellyn-Smith interview, 2008).

According to Professor Chalmers, although this research represented a critical breakthrough, it has not yet had any direct impact in terms of new treatments for cardiovascular diseases. He said, 'I don't think it has yet had a specific impact in terms of new treatments…but in terms of the physiology and the understanding of the function of the central nervous system in relation to blood pressure it's been a critical step, which has led to sort of mega development above us around the world picking up from this and applying it. So it's been very important, but I don't think you could say it has yet translated into new treatments that I could pinpoint and say that treatment wouldn't have happened without it' (Chalmers interview, 2009).

19.10 Stage 5 – adoption by practice and public

The research is now cited in textbooks and application of the research to clinical problems is now possible, so it could have broader impacts. Current understanding of the area also now lends itself to work with clinicians to try and understand what happens in the disease state.

As identified above, Dr Llewellyn-Smith has more recently started work on a heart failure project due to the interest of an honours student, which stemmed from a cardiologist who

was studying his PhD. They have established a heart failure model in rats, and preliminary results are very suggestive that the brain changes in rats with heart failure. Llewellyn-Smith is discussing these preliminary findings with clinicians – a process that is facilitated by the hospital setting of the Flinders Medical Centre.

19.11 Stage 6 – final outcomes

No wider health and economic benefits could be directly attributed to the case study research. As Professor Chalmers explained, due to the long gestation period for both clinical research and treatments in cardiovascular disease (it can take decades for new treatments to emerge from primary research), this research is perhaps best viewed as an important piece of a complex puzzle that has hidden benefits that will never be directly attributed. Chalmers said, 'It may be that people may pick up what we did with blood pressure and use it in studying bladder function or bowel function or lung function and put the same constellation of techniques together there and you see the spin-off somewhere else. That's how it all works' (Chalmers interview, 2009). The biggest impact of the case study grant is likely to be felt in outcomes of the follow-on research it influenced, which will form the basis of future treatments.

19.12 Summary of case study impacts

The payback framework describes the process from research to several categories of impact. The categories allow us to compare across different case studies. Table 19-2 shows, in point form and by impact category, some of the impacts, described more fully above, that have emerged from this grant.

Table 19-2 Payback

Payback category	Impacts from case study
Knowledge production	• Peer-reviewed publications and chapter in textbook • Techniques included in reviews, and research cited in textbooks
Benefits to future research and research use Research targeting and capacity building	• Development of specialised neuroscience techniques in cardiovascular disease • Training of two PhD students • Training of 4–5 postdoctoral fellows in the techniques • Established a new area of neuroscience that created an understanding of central nervous system involvement in cardiovascular disease • Major impact on approach used in parallel areas (eg neuronal control of other organs) • Project established recognition and credentials for the PI, which have enabled her to obtain funding for future work, build on the project with high-quality research during a successful career and participate in collaborations around the world driven by her specific expertise
Informing policy and product development	• Patent was developed but for a product with no commercial outcomes as it has limited application in a therapeutic setting
Health and health sector benefits	• Application of the research to clinical problems is now possible, which could have broader impacts • Current understanding of the area now lends itself to work with clinicians to try and understand what happens in the disease state (driven by the PI discussing research with clinicians; the hospital setting has been helpful in facilitating this
Broader social and economic benefits	• None

19.13 References

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Somogyi, P., J.B. Minson, D. Morilak, I. Llewellyn-Smith, J.R.A. McIlhinney and J.P. Chalmers, 'Evidence for an Excitatory Amino Acid Pathway in the Brainstem and For its Involvement in Cardiovascular Control', *Brain Research*, Vol. 496, 1989, pp. 401–407.

CHAPTER 20 Right-hemisphere stroke: incidence, severity and recovery of language disorders

20.1 Introduction to the case study

20.1.1 Overview

Strokes can occur in either side of the brain – in the left hemisphere or the right. It had been thought that language or communication disorders were caused solely by lesions in the left hemisphere of the brain, but in the early 1990s this view was increasingly being challenged. There was a recognition that right-hemisphere lesions, such as strokes, could also result in a communication disturbance. This case study is based on a project led by Professor Catherine Mackenzie from the Division of Speech and Language Therapy, University of Strathclyde, Glasgow. The project, funded by the Stroke Association, set out to identify the incidence and severity of language disorders caused by right-hemisphere stroke and the patterns of language recovery. The subjects were drawn from consecutive (ie all) admissions to the Acute Stroke Unit of the Western Infirmary, Glasgow, over a 14-month period from March 1994 and consisted of all those patients who met the inclusion criteria.

The project was successfully completed and the findings described in a series of publications: five directly from the project and various others from additional research by Professor Mackenzie and Dr Marian Brady, the main researcher on the project. The project has made a considerable contribution to building capacity in the field of research in speech and language therapy linked to strokes, and team members have undertaken a range of further research linked to the project. Mackenzie conducted additional research as part of her doctor of philosophy degree (PhD) and combined the findings with some of the findings from the Stroke Association-funded project. She is now a professor at the University of Strathclyde. For her PhD, Brady built on the Stroke Association-funded project and undertook a much more detailed analysis of the language disorders of a smaller number of patients after right-hemisphere stroke. Brady is now Programme Director for the Stroke Programme within the Nursing, Midwifery and Allied Health Professions Research Unit funded by the Chief Scientist Office, Scottish Government Health Directorates. One of the publications from the project is cited in the 2002 Scottish clinical

guideline on the management of patients with stroke (Scottish Intercollegiate Guidelines Network, 2002), and the project and the publications arising from it are used to inform the teaching of speech and language therapists in the United Kingdom (UK). The findings were also used in the local National Health Service (NHS) trust to produce a leaflet for patients and their families.

The impact of the findings on practice has probably not been very large, but there is anecdotal evidence of at least some greater awareness of the language problems that can arise following a right-hemisphere stroke, and local information gathered by the team indicates an increased involvement of speech and language therapists in such cases. To the extent that advice for patients and their families will have been provided by some speech and language therapists, there will probably have been a limited health gain. In 2008 Professor Mackenzie and Dr Brady brought together findings from the stream of research in a way that might increase the prospects of further benefits in the future.

20.1.2 **Background**

The background to the project was set out in both the original application (Grant Application, 1993) and in the final report to the Stroke Association, which had funded the project (Mackenzie et al., Final report, 1997). Since the mid nineteenth century it had been recognised that the left hemisphere of the brain has a major role in language processing and that aphasia, a disorder of language, commonly occurs in people who acquire left-hemisphere damage. Researchers working on language problems hypothesised a possible language role for the right hemisphere (eg Critchley, 1962, and Eisenson, 1962). Since then much research had indicated that right-sided damage may also limit competence in communication (Joanette et al., 1983, and Myers, 1986).

Basic aspects of language processing seemed usually to be retained in patients with right-hemisphere lesions, so the traditional language screening measures were unlikely to identify most patients affected with disorders following right-hemisphere strokes. Rather than experiencing difficulties with word finding, sentence construction or the sound elements of speech, the effect is primarily on the pragmatic aspects of language, ie the aspects to do with how language is used in communication situations to achieve the speaker's purposes. Amongst the described communication difficulties of patients with right-hemisphere damage are problems with topic maintenance and efficiency of expression, distinguishing significant themes from irrelevant or minor details, and integrating and interpreting information, particularly where inferencing is required (Myers and Mackisack, 1990). Because on the surface patients were not obviously aphasic, their communication difficulties were often being overlooked, and in some cases their communication difficulties were being misinterpreted as manifestation of personality disorder and social relationships were being affected (Burns, et al., 1985).

The occurrence of aphasia in patients after left-hemisphere stroke had been well documented. Although the presence of language problems in the population of patients after right-hemisphere stroke was recognised, and there had been some estimates that 50% of these patients had a communications impairment (Joanette et al., 1990), there had not been a full cohort study on what percentage of patients experienced these problems after stroke and whether there were associations with particular sites of damage or types of

stroke. Furthermore, there was a lack of knowledge about the progress of the affected patients.

Perhaps somewhat surprisingly, there was a further problem with the then knowledge state in this field, which went in the opposite direction to that of the traditional neglect of the issue. Those studies that had been conducted in patients after right-hemisphere stroke tended to focus on the most severely affected patients and, if anything, gave an exaggerated picture of the problems that would tend to occur in the affected population. Another factor possibly linked to this was that in research on communications following right-brain damage, the use of specially constructed tasks had been common. Perhaps as a result, there had been a dearth of attempts to establish whether factors such age and educational level were relevant for the communication performance of the population irrespective of damage caused by stroke.

20.1.3 The case study approach

For this case study the principal investigator (PI) and two other members of the research team were interviewed. Documentary and bibliometric analysis was conducted of: various papers from the research team describing the original research; various papers from the PI and main researcher describing a stream of research that followed on from the project; a policy document; and a patient leaflet that drew on the research in some ways. Archival review was conducted into the original application (supplied by the Stroke Association) and the final report, which was supplied by the PI following the initial interview.

20.2 Stage 0 – topic identification

Various factors led to the team deciding to make this application. These included: the potential problems that patients were experiencing but which were not currently being addressed; the unanswered scientific questions about right-hemisphere strokes; and the opportunity to conduct research in this field that was afforded by the creation of an acute stroke unit with dedicated speech and language therapy input provided by a therapist who had an interest in research. Each of these points is examined in greater depth, and the final two were linked.

20.2.1 Identifying ways to improve patient care where there was potentially an unmet need

Both as a result of knowing the literature and from their own clinical experience the applicants were aware that the communication difficulties of patients after right-hemisphere strokes were often overlooked. In their proposal the applicants referred to the various potential implications for patients whose language disorders after stroke had not been recognised. For example that they may not fully grasp explanations of their illness or treatments offered, including in 'situations where consent for inclusion in trials is required' (Grant Application, 1993). Furthermore, because the language problems of patients with right-hemisphere lesions tended not to be recognised, patients were unlikely to be referred to speech and language therapy services and, as a result, the applicants claimed that 'successful return to employment or the pursuit of hobbies and interests may be hindered' (Grant Application, 1993).

20.2.2 Addressing unanswered scientific questions

Considering the then current state of knowledge in this field, as set out above, the team felt that it was important to address the unanswered scientific questions about the incidence and severity of language disorders in patients after right-hemisphere stroke. They concluded that examination of the language skills in a representative group of subjects, with accompanying observational and contemporary objective medical data with systematic long-term follow-up, was needed.

20.2.3 Research opportunities provided by acute stroke unit

In 1990 an acute stroke service was established at the Western Infirmary, Glasgow, and all patients were admitted for a period of up to 72 hours for assessment, investigation and acute treatment. A single medical team undertook admission and early care. All patients had a computerised tomography (CT) scan and most had non-invasive assessment of cerebral perfusion. All patients were assessed by physiotherapy, occupational therapy and speech and language therapy specialists. For its time this was a pioneering service aimed at providing improved patient care. It also provided an opportunity to undertake important stroke research, as recorded by members of the stroke unit who described how the creation of the specialist unit had enabled them to recruit an appropriate number of patients for major trials at that time: 'We would have been unable to achieve this before reorganisation of our stroke care service and we therefore support an improved system of stroke care in the United Kingdom' (Morris et al., 1993). Similarly in relation to speech and language therapy, the creation of the specialist stroke unit, in which a speech and language therapist screened all the patients, created the conditions in which a study could be made of a complete cohort of stroke patients. Mrs Thia Begg, the speech therapist at the unit, and Professor Catherine Mackenzie, from the local speech and language therapy school, were keen to use the opportunity to address the unanswered questions in this field and were supported in this by Professor Kennedy Lees, Clinical Director of the Acute Stroke Unit.

20.3 Interface A – project specification and selection

Professor Mackenzie, Mrs Begg and Professor Lees worked together to develop the proposal. An initial proposal put to the Scottish Office aimed to undertake a similar approach to that used in this project but to exploit the opportunities to examine the speech and language disorders in the whole stroke population admitted to the unit. This proposal was turned down and the team decided to focus on the particular problems arising from right-hemisphere strokes. The Stroke Association had not been approached originally because, unlike the Scottish Office, they did not pay overheads to the academic institution hosting the research. Mackenzie knew of no speech therapist on the research committee of the Stroke Association, so she liaised with one member of the panel who encouraged her to submit the application and provided advice on the scale of project that might be appropriate.

The proposal set out five main research questions in relation to those patients admitted to the unit with a right-hemisphere stroke and how they would be addressed. The questions were (Grant Application, 1993):

1. 'What is the incidence of language deficit within 72 hours of stroke?

2. What changes in severity are apparent in affected patients at:
   a) one month
   b) three months
   c) six months
   d) 12 months?
3. Are distinct patterns of language deficit associated with different lesion sites?
4. Is there a relationship between improvement in language status and type of stroke (haemorrhagic versus ischaemic)?
5. What are the implications of the findings of the study for staffing requirements within the speech and language therapy service?'

The proposal made to the Stroke Association was funded to the full amount requested. No major objections to the proposal or requests for amendments were recalled by the research team.

20.4 Stage 1 – inputs to research

20.4.1 Facilitators

The grant from the Stoke Association was for £56,963. This was mainly to allow the appointment of a research officer to undertake the follow-up assessments with the patients. There was also a small amount of secretarial support provided by the grant. Professor Mackenzie used her university research time on the project, and Mrs Begg, and to a smaller extent Professor Lees, committed time to the project but this was not funded by the Stroke Association.

As described later, Professor Mackenzie also undertook some parallel research, which she used for her PhD but which was also incorporated into the findings from this study. In essence this was using her university-funded research time and private time. The acknowledgements in the final report to the Stroke Association include the following: 'Faculty of Education, University of Strathclyde for financing of C. Mackenzie's normative study which is an integral part of this report' (Mackenzie et al., Final Report, 1997).

20.4.2 Knowledge and expertise

Although this was the first externally funded project Professor Mackenzie had led, she had undertaken previous research, and published, in the field of language disorders caused by strokes. Mrs Begg had held the post of Senior Speech and Language Therapist at the Western Infirmary for many years, and with the creation of the acute stroke unit she automatically saw all the stroke patients admitted to the hospital. She conducted the initial assessments necessary for the research and decided which patients met the inclusion criteria. As noted above Professor Lees, as Clinical Director of the Acute Stroke Unit, was leading major trials to improve treatment for stroke patients (Morris et al., 1993). Dr Marian Brady, the research fellow recruited for the project, was a speech and language therapist with a particular interest in acquired neurological problems.

20.4.3 Techniques

Mackenzie identified a battery of appropriate assessment procedures to use. In themselves they were not original but, as far as is known, they had neither previously been applied in a prospective cohort study of patients with right-hemisphere stroke nor been applied to study the language recovery of patients in such a comprehensive way. Furthermore, the team thought it important that the assessments used were readily available to speech and language therapists

20.4.4 Samples/study recruits

Full advantage was taken of the opportunity to study patients consecutively admitted to a stroke unit. The involvement and support of the clinical director of the stroke unit was valuable in ensuring good access throughout the study.

20.4.5 Consumables

One of the valuable contributions from Professor Lees was not only to interpret the CT scans that were taken of every patient but also to ensure that when the scan needed to be repeated, as was often the case, this actually happened. There were resources in the project budget, under the consumables heading, to pay for these repeated scans.

20.4.6 Space

Dr Brady was given an office at the University of Strathclyde.

20.5 Stage 2 – research process

The project broadly proceeded as planned. As described in the final report to the Stroke Association (Mackenzie et al., Final Report, 1997), the experimental subjects were recruited during the 14 months from March 1994 and a battery of clinical assessments, including the CT scans, was used to determine medical status. Diagnosis and classification of right-hemisphere stroke was made on the basis of clinical presentation and radiological analysis. Strokes were categorised as ischaemic or haemorrhagic. The classification scheme proposed by Bamford et al. (1991) was used, with classes restricted to four groups in view of patient number limitation. Scans and clinical classifications were reviewed by a single observer (Professor Lees) who was unaware of language test data. Almost a quarter of the stroke sample was younger than 60 years.

The battery of verbal language assessments was selected on the basis of evidence from existing literature on communication impairment in cases of right-brain damage. To maximise the clinical applicability of the results the team ensured the assessments used were available for use in routine practice. Screening for the presence of cognitive impairment was also conducted. As noted above, what was so innovative about this study was not the specific tests used but rather the comprehensive way in which they were being applied. This took various forms: all stroke patients who met the entry criteria were entered into the study, so as to assess incidence; the study looked at recovery as well as severity; and a parallel study was conducted on a comparable non-stroke population to allow proper assessments to be made of the level of impairment of the stroke patients.

Stroke subjects were excluded when there was evidence of: a previous neurological incident; known psychiatric history; old age accompanied by a level of frailty that precluded cooperation in the assessment schedule; dementia; or hearing or vision considered insufficiently acute to cope with the assessment materials.

The results from the parallel study were included in the final report to the Stroke Association, even though it had not funded that study, because they formed an integral part of the findings. Satisfactory inter- and intra-judge reliability and test–retest reliability was established for a section of the non-brain-damaged population representing the same age range as the experimental group (Mackenzie et al., Final Report, 1997).

20.6 Stage 3 – primary outputs from research

20.6.1 Knowledge

The list of articles below are the five that came directly from this project.[1] The brief account of each paper outlines key points from the knowledge produced by the study.

1. Mackenzie, C., T. Begg, M. Brady and K.R. Lees, 'The Effects of Verbal Communication Skills on Right Hemisphere Stroke in Middle Age', *Aphasiology*, Vol. 11, 1997, pp. 929–945.
2. Mackenzie, C., T. Begg, K.R. Lees and M. Brady, 'The Communication Effects of Right Brain Damage on the Very Old and the Not So Old', *Journal of Neurolinguistics*, Vol. 12, 1999, pp. 79–93.
3. Mackenzie, C., M. Brady, T. Begg and K.R. Lees, 'Communication Ability Following Right Brain Damage: the Family Perspective', *Advances in Speech-Language Pathology*, Vol. 3, 2001, pp. 81–96
4. Mackenzie, C. and M. Brady, 'Communication Ability in Non-Right Handers Following Right Hemisphere Stroke', *Journal of Neurolinguistics*, Vol. 17, 2004, pp. 301–313.
5. Mackenzie, C. and M. Brady, 'Communication Difficulties Following Right-Hemisphere Stroke: Applying the Evidence to Clinical Management', *Evidence-Based Communication Assessment and Intervention,* Vol. 2, 2008, pp. 235–247.

In the first paper Mackenzie et al. (*Aphasiology*, 1997) describe the results from the assessment of 81 right-handed middle-aged subjects (64 non-brain-damaged and 17 right-hemisphere strokes) on a series of verbal comprehension and spoken discourse tasks. Educational level was found to affect the performance of the non-brain-damaged subjects. The results highlight the requirement for language task control data to be education

[1] Because of the way the case studies in the Retrosight study are structured, and because the production of knowledge as contained in publications is viewed as an important impact, the focus here is on describing the key points in the articles. As noted by a peer-reviewer these do not fully map onto the specific list of research questions for the project described here. However, the final report to the Stroke Association did address the research questions much more specifically.

referenced and that there is an association between right-brain damage and some deficits in verbal communication.

The second paper (Mackenzie et al., 1999) describes the communication behaviour one month after stroke of two groups of right-brain-damaged patients of different ages (Group 1 included those younger than 75 years and Group 2 those 75 years and older) and two equivalent non-brain-damaged groups. All subjects had received basic education only, until the age of 14 or 15 years. In Group 1 of the patients with right-brain damage, many of the commonly reported deficits were observed, including comprehension of metaphor and inference and reduction of the amount of descriptive content. Overall, the performance of the group was similar to that of non-brain-damaged subjects aged 75 years and older.

The third paper (Mackenzie et al., 2001) is also an important paper describing the perspective of family members who knew about the everyday communication function of the stroke patients before, as well as after, their right-hemisphere stroke. Thirty-five subjects with right-hemisphere brain damage were evaluated by family members at one, three, six and 12 months after the stroke. Performance on a set of clinical communication measures indicated clear communication impairment in 23 of these subjects. For these 23 subjects, reduction in communication ability from pre-stroke level was usually judged by family members to be minimal, but at least 25% identified impairment in aspects of discourse such as conversational participation, topic and referencing; following directions; understanding writing; and communicating emotions. Various possible reasons are discussed for the differences between the assessments and the suggestion was made that efficacy studies should be conducted to establish the benefits of communication intervention for the population with right-brain damage.

The fourth paper (Mackenzie and Brady, 2004) describes an area in which performance data had never previously been reported on: the communication effects associated with non-right handers sustaining right-hemisphere strokes. The results for a small group of non-right handers in the overall study were compared with those for right-handed subjects, and on all communication measures there was a striking similarity between the scores. The team suggested this is an area that needs further research.

The final paper (Mackenzie and Brady, 2008) was published only at the end of 2008. This paper draws on the Stroke Association-funded project, the parallel project by Professor Mackenzie and follow-on work described below. It acknowledges the Stroke Association funding and summarises the findings from the various studies. It then draws on them, and the work of others, to make recommendations for clinical management of right-hemisphere stroke. The authors suggest the findings could guide future outcome research because there is an urgent need for the evaluation of communication management programmes to determine whether therapists may, with confidence, offer an effective intervention service to those people whose communication skills are affected by right-hemisphere strokes. The call for further research to show the benefits of interventions indicates that the extent to which improvements can be achieved still needs to be established.

Table 20-1 and Figure 20-1 show the results for this case study of the standard bibliometric analysis that is being conducted for all case studies. Two out of the five journal articles were in peer-reviewed journals that are not included in the Web of Science

(WoS) used to conduct the bibliometric analysis. In general, peer-reviewed journals in areas such as the allied health professions are less well represented in the WoS than most other medical fields.

Although a good range of papers was produced, the authors felt that there would have been only limited scope for them to receive citations because there are very few, if any, researchers doing further research in this area in the UK, and researchers in the United States, where there is more research, rarely cite UK research. This suggestion is supported by the bibliometric report for the two articles that were included as being indirectly linked to the project. These two come from additional analysis that Mackenzie undertook for her PhD. As described below, the findings from this analysis were linked to this project, and were important for the final report, but are classified as being indirectly linked to the project because they were not funded by the Stroke Association and were not specifically on right-hemisphere strokes. The bibliometric analysis shows that one of these two papers on this more general topic (Mackenzie, *International Journal of Language and Communication Disorders*, 2000) was in the top category for citations, when account is taken of the research field.

Table 20-1 Publication output and impact of directly related publications[1]

Number of journal articles:	5
Number of articles included in citation analysis:	3
Total number of citations (all papers):	13
Aggregate relative citation impact:	0.24 (Class II)
Self-citations:	31%

Distribution of publications across relative citation impact classes:

	Class I (uncited)	Class II (>0 and <0.8 citations)	Class III (0.8 to 1.2 citations)	Class IV (1.2 to 2.0 citations)	Class V (>2.0 citations)
Number of publications	1	2			
Proportion of total output	33%	67%			

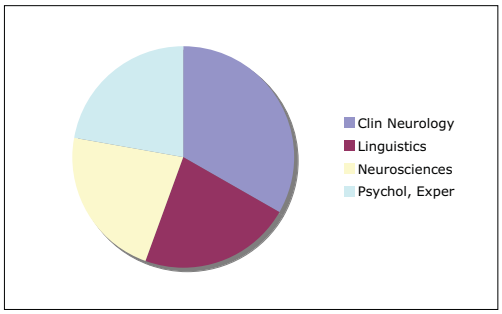
Most highly cited publication[2]:	Mackenzie, C., T. Begg, M. Brady and K.R. Lees, 'The Effects of Verbal Communication Skills on Right Hemisphere Stroke in Middle Age', *Aphasiology*, Vol. 11, 1997, pp. 929–945
Times cited:	9

[1] In addition, two publications were indirectly linked to this grant. Both these publications were indexed in WoS and received 25 citations in total, giving a relative citation impact of 1.61. One publication was in relative citation impact Class II and the other in Class V. Their self-citation rate was 20%.

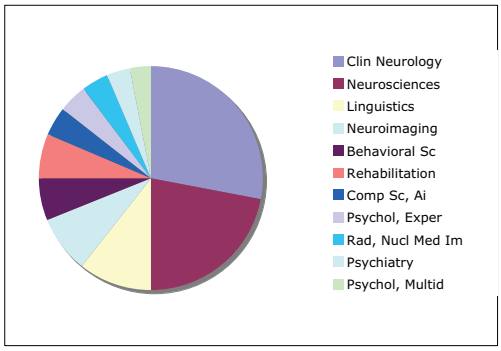
[2] Citation count extracted April 2009.

Figure 20-1 Knowledge diffusion: fields of research of output (a), fields of citing papers (b) and countries of citing papers (c)

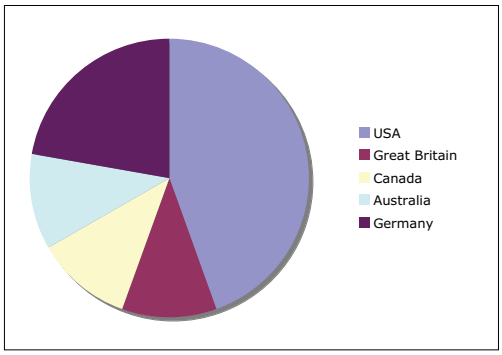
(a)



(b)



(c)



20.6.2 Benefits to future research and research use

Capacity building and career development

The project has made a considerable contribution to capacity building in the field of speech and language therapy research related to strokes, especially through the PhDs obtained by the PI and the research officer and their subsequent career developments. As noted above, Professor Mackenzie undertook a study of communication in a section of the non-brain-damaged population representing the same age range as the experimental group. They provided a control group for the main study, and Mackenzie used data from both studies for her PhD thesis. Her PhD study, therefore, became an integral part of the Stroke

Association project, but this part of the study did not receive any funding from the Stroke Association; she relied on her university-funded research time and private time. Probably in contrast to researchers in most other medical fields, this is an example of the PI in an allied health professions field who has herself received a PhD based at least in part on the findings from a funded project. As noted above, Mackenzie described this additional work undertaken for the PhD in two publications:

1. Mackenzie, C., 'Adult Spoken Discourse: the Influences of Age and Education, *International Journal of Language and Communication Disorders,* Vol. 35, No. 2, 2000, pp. 269–285.
2. Mackenzie, C., 'The Relevance of Education and Age in the Assessment of Discourse Comprehension', *Clinical Linguistics and Phonetics,* Vol. 14, No. 2, 2000, pp. 151–161.

Through her involvement in the project, Dr Marian Brady, who had been recruited from clinical practice to be the research officer on this study, became very interested in undertaking a higher degree. She registered with Professor Mackenzie at the University of Strathclyde, originally for a master of philosophy (MPhil), as is standard registration process for undertaking PhDs in that institution, and subsequently transferred that registration to a PhD. The topic for her PhD grew directly out of the Stroke Association project but was not part of it. She felt that when she saw some of the right-hemisphere patients in the project she would perceive interesting patterns of communication and yet she was not picking this up on the scoring in the battery of standard tests that she was using. For her PhD she undertook a much more detailed analysis of communication at a discourse level with a much small number of people than in the Stroke Association project. After the end of the project she was pursuing her PhD at the same time as holding down a more senior clinical post in a specialist field; a post that she felt she had secured partly on the basis of having successfully conducted the research officer role. To complete the PhD she gave up her clinical post and secured a studentship from the University of Strathclyde. Four further publications came from Brady's PhD, and Mackenzie was a co-author on all of them (Brady and Mackenzie 2001, and Brady et al., 2003, 2005 and 2006).

The project and the PhD were extremely important to Dr Brady's subsequent career. In interview she stressed how few opportunities there were, especially at the time of the project, for allied health professionals to obtain PhDs and develop a research career. At the end of her PhD studentship, she obtained a research post at what is now called the Nursing, Midwifery and Allied Health Professions Research Unit (NMAHPRU) located at Glasgow Caledonian University and funded by the Chief Scientist Office (CSO) of the Scottish Government Health Directorates.

Originally, the research post was not focused on either speech and language therapy or stroke. Soon after Dr Brady's appointment, however, the NMAHPRU underwent a restructuring process. Around this time, stroke research was regarded as being underfunded in many countries of Europe (Pendlebury et al., 2004) and it had been identified as a national research priority for Scotland. Brady was naturally keen to encourage research capacity related to stroke in the nursing and the professions allied to health. Brady was appointed Programme Leader (and later Director) of the newly established Stroke Programme within the re-structured unit. The remit of the programme goes much wider

than just speech and language therapy, but she has been able to continue working in this field.

From the perspective of career development, Professor Mackenzie felt the project had been of importance to her and was a major step in securing a chair at the University of Strathclyde. She has gone on to undertake various other research projects and stated: 'A lot of the other things I've gone on to do I might not have done if I hadn't done this particular project' (Mackenzie interview, 2008). Mrs Begg was not far off retirement age when the project finished and so it had little impact on her career, but she did undertake some further research.

Targeting further research

As noted, the work undertaken for Dr Brady's PhD grew directly out of the Stroke Association project. In turn, that PhD research led to a successful application to the NHS New and Emerging Applications of Technologies (NEAT) programme for funding for a project to develop a technology-based transcription-less approach to discourse analysis. The work described above from the NEAT project was disseminated through a specialist colloquium on discourse analysis hosted by the NMAHPRU. The papers from this colloquium, and other work, formed the basis of a special edition of the journal *Aphasiology*. A paper by Mackenzie et al. (2007) described further analysis of the normative data originally gathered by Mackenzie in her parallel study to the Stroke Association project and by Brady in her PhD work.

20.7 Interface B – dissemination

The findings from the Stroke Association project were presented at various conferences and meetings, some of them international:

- Mackenzie, C., T. Begg, K.R. Lees and M. Brady, *Communication Deficits in the Middle Aged Right Hemisphere Stroke Population*, British Aphasiology Society Biennial International Conference, York 1995.
- Mackenzie, C., *Communication Deficits in Right Hemisphere Stroke*, Eleventh Annual British Stroke Research Group Meeting, Bromley, Kent, 1997.
- Mackenzie, C., T. Begg, K.R. Lees and M. Brady, *Right Hemisphere Stroke: Incidence, Severity and Recovery of Language Disorders*, Third Stroke Association Scientific Meeting, Oxford, 1997.
- Mackenzie, C., *Research into the Communication Effects of Right Hemisphere Stroke*, Acquired Language Disorders Special Interest Group, Glasgow, 1997.
- Mackenzie, C., *Identifying the Experimental Group in Stroke Research*, Postgraduate Rehabilitation Studies Group, University of Edinburgh, 1997.
- Mackenzie, C., T. Begg, K.R. Lees and M. Brady, 'Verbal Communication in Right Hemisphere Stroke: an Age, Education and Time Controlled Study', Seventh 7th European Stroke Conference, Edinburgh), 1997.

Then in 2007 Professor Mackenzie was invited to give the keynote address on this stream of work to a Dutch conference:

- Mackenzie, C., *Communication Disorders in Right Hemisphere Stroke*. Dutch Association for Speech Therapy en Phoniatry (NVLF) 80th birthday jubilee congress, Amsterdam, 2007.

Professor Mackenzie included the findings of the study in her lectures to speech and language therapy students at the University of Strathclyde. She was also invited to lecture on this at Edinburgh to students at the only other speech and language therapy school in Scotland. She also wrote a piece based on the study for *Stroke Matters,* the Stroke Association's journal.

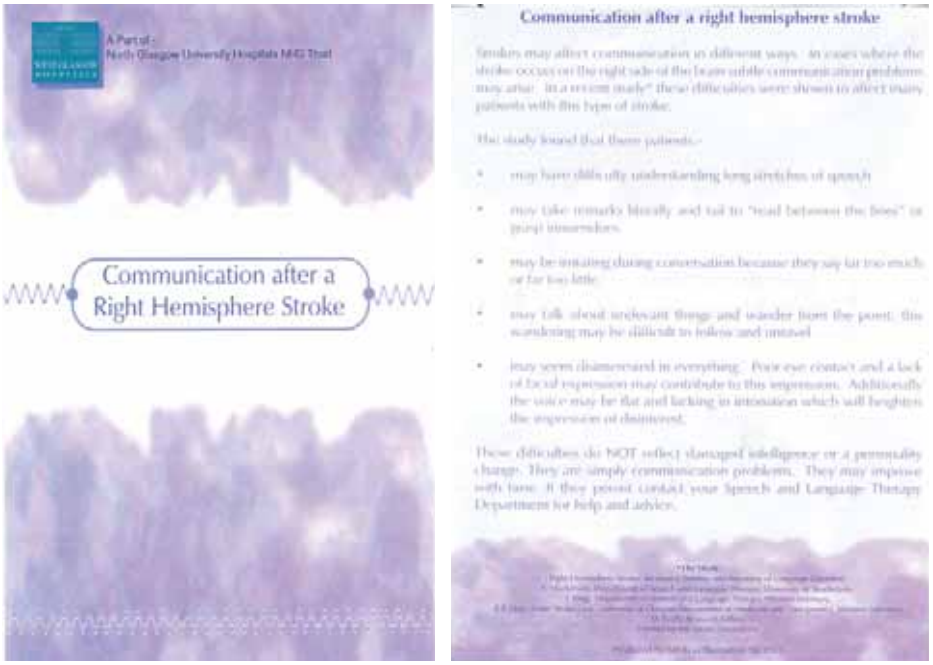
20.8 Stage 4 – secondary outputs

Professor Mackenzie was invited to be the speech and language therapist on the committee drawing up guideline 64 on the management of stroke for the Scottish Intercollegiate Guideline Network (SIGN) (SIGN, 2002). This, however, was because of her general position as a senior figure in the speech and language therapy community in Scotland rather than being to do with this specific research project. Guidelines produced by SIGN are regarded as the most authoritative produced in Scotland. In the section on language the guideline cited Mackenzie et al. (1999) and stated that: 'Aphasia is usually associated with left hemisphere damage, but symptoms such as subtle communication deficit, affecting communication interaction, notably non-verbal communication, and communication of non-literal or inferred information, may also occur following right hemisphere stroke' (SIGN, 2002). The influence of Mackenzie in ensuring that the guideline included this information is indicated by the fact that the equivalent guideline in England at that time made no reference to communication problems resulting from right-hemisphere stroke. Nevertheless, there was no scope in the SIGN guideline to go further and make specific recommendations for treatment.

In addition to including references to the project's findings in her lectures to students, Professor Mackenzie also ensured that the topic was included in the curriculum of the University of Strathclyde's course on speech and language therapy. Mackenzie also believes the bigger picture is that the publications arising from the project have probably played a role in the inclusion of the topic in other speech and language therapy courses in the UK: she knows from academic contacts in these institutions that the publications are referenced in lectures.

Mrs Begg, in consultation with the research team, developed a leaflet that was produced by the local NHS trust and was given out to patients and their families to provide them with appropriate information based on the project's findings. The two sides of this leaflet are reproduced as Figure 20-2.

Figure 20-2 Leaflet from NHS trust on communication after a right-hemisphere stroke



It is claimed that the stream of work culminating in the NEAT-funded project has led to 'the potential availability of a valid and reliable transcription-less approach to analysis that speech and language therapists can apply to analyse their clients' discourse' (Armstrong et al., 2007). Using the standard categorisation of impacts applied in these case studies, perhaps this could be considered to be a form of product development because the aim is to develop something that could be used in practice, not just in further research.

20.9 Stage 5 – adoption by practice and the public

In their 2008 paper, Mackenzie and Brady claim that in the UK, referral of right-hemisphere damaged patients to speech and language therapists 'because of communication difficulty, rather than motor speech or swallowing disorder, is still relatively unusual' (Mackenzie and Brady, 2008). The situation is reported to be rather different in the United States, where increasing numbers of adults with right-hemisphere damage are appearing in the case loads of speech and language therapists (Tompkins, 1995).

During interviews, the members of the research team reported some anecdotal evidence of greater awareness by speech and language therapists of the problems that can arise following a right-hemisphere stroke. They suggested that this could be as a result of the teaching, presentations, articles and guidelines etc describing the findings from the stream of research. Furthermore, although right-hemisphere stroke patients were not, until recently, regularly being referred to a therapist, there is some local information that this is slowly changing. The speech and language therapy resources available were traditionally concentrated on patients with left-hemisphere stroke and dysphagia, where the need is generally greater.

There were some things that speech and language therapists who knew about the research could do to help those patients who were referred, and the recent article by Mackenzie and Brady (2008) contributes to the literature that provides guidance on the management of patients.

20.10 Stage 6 – final outcomes

Thus far the project is likely to have resulted in some limited health and broader economic benefits. These will have come about as a result of the advice that some speech and language therapists who know about the research findings will have been giving to patients and their families. In addition to the health gain that some patients will have received, in a few cases such health gain could also have made a contribution to stroke patients being able to return to work. However, it is impossible to make any assessment of the scale of the benefits.

If the recent article (Mackenzie and Brady, 2008) results in a greater application of the evidence in clinical management in future, this should lead to somewhat greater health benefits and possibly in a few more patients being able to return to work who might not otherwise have been able to do so.

20.11 Additional comments

Dr Brady argued that there is a very great need to ensure the availability of funding streams to support interested speech and language therapists and other allied health professionals in developing a clinical research career. Although there are some newly developed funds such as CSO predoctoral and Stroke Association fellowships, the opportunities are few given the number of allied health professionals in clinical practice. Ensuring clinicians from these fields can find a good path into research careers (ie good training in that path with good support) will ensure the clinically important questions are asked and addressed.

20.12 Payback

The payback framework describes the process from research to several categories of impact. The categories allow us to compare across different case studies. Table 20-2 shows, in point form and by impact category, some of the impacts, described more fully above, that have emerged from this grant.

Table 20-2 payback

Payback category	Impacts from case study
Knowledge production	• Five peer-reviewed articles directly linked to the project. • Other peer-reviewed articles indirectly linked to the project.
Research targeting and capacity building	• Made a substantial contribution to the PI's PhD and subsequent career. • Provided important research training for the Research Officer who went onto to undertake a PhD on a related topic and is now the Programme Director for the stroke programme at a national research centre. • The research team have built on the original project in a stream of further work.
Informing policy and product development	• Was cited in the relevant national guideline on stroke from the Scottish Intercollegiate Guideline Network, but this section of the guideline highlighted the issue rather than suggesting possible treatments. • Informed curriculum development in at least one school of speech and language therapy. • The local NHS hospital trust produced a leaflet for patients and their families describing the findings. • A follow-on projects led to the potential development of a transcription-less approach to analysis that speech and language therapists can apply to analyse their client's discourse.
Health and health sector benefits	• Thus far there have probably been a very small level of health gains • It is possible that there could be more gains in the future if the evidence is applied in clinical management.
Broader social and economic benefits	• It is possible that a few people have been able to return to work who might not otherwise have done so.

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CHAPTER 21 To study secondary prevention of hypertension

21.1 Overview of case study grant

This case study examines the evolution and impacts of the National Heart Foundation of Australia (NHFA)-funded clinical research grant, *Secondary Prevention of Hypertension,* awarded to Professor Trefor Morgan for the period 1988–1990 (grant reference G2319).

Much evidence in the past 30 years has implicated diet and lifestyle as major contributing factors to the development of cardiovascular disease (CVD). Risk factors such as cigarette smoking, excess intake of salt and saturated fats, and low-fibre diets have been shown to potentiate the disease process. The current recommendation from the National Heart Foundation of Australia and the National Health and Medical Research Council (NHMRC) is to limit salt consumption to less than 2,300mg of sodium daily (National Heart Foundation of Australia, website as of 2010; National Health and Medical Research Council, Nutrient Reference Values for Australia and New Zealand, 2006), so as to maintain healthy blood pressure and reduce CVD risk. However, past studies and meta-analyses assessing the impact of dietary sodium intake on blood pressure have presented conflicting views, providing a point of disagreement between different scientists. Much of the research undertaken by Professor Morgan throughout his successful career has contributed to our understanding of the dynamic relationship between salt restriction and blood pressure (Morgan et al., 1978, and Morgan and Nowson, 1986). This particular clinical research project was an extension of Morgan's previous work but failed to support clearly his earlier findings regarding sodium restriction and management of hypertension. Secondary unexpected findings of this study did, however, have an impact on future clinical research and public health.

Hypertension is a common disorder among the Australian community, and blood pressure reduction has been shown to prevent vascular events such as heart attack and stroke. Although medications are available to reduce blood pressure, these are not free from side-effects and need to be taken long term, if not indefinitely. This study aimed to determine the impact of non-drug therapies on blood pressure – specifically whether or not a low-sodium diet could be effective in maintaining already controlled hypertension. If this hypothesis were proven to be true, both public health and associated financial implications would be considerable.

This study monitored patients with well-controlled hypertension as they were transferred to a low-sodium diet and their drug therapy was stopped in order to observe the rate at which high blood pressure returned. Approximately 200 patients completed the study. The results showed that although patients following a reduced sodium diet were slower to return to drug therapy than patients with no sodium restriction, there was eventually still a need to return to drug therapy. It thus seemed that salt restriction had an impact on blood pressure control but was not enough to control hypertension completely. According to Professor Morgan, a major contributing factor to this somewhat frustrating result was poor patient compliance in adhering to a low-sodium diet in the long term.

An unexpected outcome was perhaps the most clinically significant finding of this particular study. The study discovered a higher than anticipated prevalence of left ventricular hypertrophy (LVH) in patients with well-controlled hypertension. This contradicted previous beliefs relating to cardiac health. Prior to the study, it had been understood that most patients with hypertension that was well controlled by commonly used drug therapy for at least two years would have normal cardiac size, but echocardiography in this study showed that 50% of these patients still had LVH. The study thus exposed the apparent risks associated with some antihypertensive drugs in failing to regress positive feedback mechanisms associated with progression of hypertension (ie cardiac hypertrophy). This outcome led Professor Morgan to believe that some populations, such as elderly people, could experience higher mortality with hypertensive treatment due to worsening of cardiac hypertrophy. In light of this finding, Morgan proposed that the use of drugs to treat hypertension should be carefully considered given that cardiac hypertrophy was an independent risk factor for sudden death from coronary artery disease. He also reflected that this finding may explain some of the inefficacy of commonly used antihypertensive drugs in preventing heart attack. This finding had implications for future pharmacological treatment of hypertension, as it suggested that some drug therapies did not reverse the effects of hypertension, ie LVH, as well as others. This discovery left a strong impression on Morgan and had implications for his career and further research concerned with the treatment of elderly people with hypertension; in particular, it led to his decision not to partake in the then upcoming high-profile Hypertension in the Very Elderly Trial (HYVET) study (HYVET Press Office, 4 May 2009), as the trial conflicted with his views that antihypertensive drugs should be used with caution in the elderly population.

21.2 Introduction to case study

21.2.1 Scientific background

In medical terms, hypertension refers to sustained, abnormally high blood pressure in the arteries. When blood pressure is checked, two values are recorded. The higher value reflects the highest pressure in the arteries, which is reached when the heart contracts (systolic pressure). The lower value reflects the lowest pressure in the arteries, which is reached just before the heart contracts again (diastolic pressure). Blood pressure is written as systolic pressure over diastolic pressure, and 'normal' blood pressure is referred to as 120/80mmHg. Hypertension is defined as a reading at rest persistently higher than 140/90mmHg.

In most cases, hypertension does not cause symptoms until it begins to damage body organs. For this reason, hypertension is sometimes referred to as the 'silent killer'. Uncontrolled high blood pressure increases the risk of problems such as stroke, aneurysm, heart attack, heart failure, kidney damage and blindness (Lab Tests Online, 2007). The higher the blood pressure, the greater the associated risk to health.

Today, hypertension contributes to more deaths and disease than any other biomedical risk factor worldwide (National Heart Foundation of Australia, 2009). Almost one third of Australians older than 50 years have high blood pressure, and nearly half of the Australian population will have high blood pressure before they reach the age of 70 years (Baker IDI, 2009). For the majority of these hypertensive patients, both genetic and lifestyle factors play a role. Despite the extensive presentation of hypertension and its impacts on public health, a plethora of research in the past decades has indicated that diet and lifestyle factors can have a significant impact on preventing and controlling hypertension.

The intention of the research project examined in this case study was to investigate whether a low-sodium diet could prevent the re-emergence of hypertension in people whose blood pressure has been well controlled for 12 months or longer on drug therapy, ie could the hypertension disease process be 'reset' through adoption of a low-salt diet?

Blood pressure is normally kept within a range of normal values via homeostatic mechanisms including negative feedback. These negative feedback mechanisms 'negate' the impact of changes experienced by triggering counter responses to restore the blood pressure to a normal undisturbed state. In contrast, positive feedback mechanisms increase or amplify a change in the body's internal conditions. In the case of hypertension, the positive feedback mechanisms involve cardiac hypertrophy (ie thickening of the heart muscle) and increased vessel stiffness. These positive feedback mechanisms are also called 'amplifiers', as they potentiate and accelerate the cardiovascular disease process.

This research paper proposed that if high blood pressure is controlled for a prolonged period, the positive feedback mechanisms of cardiac hypertrophy and increased vessel stiffness would regress and the patient would return to an earlier point in their hypertension development cycle. It was proposed that negative feedback mechanisms might subsequently be sufficient to maintain blood pressure within the normal range, as long as certain limits of regulation are not exceeded.

At the time of research, it was widely accepted that regulation of blood pressure was under a variety of negative feedback controls; in the proposal, Professor Morgan referenced Guyton (1980) as having summarised these well. Some previous studies (Korner, 1985, and Folkow, 1982) had suggested that hypertension was accompanied by hypertrophy and stiffening of blood vessels, which acted as amplifiers to perpetuate the increased blood pressure and resulted in malignant hypertension. Arguments were made (Folkow, 1982, and Lever, 1986), with which Professor Morgan agreed, that positive feedback mechanisms also occurred early in the development of hypertension and once started, if not checked, would ultimately lead to malignant hypertension. This chain of events was understood to be triggered when the capacity of the negative feedback mechanisms was exceeded, such that the normal blood pressure could no longer be maintained. Thus it was expected that hypertension would be perpetuated and would worsen. Was it possible that sodium intake could influence these events?

The application pointed to three recent studies that had supported the concept that if blood pressure could be brought under control for a period of time with pharmaceutical therapy, then it may be possible to preserve that controlled state with non-pharmacological therapy such as diet and lifestyle changes (Morgan and Anderson, 1987; Stamler et al., 1984; and Jennings et al., 1986). Professor Morgan himself had been involved in studies investigating this concept, and his primary research focus had been sodium restriction and blood pressure control. In contrast, Stamler et al. (1984) explored a salt- and calorie-restricted diet, and Korner et al. (1985) and Jennings et al. (1986) explored the role of exercise. None of these studies had at the time shown success in preventing a return to drug therapy.

At the time of this study, epidemiological studies had shown that there were striking individual and inter-population differences in blood pressure, yet there were conflicting views on the benefits and risks of salt restriction. The predominant opinion in the United Kingdom at the time was from Sir George Pickering and his colleagues, who strongly opposed the concept of treating hypertension with salt restriction (Beevers and Stamler, 2003). A leading figure in renal research in Australia, Dr Kincaid-Smith, similarly shared this view, additionally reminding readers of the possible dangers of a low-sodium diet (Kincaid-Smith, 1997).

21.2.2 Principal investigator's background

The principal investigator (PI) on this project was Professor Trefor Morgan. Morgan was Head of Physiology at the University of Melbourne and, at the time, conducted much of his research at the Department of Veterans Affairs and its repatriation hospital at Heidelberg, Victoria. Morgan's research experience included both biomedical and clinical research; he also had clinical experience. His background was in the renal field, including time in the United States in the mid 1960s conducting research on renal function in relation to sodium and water. It is important to note here that the kidneys provide a major mechanism of blood pressure control and regulation via the renin–angiotensin–aldosterone system (RAAS). This system responds directly to changes in blood pressure by regulating sodium excretion and retention in the kidneys.

The Department of Veteran Affairs is an Australian Government Department that provides support and information for Australia's war veterans and their dependents. Among the services provided are health care, rehabilitation and counselling services, as well as pensions and compensations, home-care assistance, commemorative activities and historical information.

Heidelberg Repatriation Hospital served as a military hospital during the Second World War and was handed over from the Australian Army to the Repatriation Commission, or Department of Veteran Affairs, in 1947. It became a state hospital on 1 January 1995. The hospital had a particular focus on the treatment and care of war veterans and war widows.

21.2.3 The case study approach

The case study based on this research grant involved a combination of: face-to-face interviews with the PI; interviews with other members of the research team; review of the

original grant application and supporting documents including assessor comments; and documentary analysis of various papers produced by the PI from this research.

21.3 Stage 0 – topic/issue identification

Morgan said, 'The research and the clinical work I had done before seriously influenced the topic' (Morgan interview, 2008).

The idea for this research arose from a stream of research that started in the 1960s and was very much influenced by Morgan's previous research in addition to his clinical experience:

- PI's previous research
- PI's clinical experience
- disproving others
- diet and lifestyle factors could replace medication for hypertension.

21.3.1 PI's previous research

Professor Morgan had been involved in biomedical and clinical research since the 1960s. He had been interested in the relationship between sodium intake and hypertension for many years prior to this project, with his first grant submission to the NHF in the early 1960s concerning the effect of sodium intake on hypertension. In 1965 he spent time in the United States conducting research on renal function in relation to sodium and water. His extensive clinical experience pointed to a strong relationship between sodium intake and regulation of blood pressure via the RAAS.

Professor Morgan had conducted a number of studies whose results had been published in a number of prestigious journals including the *Lancet* (Morgan et al., 1978) and *Canadian Journal of Physiology and Pharmacology* (Morgan and Nowson, 1986). His previous papers had looked at blood pressure in relation to sodium restriction and also the inter-relationships and impacts between sodium and other minerals such as calcium, magnesium and potassium on blood pressure. The grant application notes 20 relevant publications authored or co-authored by the applicant (including Chalmers et al., 1986); this contrasts with six publications from other groups and three by the co-applicant (Anderson) but without Morgan's involvement. Funders of this body of work included the National Heart Foundation of Australia, Department of Veterans Affairs and National Health and Medical Research Council (NHMRC); indeed one of the relevant publications cited in the application was titled 'Australian NHMRC Dietary Salt Study in Mild Hypertension' (Chalmers et al., 1986), which was in press at the time the application was submitted and acknowledged Morgan as a contributor. Morgan's commitment to the subject was further reflected by his position of chairman on the NHMRC working party for sodium in the Australian diet.

At interview, Professor Morgan noted of particular relevance his involvement in clinical trials starting in 1971, which he believed were the first to show that sodium restriction influenced blood pressure. The resultant paper was published in 1978 in the *Lancet* – one of the most important forums for this work (Morgan et al., 1978); this paper has been

cited more than 300 times to date. This study was partially designed to be a prognostic study. It was a controlled study investigating the effects of reduced sodium intake on blood pressure in males aged 55–75 years being treated for mild hypertension (as compared to a drug-treated group and a control group). It had the following findings: '31 patients with a diastolic blood-pressure between 95 and 109 mm Hg have been treated for two years with a regimen involving a moderate restriction of salt in the diet. The results are compared with those in a control group and in a drug-treated group. Salt restriction has reduced the diastolic blood-pressure by 7.3+/-1.6 mm Hg, a result similar to that in patients treated with antihypertensive drugs. In the untreated group the diastolic blood-pressure rose by 1.8+/-1.1 mm Hg. Most patients did not achieve the desired amount of salt restriction and a stricter adherence to the diet might have caused further falls in blood-pressure. Excessive salt intake is probably a major cause of the epidemic of hypertension in "civilised" countries and a reduction in salt intake may help to control the epidemic. In persons with a diastolic blood-pressure between 90 and 105 mm Hg salt restriction should be tried before drugs' (Morgan et al, 1978).

The intention of the grant in question was to build directly on a pilot study undertaken by Professor Morgan. The pilot study had shown that a reduction in salt intake could delay the return to drug therapy, and the research proposed in the grant sought to confirm these preliminary findings on a larger scale. The pilot study examined 20 well-controlled patients as their sodium intake was normalised or reduced for 8–12 weeks, after which their drug therapy was stopped. Six months after drug therapy was initially stopped, nine of the ten patients following a normal diet had returned to drug therapy, while four of the ten patients on a reduced sodium intake had returned to drug therapy. The numbers returning to drug therapy and the rises in blood pressures were significantly different between the two groups. These results were published in the *Canadian Journal of Physiology and Pharmacology* (Morgan and Nowson, 1986) and the values from the pilot study were projected to the proposed study to enable statistical analysis. Both the paper published from the pilot study (Chalmers et al., 1986) and the *Lancet* publication (Morgan et al., 1978) are referenced in a systematic review published in the *British Medical Journal* (Hooper et al., 2002).

21.3.2 PI's clinical experience

Professor Morgan's background combined biomedical and clinical research in addition to clinical experience and teaching. Morgan said, 'I don't distinguish between research, patient care and teaching' (Morgan interview, 2008).

Professor Morgan believed that his clinical experience enabled him to form hypotheses to test and also that it added to his conviction to challenge thinking, as he was able to observe for himself correlations between sodium-controlled diets and blood pressure through experience in renal clinics.

Key to this project was Professor Morgan's clinical experience in the renal field – observations he made while based at renal clinics created strong hypotheses to test at a time when sodium was believed, in the United Kingdom and Australia at least, to have minimal involvement with blood pressure. While working in a clinic in the United Kingdom, he noticed an absence of high blood pressure among dialysis patients following sodium-controlled diets. At another renal clinic, this time in Melbourne, Australia, a high-sodium

diet was advocated and patients had elevated blood pressure that was difficult to control. It was these experiences that provided him with hypotheses to test.

21.3.3 Disproving others

As noted above, the concept that sodium restriction had a role in controlling high blood pressure was controversial. Disproving 'doubters' provided motivation to Professor Morgan and his undertaking of the study; indeed, Morgan had a letter on this topic published in the *Lancet* (Morgan et al, 1986).

21.3.4 Diet and lifestyle factors could replace medication for hypertensives

The premise of the research was that pharmaceutical therapy for hypertensive patients could be replaced by diet and lifestyle therapy. From Professor Morgan's perspective, this added additional impetus for his research.

At the time, other research groups were working on similar hypotheses – notably the group led by Graham Macgregor from the United Kingdom, which looked at primary prevention and initial treatment.

21.4 Interface A – project specification and selection

The grant was prepared and submitted by Professor Morgan, with statistical advice provided by John Hopper. It was accepted by the NHF.

The research project had two aims:

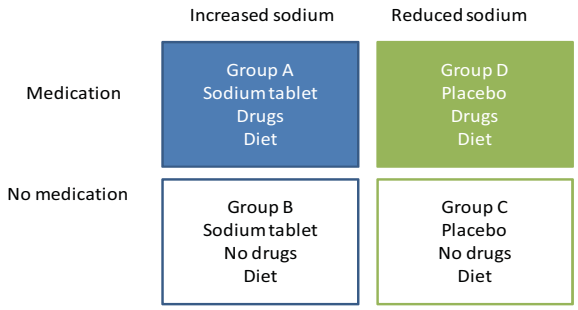
- to determine if non-pharmacological measures (in this case a decreased sodium diet) can prevent the re-emergence of hypertension in people who have been well controlled for 12 months or longer on drug therapy
- to determine the role of secondary amplifiers (cardiac and vascular) in this response.

Of particular importance to this study, and a differentiator from other studies at the time, were the qualification criteria for patients entering into this trial. All patients were to have had proven hypertension prior to the initiation of drug therapy and to have had good control of their hypertension with said drug therapy for at least two years prior to this study. The protocol called for pre-therapy diastolic blood pressure >100mmHg on at least two occasions, which had reduced with drug therapy to <85mmHg over the last 12 months at every visit. The protocol also required all patients to have no evidence of cardiac hypertrophy on electrocardiography (ECG) and echocardiography when they entered the study.

Three key phases were planned in the research programme. The first phase was pre-intervention, when subjects continued with their current drug treatment. In this time, subjects were monitored and screened to meet study entry requirements and were randomised to a study group. The second phase was that of intervention. Qualifying subjects were placed on a diet to reduce sodium intake and increase potassium intake for three months, with monthly monitoring. In the third phase, drug therapy was stopped and sodium intake was modified, with subjects allocated to one of four groups (Figure 21.1).

Two groups received a slow-release sodium tablet daily and two groups received a matching placebo instead. Within each table group, one subgroup continued with their previous medication, while the other subgroup did not. In phase 3, subjects were seen two days, one week, two weeks and then monthly after stopping therapy, depending on their blood pressure response. If an increase above 90mmHg was seen on two occasions, the trial for that individual was stopped and drug therapy reintroduced. The research proposed to follow subjects for 24 months to enable longer term comparisons to be drawn. It also proposed that if the results of the pilot study informing this study were confirmed in the first 48 subjects to reach six months, the whole group of patients would then continue for a further six months according to the protocol. After this time, the tablets (both sodium and placebo) would be withdrawn and the patients followed to determine the time to increase in blood pressure.

Figure 21.1 Four-group structure for modification of sodium intake



The routine examination at each visit involved weighing the patient, monitoring supine and erect blood pressure, testing urine electrolytes and completing a side-effect questionnaire. Diet counselling occurred at each visit in phase 2 and at three months in phase 3. A tablet count was undertaken in phase 3. Special tests in each phase, usually conducted at the last visit, were scheduled to include: creatine, urea, sodium, potassium, bicarbonate, calcium, magnesium, cholesterol and lipids, plasma renin activity (PRA), atrial natriuretic peptide (ANP), standardised ECG, echocardiography and vascular compliance (venous plethmysography and arterial wave velocity). At six months, 24 months and withdrawal, all patients had set tests: ECG, echocardiography and vascular compliance.

The sample was designed to enable key ideas to be explored. Of particular interest were the comparison of results from patients in Group B and Group C, which would show whether a difference in sodium intake could alter the rate at which blood pressure increases after drug therapy has been stopped, and the comparison of results from patients in Group C and Group D, which would show the continuing effect of drugs.

The analysis was to focus on three principal endpoints: return to drug therapy, blood pressure increase and length of time until diastolic blood pressure >90mmHg. Predictions were made based on the results of the pilot study. Further planned analysis included whether the return of hypertension is influenced by vascular compliance, cardiac size, level of sodium intake and previous drug therapy to provide information about the mechanisms

that cause re-emergence of high blood pressure and information related to the hypothesis of Folkow (1982) and Lever (1986) of a positive feedback mechanism operating early in the development of hypertension. Finally, the study also looked at the effect of stopping drug therapy on heart and vessels over a six-month period.

The potential value of the proposed research was noted in the review process: 'Whichever result is obtained, the data should be a significant contribution to our understanding of the relationship between long-term treatment, reversal of hypertensive cardiovascular changes and progression of hypertension' (Grant-in-Aid Assessor Report, 1987).

Reviewers had little impact on the project specifications and there were no major conceptual changes from this process, although there was some clarifying and fine-tuning of the project. The assessors raised a number of questions about the study design, in particular relating to dietary intake, body size, type of medication used, age of patients ('Why are patients below the age of 45 excluded? I imagine this is because of the applicant's connection with the Repatriation Service' (Grant-in-Aid Assessor Report, 1987)) and the analysis proposed. Questions raised at the review interview were 'well answered' according to the interview report. However, funding for the half-time technical officer was declined, as the committee were not convinced of the value of ECGs and vascular compliance.

The review committee seems to have been very positive about the proposed research, rating it fundable, with a score of 4.25, and noting:

- 'An exciting project well worth support' (National Heart Foundation of Australia Scientific Review Committee Report, 1987)
- 'Committee enthusiastic for this project. Perhaps should have been rated higher' (National Heart Foundation of Australia Scientific Review Committee Report, 1987)
- 'Interesting proposal' (Grant-in-Aid Assessor Report, 1987).

This research application was approved for funding by the NHF. It had also been submitted to the NHMRC but was declined – Professor Morgan believes this was because the NHMRC at the time was funding more basic research rather than clinical research projects and this, of course, was a clinical project. Had funding from the NHF not been forthcoming, Morgan believes it is likely that this contribution would have been found elsewhere; failing that, the research would have probably have gone ahead anyway but on a smaller scale.

21.5 Stage 1 – inputs to research

The most important inputs to the research were, in the view of Professor Morgan, knowledge and expertise. Time, space and consumables were important and necessary to the research going ahead.

21.5.1 Financial

Grant funding

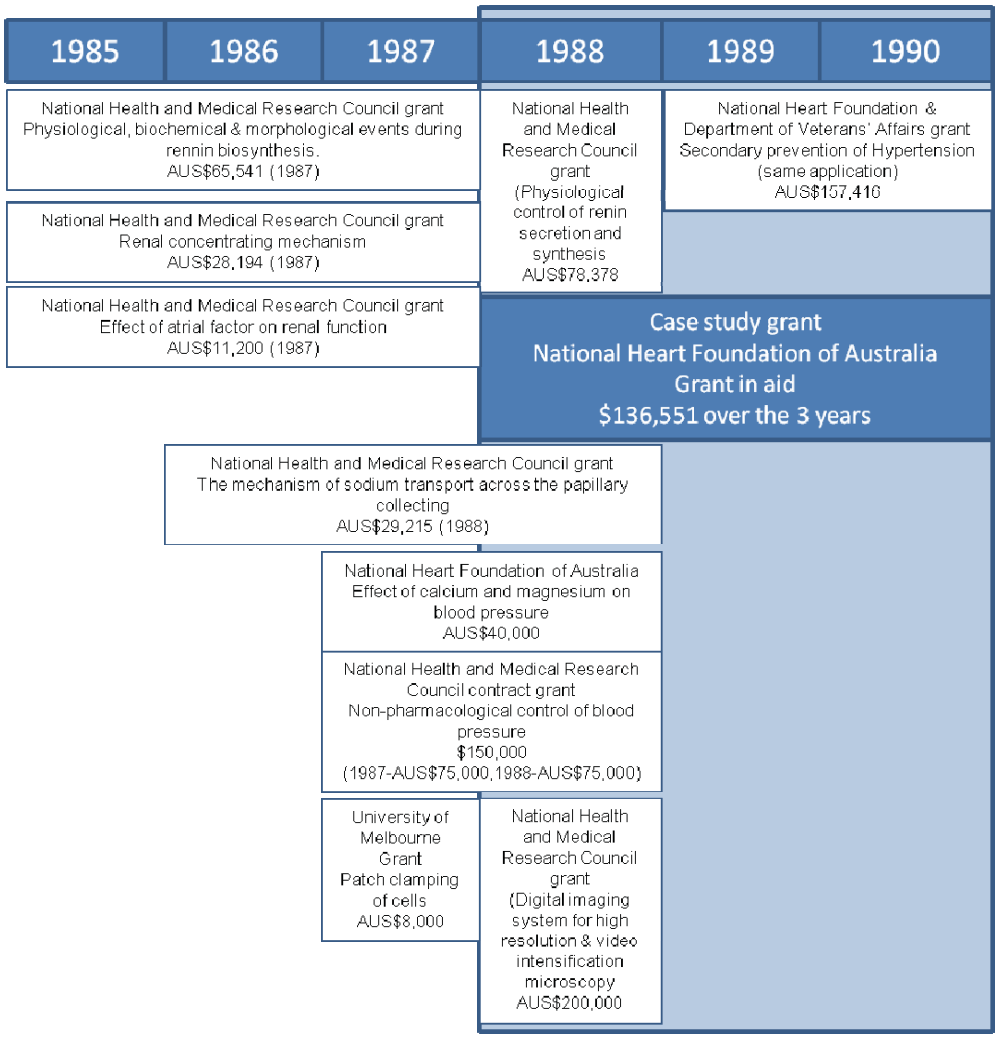
The grant provided funding for staff assistance and some maintenance costs for the three-year period 1988–1990.

Funding for a full-time research nurse was originally approved but was revised in the course of the project at the request of Professor Morgan to instead allow for part-time nursing, secretarial and technical help – ie several staff to cover the duties required and enable the required procedures to be run. The grant also funded a sessional dietician; however, funding requested for a half-time technical officer was declined. The maintenance allowance was also less than requested, with the project receiving just under two thirds of the amount originally requested.

At the time of the grant application, Professor Morgan was receiving one grant from the NHF and five grants from NHMRC, one of which was a contract grant. Three of these grants were for research in the renal area. The contract grant provided funding for research on the non-pharmacological control of blood pressure. Details of funding held by Morgan at the time of the application are shown below in Figure 21.2. Morgan was also requesting a digital imaging system (Aus$200,000 requested from NHMRC), two further research projects (one from NHMRC and one from NHF) and equipment for patch clamping of cells (shown in grey in Figure 21.2).

It should be noted that much of the funding at Professor Morgan's disposal at the time came from sources other than the NHF and, according to Morgan, may have benefitted this grant.

Figure 21.2 Grants and funding held and applied for (grey) by PI at time of application



Other types of funding (overheads paid, salary coverage, voluntary contribution, research assistants provided and consumables provided)

A considerable amount of support was provided by the Department of Veterans Affairs through the repatriation hospital, including facilities, equipment required for tests involved, a nurse and a dietician from the hypertension clinic, a technician in the cardiac unit and a technician in the vascular unit. Although these resources were shared with other projects, Professor Morgan suggested that this was perhaps more significant than the contribution from the NHF.

21.5.2 Knowledge and expertise

Research experience (of PI and team)

Knowledge and expertise was a very important input to this research. As noted above, Professor Morgan had extensive experience in this field and was involved in a number of associated projects at the time, although not necessarily specifically in the CVD arena. This

project built directly on his previous work. Morgan was the most senior member of the research team.

Professor Caryl Nowson was named on the application as associated with the project. Professor Morgan believes that whether she was named or not she would have somehow been involved in the project. Nowson was completing her doctor of philosophy (PhD) degree and was working part-time at the hospital as a dietician, charged specifically with providing dietary advice for low-salt diets. She had been in this role since 1983 and believes she refined her techniques over time. As a dietary counsellor, she not only provided dietary advice but also monitored patients and tried to 'keep them on the straight and narrow' (Nowson interview, 2009), developing motivational techniques through her experiences. At the time of this particular grant, Nowson was certainly qualified to provide practical dietary advice, with a published low-salt cookbook to her name[1]. Prior to and during this project, Nowson was involved with a number of other research projects run by Professor Morgan and other researchers. It seems that Nowson's input to this particular project was limited to dietary counselling: by her own admission, her intellectual input to this project was limited as her efforts were focused elsewhere, notably to the completion of her PhD and preparing a grant application (with others) to explore calcium and osteoporosis.

Dr Adrianne Anderson, a visiting medical officer at the repatriation hospital, was named on the application as an associated senior investigator. She was a clinician at the hypertension clinic and was actively involved in this project, as were a number of nursing sisters from the clinic.

Rosemary Snowden was a level 3 (year 2) registered nurse at the time and was based at the repatriation hospital. It was her salary that was sought as part of the funding; however, this was subsequently amended to a series of part-time assistants' positions.

John Hopper, PhD, from the University of Melbourne, was named on the application and provided statistical advice and analysis for both the application and the project. At the time of the application he was an NHMRC Senior Research Officer and held the honorary position of Statistical Consultant to the Royal Melbourne Hospital.

Techniques

The project involved a double-blind study wherein participants were given either sodium chloride tablets or sodium chloride placebo tablets. Participants were recruited according to strict criteria concerning hypertensive history and heart health; the fact that all participants were recruited through the Department of Veterans Affairs' clinic helped to ensure medical history was known. There were three key phases – pre-intervention, intervention and the double-blind study – as detailed above. Specified tests were conducted at routine visits and other set intervals, as detailed above, and throughout Phases 2 and 3, all participants were given the same dietary advice to minimise environmental dietary

[1] In 1986 Nowson published a cookbook with *Women's Day* magazine, with a print run of 11,000. The rights were bought by the Australian Broadcasting Corporation (ABC) and the book was re-released in 1988 to accompany the TV series *Bodyshow;* the second edition included a foreword by the PI and some more recipes from Nowson.

effects. Statistical analysis was undertaken including variance and chi-squared tests to compare differences.

21.5.3 Equipment, infrastructure and space

At the time of this research, Professor Morgan was Professor of Physiology at the University of Melbourne. In this role, he controlled laboratory space at the university, which he was able to use, and could access facilities. However, for this particular research, the majority of the work was in fact conducted at the Department of Veteran Affairs' repatriation hospital, which supported much of his clinical work in a practical sense.

The repatriation hospital was important to the recruitment of patients for the study. Professor Morgan had an established relationship with this hospital and conducted a number of studies in conjunction with clinics there. The history of the clinic's patients was known, and this was a valuable benefit to this research project given the importance placed on the protocol criteria of all participants being true hypertensives. Indeed, reviewers noted: 'Good patient population well researched' (National Heart Foundation of Australia Scientific Committee Review Report, 1987).

21.5.4 Research samples, datasets, patient cohorts, etc

Given that this project was clinical research, patients were involved. Patients were recruited through attendance at the clinic at the repatriation hospital, where they were asked if they would be prepared to be involved in the study. Professor Morgan believes that the prospect of an alternative to drug therapy and being able to safety stop using medication was an attractive proposition to many and encouraged their involvement. He also felt this was a motivating factor for researchers.

Professor Morgan noted that recruiting through the clinic gave a greater certainty to patient history. It was important to this study that participants were truly hypertensives, and this could be known easily through the patient's relationship with the clinic.

Professor Nowson believed that patients at the repatriation hospital, having been soldiers and served their country, were influenced by a 'serve-your-country' mentality and thus were happy to be involved and help (Nowson interview, 2009).

21.6 Stage 2 – research process

The project proceeded broadly as planned; however, some modifications were made in response to outcomes that emerged in the recruitment phase. For example, following commencement of the research it seems that a change was made to the study design, with the unforeseen discovery that 50 percent of the patients screened had significant LVH despite good control of hypertension for at least two years prior – a higher prevalence than expected. As a consequence, patients were stratified into groups with and without LVH. Another report talks about using three groups: 1) hypertension well controlled and no cardiac hypertrophy; 2) hypertension well controlled but continue to have cardiac hypertrophy; and 3) hypertension has not been as well controlled as the first two groups.

It seems that the recruitment process was lengthy, with a progress report noting that while 300 patients had had echocardiograms performed and 120 had proceeded to the study,

recruitment and randomisation would continue for three more months to enable analysis of the study and completion by the end of the year.

Professor Morgan believes that although the methods used were appropriate at the time of the research, other relevant techniques that have become more accepted since this time would also have been used if the research was being conducted today. For example, the study used clinical blood pressure measurements. Today it is accepted that this technique has a white-coat effect in a large part of the population and ambulatory blood pressure monitoring is favoured. At the time, however, ambulatory blood pressure monitoring was not well established and equipment was very expensive. It is also accepted today that sleep blood pressure is the most important blood pressure measure and the measure in which changes occurring as a result of high salt intake first will be seen; indeed, it is known today that treatment administered during sleep can reverse LVH. If this project was to be done today, blood pressure would be monitored 24 hours a day.

In addition, the research focused on sodium intake, while high salt intake today refers to and acknowledges the interaction of sodium and potassium; generally sodium intake increases while potassium decreases with processed foods and potassium increases while sodium decreases with fresh foods. Sodium chloride tends to be added to foods, and cooking can cause potassium to leech out.

21.7 Stage 3 – primary outputs from research

21.7.1 Knowledge

Ultimately, the research did not prove the research objective – ie that a low-salt diet can prevent the re-emergence of hypertension in people whose blood pressure has been well controlled for 12 months or longer on drug therapy. There was no clear outcome, and the research raised more questions to be answered.

According to Professor Morgan, the research disproved dominant thinking at the time that it was possible to reverse hypertension and stop drug therapy by reducing sodium intake. As Morgan suspected from the pilot study, although patients following a reduced sodium diet were slower to return to drug therapy than patients who were not following such a diet, nonetheless there was a need to eventually return to drug therapy. This discovery had implications for Morgan's career and for further research concerned with the treatment of elderly people with hypertension.

Another discovery made in this research was a greater than anticipated prevalence of LVH in elderly people (aged 45–81 years) with proven and subsequently well-controlled hypertension, which contradicted previous beliefs relating to cardiac health. Prior to the study, it had been believed that most patients with hypertension that had been well controlled by commonly used drug therapy for at least two years would have normal cardiac size. In contrast, echocardiography performed in this study showed that 52% of these patients still had LVH. The research could not identify the nature of the hypertrophy. In conclusion, it was proposed that, as cardiac hypertrophy was an independent risk factor for sudden death from coronary artery disease, the use of drugs to reduce blood pressure should be carefully considered, and that this finding may reflect

some of the poor results of preventing myocardial infarction by treating hypertension with commonly used drugs. This had implications for pharmacological treatment of hypertension, as it suggested that some pharmacological therapies did not reverse the effects of hypertension, ie LVH, as well as others.

A key barrier to success, according to Professor Morgan, was the difficulty in achieving long-term compliance with reduced sodium in diets; certainly it was hard to get the foods required to facilitate this on a sustained basis at the time of this study, as the food industry was not geared towards this, and Morgan believes this remains an issue today. However, the dietician involved believes that a major supermarket already had a clearly labelled and affordable range of low-salt foods, including bread, to which she was able to direct patients; only patients without easy access to this supermarket chain may have found it harder to comply.

It seems that two papers were published as a direct consequence of this specific grant, with a further 10 publications indirectly linked to this grant (Morgan et al., 1994, and Jones et al., 1990). Professor Nowson made an important note that Professor Morgan did not make a lot of publications at the time as this was not the primary output; Professor Morgan agreed with this, noting that he would only publish something once. It may therefore be misleading to assess the impact of this grant on publications alone. Nowson said, '[Trefor] didn't actually take an awful lot of things to publication. It was a different time, it wasn't the sort of primary outcome the way it is now, that every one counts your publications and that it's your publications that count for everything. At that time it wasn't a major thing; that was my impression anyway…different to how things work these days' (Nowson interview, 2009).

The two papers directly linked to this grant are:

- Morgan, T., J. Hopper, A. Anderson, L. Carricks, E. Jones, J. Johns, R. Green and C. Nowson, 'Can Drug Therapy Be Stopped in Elderly Hypertensive Patients', *Cardiology in the Elderly*, Vol. 2, 1994, pp. 119–125.
- Jones, E., T.O. Morgan, P. Califiore and J. Johns, 'Prevalence of Left Ventricular Hypertrophy in Elderly Patients with Well Controlled Hypertension', *Clinical and Experimental Pharmacology and Physiology*, Vol. 17, 1990, pp, 207–210.

The first paper (Morgan et al., 1994) has been cited twice in the time from publication to April 2009; unfortunately the paper could not be accessed for review itself. It has been cited in the systematic review entitled 'A Systematic Review of Predictors of Maintenance of Normotension After Withdrawal of Antihypertensive Drugs' (Nelson et al., 2001). This review was undertaken by Australian researchers based at the Victorian Institutes of Monash Medical School and the Baker Medical Research Institute and was published in the *American Journal of Hypertension* in 2001. The other citation arising from this publication was in a paper reporting on a study comparing the effectiveness of different approaches to participant enrolment in a behaviour modification trial (Whelton et al., 1997). In addition, while not emerging in Web of Science citations, this paper has been found to be cited in guidelines published by the National Institute for Health and Clinical Excellence (NICE) in the United Kingdom (NICE, 2004) as described in a later section. Professor Morgan noted that the publication *Cardiology in the Elderly* was new at the time.

He believes it had been seeking papers to publish and felt it was likely that he had been approached to submit a paper. The publication is not in press today.

Table 21-1 and Figure 21-3 illustrate the publication output attributed to the case study grant application, its impact and the extent of the knowledge diffusion.

Table 21-1 Publication output and impact of directly related publications[4]

Number of journal articles:	2
Number of articles included in citation analysis:	1
Total number of citations (all papers):	2
Aggregate relative citation impact:	0.11 (Class II)
Self-citations:	0%

Distribution of publications across relative citation impact classes:

	Class I (uncited)	Class II (>0 and <0.8 citations)	Class III (0.8 to 1.2 citations)	Class IV (1.2 to 2.0 citations)	Class V (>2.0 citations)
Number of publications		1			
Proportion of total output		100%			

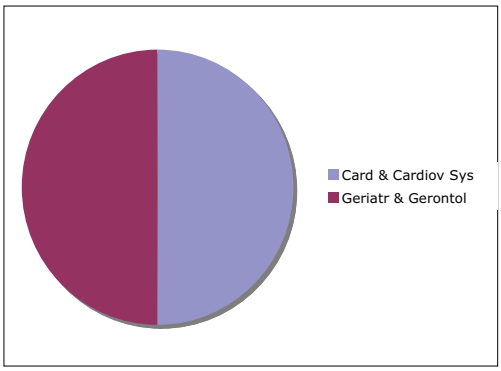
Most highly cited publication[5]:	Morgan, T., J. Hopper, A. Anderson, L. Carricks, E. Jones, J. Johns, R. Green and C. Nowson, 'Can Drug Therapy Be Stopped in Elderly Hypertensive Patients', *Cardiology in the Elderly*, Vol. 2, 1994, pp. 119–125
Times cited:	2

[4] In addition, ten publications were indirectly linked to this grant. These publications were all indexed in Web of Science and received 83 citations in total, giving a relative citation impact of 0.47. All were in relative citation impact Classes I and II, except for one in Class IV, and their self-citation rate was 18%.

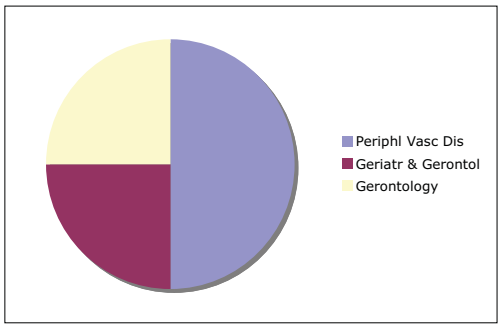
[5] Citation count extracted April 2009.

Figure 21-3 Knowledge diffusion: fields of research of output (a), fields of citing papers (b) and countries of citing papers (c)

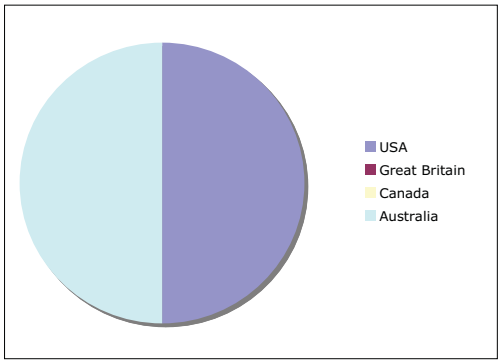
(a)



(b)



(c)



The second paper directly attributable to this specific grant is officially described as a note and was provided with a progress report submitted by Professor Morgan to the funder (Jones et al., 1990). It does not appear in Web of Science and for this reason is not reported in the publication output and impact data shown in Figure 21-3 above. This paper reported the finding of a greater than expected prevalence of LVH among the study population, noting too that the 52% incidence recorded was possibly an underestimate due to criteria used in the study. No relationship between level of treated blood pressure and

prevalence of LVH was found, which was taken to lend support to the idea that the development, maintenance and regression of LVH in hypertension was influenced by factors other than blood pressure, of which age could be one. In contrast to previous studies that showed that angiotensin-converting enzyme (ACE) inhibitors, beta-blocking drugs and calcium antagonists had a positive effect on the regression of LVH in hypertension, no evidence was found to suggest that use of any particular antihypertensive drug was associated with a lower prevalence of LVH, although it was espoused that this could be due to the older age of this study population. The paper concluded that if reversal of LVH in hypertension was to be attempted, drugs that could do this, as well as controlling hypertension, in all age groups should be identified, and ongoing study of larger numbers of patients and prospective trials of the effects of specific drugs on LVH could help with this. This paper has subsequently been cited four times.[2]

21.7.2 Benefits to future research and research use

Capacity building and career development

The PI

This project was one of a number of studies Professor Morgan undertook to explore the role of sodium in hypertension. Almost 20 years on, Morgan continues to be involved actively in the fields of hypertension and CVD; he is convinced that hypertension and blood pressure are greater risk factors for CVD than obesity and believes that this can be stopped if something can be done before disease sets in. In the interim since this grant, he has conducted, and continues to conduct, both clinical and basic research looking at the impacts of both pharmacological and non-pharmacological inventions to hypertension. He has also made significant contributions to teaching; today he lectures at the University of Melbourne in the Department of Physiology. His career includes internationally recognised contributions on the pathogenesis, treatment and prevention of hypertension, which have shaped clinical practice, and significant roles in two Australian national blood pressure trials, which have been international landmarks in the field. He has been invited to speak at a number of major conferences – for example, recently convening a workshop at the conference of the International Society of Hypertension in Beijing in 2007 titled 'Hypertension in the Asian Pacific Region – the Problem and the Solution' (International Society of Hypertension, 2007). He has also written books, including '*Case-Based Medical Physiology*' with Bell and Kidd (Bell, Kidd and Morgan, 2005), which was aimed at the medical profession, and book chapters on the subject of hypertension, including a chapter called 'Principles of Individualized Hypertension Management' in *Hypertension: Principles and Practice* (Morgan, 2005).

His research experience, including but not specific to this grant, has led to his involvement in a number of committees, including:

- NHMRC working party on sodium in the Australian diet (Chair)
- Asia–Pacific Society of Hypertension (Secretary)

[2] Citation count from Google Scholar; it should be noted that Google Scholar tends to identify a wider range of citations than Web of Science, which has been used to identify citation counts across directly attributable publications in this broader study.

- Australian Physiological Society (honorary member)
- High Blood Pressure Research Council
- International Renal Commission on Physiology (President).

Professor Morgan is known among his peers for promoting the importance of salt and blood pressure, being a forerunner in this field: 'His advocacy of the importance of salt and blood pressure predated the contemporary resurgence of interest in this topic and his novel hypotheses regarding salt and the vascular and cardiac complications of hypertension help define a new chapter of basic and clinical research' (Harrap, 2006). However, it is difficult to find Professor Morgan directly referenced in professional papers on the topic of salt and hypertension. For example, he is not referenced in a recent professional paper from the National Heart Foundation of Australia entitled *Salt and Hypertension* (National Heart Foundation of Australia, 2007). This is perhaps, in part, through his involvement in a number of relevant committees, which are themselves referenced.

It is likely that the particular grant in question probably did not make a major contribution to Professor Morgan's subsequent career. He continued to be active in the field of hypertension research, and his publication list shows many and varied papers after this grant, including the effects of antihypertensive drugs, but the extent to which this has been influenced by the grant in question is uncertain. Morgan recalled a continuation of sodium and potassium studies with John Chalmers as the PI, as Chalmers was better placed to secure funding from the NHMRC.

An interesting observation made by Professor Morgan is that his involvement in the grant in question in fact stopped his involvement in another high-profile study that has recently been completed. Morgan was initially approached to be involved in the Hypertension in the Very Elderly Trial (HYVET), which was 'the first morbidity/mortality trial to answer the questions of antihypertensive treatment benefits in very elderly hypertensives' (HYVET, 2007). This study was the largest ever to address these questions, looking at 3,845 patients, and was funded in main by the British Heart Foundation and the Institut de Recherches Internationales Servier. However, following the findings from the grant in question, Morgan withdrew his involvement in this study on the grounds that he did not believe that elderly people with true hypertension could be safely controlled through non-pharmacological methods and, as such, that it was unsafe to stop drug therapy.

HYVET went ahead, but, after a lengthy recruitment process, was stopped early in July 2007 on the recommendation of an independent data monitoring committee after observation of significant reductions in overall mortality and stroke in those receiving treatment. The study received a number of accolades: being voted 2008 trial of the year by the ImpACT/Society for Clinical Trials and judged by these bodies to be a landmark clinical trial for design, execution and results; voted to be among the top 10 major advances in heart disease and stroke research for 2008 by the American Heart Association; nominated as most important clinical trial of the year by Medscape (an online resource for clinicians); and identified as exceptional among the all-time top 10 list for clinical trials compiled by Faculty of 1000 Medicine.

Other team members

Professor Nowson is still active in research and is currently Professor for Nutrition and Ageing at Deakin University, Victoria. She continues to be involved with dietary counselling and has a particular focus on the older population. Her specific research interests today are based around dietary approaches to prevent hypertension and osteroporosis, and she now works at the population level and has undertaken a number of community-based intervention studies assessing the impact of dietary modification on blood pressure and risk factors of osteoporosis. Although Nowson was not immediately further involved with low-salt diets following this grant, she believes she has since come full circle and returned to this theme, and she is committed to reducing the salt intake for Australians, which she believes will have a significant impact on mortality and disease burden due to CVD.

The exact extent of the impact this particular grant has had on Professor Nowson's own career is, through her own admission, incredibly hard to determine. She strongly believes that her involvement with the work of Professor Morgan, ie involvement with this and other projects, has had a significant influence on her own thinking and has been an influence in her building a programme of research around effecting a reversal of hypertension through dietary intervention. Showing her commitment to this idea, further to her work with Professor Morgan and a sabbatical in the UK, Nowson became involved in World Action on Salt and Health (WASH)[3] and was responsible for setting up the Australian division, AWASH, in 2005. From inaugural chairperson, her involvement continues today as a member of the secretariat and working committee.

Dr Anderson today is a clinician and practices in Heidelberg, Victoria.

Dr John Hopper provided statistical advice and analysis for both the application and the project. Hopper trained as a mathematician and statistician and brought these skills to medical research 'because I'd rather put my talents to that use than go and help some company make bigger profits' (NHMRC, 2008). Today, Hopper is a world-leading researcher in genetic epidemiology. He is one of nine inaugural Australia Fellows awarded by the NHMRC in 2007. He is a professorial fellow and Director of Research of the Centre of Molecular, Environmental, Genetics and Analytic Epidemiology in the Department of Population Health at the University of Melbourne. This research project would have been one of many that he was involved with, and although it may not have had a significant impact on his career development, it will have made some contribution.

Targeting of future research

The research raised a number of questions for further research. The research indicated that it was not possible to permanently stop drug therapy among older hypertensive patients, raising the question of what was the best treatment, albeit pharmacological, for elderly people with hypertension in the long term. That said, the search for non-pharmacological

[3] WASH is a group of leading worldwide experts who aim to reduce salt in the diet worldwide by influencing the media and food industry. It exerts pressure on multinational food companies to reduce the salt content of their products, aims to influence government policy on salt reduction in different countries by highlighting the need for a salt reduction strategy and works closely with the World Health Organization to develop a more coherent strategy towards salt reduction worldwide.

lifestyle control of hypertension continued, with teams at the Baker Heart and Diabetes Institute (Baker IDI) looking at the role of exercise in hypertension control.

The research also found LVH to be more prevalent than expected among elderly patients with pharmacologically well-controlled hypertension, raising the question of why there was so much LVH, and, in response to this, raised questions about the long-term effects on cardiovascular health of different classes of hypertensive drugs in people with hypertension. Professor Morgan was among the researchers who went on to explore this question further.

Professor Morgan also took further research questions concerned with the process from this research – for example, definitions used in studies and protocols; how people with hypertension were to be defined and how many visits and measurements were required to determine this; and what level of fall in blood pressure was required for blood pressure to be considered controlled and in what population (ie individuals versus community). The research also occurred at a time when ambulatory blood pressure monitoring was becoming more available and accepted, which had implications for sample sizes required in studies, as it enabled a greater accuracy of blood pressure readings at the individual level, reducing the sample sizes required from community studies.

A study undertaken by Professor Morgan following this grant considered several of these questions. Although not focusing on sodium reduction, the study looked at people with hypertension who had never been treated for hypertension and examined the impacts of four different classes of drug. Although blood-pressure measurements were still taken clinically, ten measures were taken in every one visit and some ambulatory monitoring was also employed.

Professor Morgan believes that this research also played a role in changing thinking in Australia regarding the role of sodium in diets. It supported other research in demonstrating an effect on blood pressure through reduction of sodium, and although this was not sufficient to stop drug therapy among elderly people with hypertension in the long term, this was an idea that was taken up and explored further among other populations. Morgan also believes that this research drew attention to the significance of the interaction of sodium and potassium.

Professor Morgan believes that this research continues to have an influence, with similar studies being conducted today, albeit using different techniques and also looking at the interaction of sodium, potassium and renin–angiotensin rather than as absolute terms. Indeed, ten papers published between 1997 and 2005 by Morgan are identified as an indirect output of this grant.

21.8 Interface B – dissemination

Beyond publications, dissemination has involved talks, letters to newspapers in response to statements and involvement in organisations of several international committees. Related to Professor Morgan's involvement with committees is a key paper that was pending publication at the time of interview but has since been published (Bakris et al., 2008).

21.9 Stage 4 – secondary outputs

The paper by Morgan et al. (1994) produced from this research has been cited in a systematic review undertaken in Australia. It was also referenced in the evidence-based *Clinical Practice Guideline. Essential Hypertension: Managing Adult Patients in Primary Care* (North of England Hypertension Guideline Development Group, 2004) as a study reporting on the withdrawal of antihypertensive medication (along with 14 other papers referenced on pages 529–533 of the guideline), in which between 10 percent and 60 percent of patients remained normotensive for at least a year after drug therapy was stopped. The guideline also cites several other papers from Morgan's stream of research on this topic, suggesting that this grant is part of a bigger story.

As previously discussed, it is difficult to find Professor Morgan directly referenced in professional papers on the topic of salt and hypertension, potentially because he has been involved in a number of committees that are themselves referenced.

Professor Morgan has, however, been trying to influence government policy on this matter, with an excellent example being his involvement in a recent workshop in Beijing 'Hypertension in the Asian Pacific Region – the Problem and the Solution' (International Society of Hypertension, 2007), which involved discussion on how to influence governments, food manufacturers and the World Health Organization.

As noted above, the dietician from the research project, Professor Nowson, has not only continued to be involved with dietary counselling with elderly populations, with a view to preventing hypertension, but has also established AWASH, the Australian division of WASH. Although not necessarily directly attributable to her involvement with this grant, the grant was one of several projects that gave Nowson exposure to Professor Morgan's work, and she believes that these experiences have significantly influenced her own thinking and her own research around effecting a reversal of hypertension through dietary intervention.

Finally, the research drew attention to the fact that drug therapy could, in fact, contribute to the development of independent risk factors for CVD, as a higher than expected prevalence of LVH was found among the population with controlled hypertension. This led to further investigation, with implications for the pharmaceutical industry.

21.10 Stage 5 – adoption by practice and the public

It is extremely difficult to determine the exact impact of this particular grant on adoption by practice and the public. It is evident that the school of thought about the impact of dietary sodium on health has changed, but the role this grant played in this evolution is unclear.

Today, it is widely accepted that high levels of salt are bad for health, regardless of age, although exactly why salt is bad for health is perhaps not understood. National guidelines for the recommended maximum daily intake of salt have been established. Popular marketing messages from food manufacturers include 'low salt' and 'reduced salt', and research from AWASH (2007) suggests that awareness among the Australian population that salt is bad for health is high, although awareness of the recommended level is low. A

consumer research project commissioned by AWASH (2007), which surveyed 1,084 Australian adults, reported that around two thirds of those surveyed knew that salt was bad for health; although most were aware that high levels of salt are a cause of high blood pressure, around one quarter did not understand that salt increased the risk of heart attack and stroke, and fewer were aware of the adverse effects of salt on the kidney. Dietary salt was found to be a concern for the majority of those surveyed (nearly three quarters shared this concern), which made it the third food-content concern after saturated fat and sugar. Most respondents recognised processed foods as a main source of salt in diets, but just one third claimed to regularly try to buy 'low salt' or 'no added salt' foods and around one fifth of those surveyed still added salt to food in the home during cooking and at the table.

Campaigns by AWASH aim to realise a reduction in the average salt intake of the Australian population to meet the NHMRC's recommended maximum daily intake for adults of 6g by 2012. The Australian Food and Grocery Council has pledged support, as have several key companies, with Coles and Smiths Snackfood Company committing to reduce salt in their products by 25% over the next five years.

Although not a direct consequence of this research funding, it would be fair to say that this research grant has had an influence on this. The research, and the broader body of research with which Professor Morgan was involved, is just part of this continuing story.

21.11 Stage 6 – final outcomes

It is unlikely that this research has itself had a direct impact on wider health benefits; however, despite low publication and citation outcomes, it would seem there is a legacy from the research though its unexpected outcomes and, in combination with a broader body of work, an influence on Professor Morgan and a team member.

The research found that salt restriction had an impact on blood pressure but not to an extent that it could control hypertension without the need for drugs – at least in an elderly hypertensive population. The research may not have informed public awareness but supports the argument for low-salt diets. Furthermore, it suggested a need to examine more closely the effects of pharmacological hypertensive treatments, as it seemed to show that some drug therapies did not reverse the effects of hypertension as well as others and, in fact, could be contributing to the development of other independent risk factors such as LVH.

Today, although not a direct result of this research, guidelines for daily salt intake have been established, and organisations are exerting pressure on food manufacturers to review their products with a view to reducing salt levels. Indeed, a business is developing around low-salt products as companies seek to adapt. For example, Cole and Smiths Snackfood Company reformulated 11 products in its first year of pledging to address salt issues, and Unilever Australia's salt-reduction programme has developed sodium [salt] criteria to guide new product development and employs an ongoing technical research programme in research centres in Germany and the Netherlands (AWASH, 'The Food Industry', 2007).

21.12 Summary of case study impacts

The payback framework describes the process from research to several categories of impact. The categories allow us to compare across different case studies. Table 21-2 shows, in point form and by impact category, some of the impacts, described more fully above, that have emerged from this grant.

Table 21-2 Payback

Payback category	Impacts from case study
Knowledge production	• One peer-reviewed article • One note in a peer-reviewed journal
Research targeting and capacity building	Capacity building: • Contribution to team member's career in conjunction with other projects undertaken with Professor Morgan Research targeting: • Raised several new questions: how best to treat elderly people with hypertension in the long term, the impact of different drug therapies on the effects of hypertension, how to define hypertension for the purposes of research, and potential to employ new measurements such as ambulatory blood pressure monitoring
Informing policy and product development	• Research referenced in one systematic review and two guidelines • Contributed to Professor Morgan's withdrawal from a major international clinical trial • Indirect output with team member going on to establish AWASH • Questioning the impact and efficacy of different antihypertensive drug therapies
Health and health sector benefits	• Salt restriction can reduce blood pressure and thus the risk of CVD
Broader social and economic benefits	• Contributing to understanding the impact of dietary salt on heart health • Contributing to body of evidence influencing the food manufacture industry

21.13 Observations on process

This grant was classified by Professor Morgan as high impact, but several factors made it very difficult to qualify this including:

- very limited publications at the time, as publishing was not seen as the primary output
- funding being part of a bigger resource pool
- involvement in a number of projects, which makes it difficult for team members to unpick the case study.

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CHAPTER 22 The effects of lean meat diets on plasma lipids and haemostatic functions

22.1 Overview of case study grant

In 1989, Professor O'Dea (the principal investigator (PI)) received Aus$64,677 over two years from the National Heart Foundation of Australia (NHFA) for a grant titled 'The Effects of Lean Meat Diets on Plasma Lipids and Haemostatic Function' (grant reference: G88M2513). The research project was conducted at Deakin University in collaboration with the University of Melbourne (Department of Medicine, Royal Melbourne Hospital), Royal Melbourne Institute of Technology and the State Health Laboratories.

Studies by the research investigators not long before the case study grant began had shown that low-fat diets rich in very lean beef (500g/day) were as effective at lowering blood cholesterol levels as similarly low-fat fish-supplemented or vegetarian diets. However, this beneficial effect was reversed by the addition of beef fat (dripping) to the diet. In essence, it was found that it is the fat consumed together with red meat, rather than the red meat per se, that is responsible for the apparently detrimental effects for cardiovascular health.

The initial aim of the case study grant research was to determine the effect in normal healthy people of three other commonly used dietary fats (butter, safflower oil and olive oil) on the cholesterol-lowering effects of low-fat diets rich in lean beef. The second aim was to assess the use of low-fat diets rich in lean meat in the treatment of high blood cholesterol levels and to test the possibility of liberalising the fat intake of people with hypercholesterolaemia (high blood levels of cholesterol) by including particular fats such as olive oil and/or safflower oils in their diets. The third aim was to investigate the relationship between the changes in polyunsaturated fatty acid composition of plasma fats and haemostatic function (blood flow and blood clotting) when people consume low-fat diets rich in certain very lean meats (such as kangaroo) and different types of Australian fish. The expectation of the research investigators was that this research programme should help improve understanding of the relationships between different types of dietary fat in commonly eaten foods and the risk of heart disease and stroke. The research programme included human and rat studies.

In the end, the case study grant was only a small part of a much larger pool of funding that contributed to the research programme. In addition, although the case study grant application was awarded with the rat studies excluded, our interviews and the funding

allocation, which was largely for the salary of the graduate research assistant, on-costs and maintenance costs, suggests that the grant funding was, in fact, largely used for the rat studies.

The primary outputs from the case study grant were three publications. The key findings included that red meat is quite compatible with cholesterol lowering as long as the background diet is low in saturated fat and that olive oil and safflower oil may be included in the diets of some people with elevated cholesterol levels. The finding specific to the rat studies was that supplementation of arachidonic acid was more effective than supplementation of linoleic acid in reversing the effects of prostanoid production and phospholipid fatty acid composition in rats fed diets enriched with butter.

Beyond contributing to the larger meat research programme conducted by the research team, the smaller case study grant was thought to have no or limited impact beyond the direct funding on the career of the graduate research assistant Mrs Steele. Similarly, although the larger meat research programme had a direct impact on the meat industry's marketing towards lean meat and there was significant interest in the data among dieticians and other practitioners, etc, it is unclear how definitely and directly the research programme impacted on nutritional policies and guidelines and wider health and economic benefits.

22.2 Introduction to case study

22.2.1 Overview

The major objectives of this research programme, as described in the grant application, were: firstly, to determine the effects of the addition of different dietary fats on the cholesterol-lowering effect of low-fat diets rich in lean beef; secondly, to assess the use of low-fat diets rich in lean meat in the treatment of hypercholesterolaemia and test the possibility of liberalising the fat intake of people with hypercholesterolaemia, focussing on the addition of particular fats (olive oil and safflower oil); and thirdly, to investigate the relationship between the changes in polyunsaturated fatty acid composition of plasma fats and haemostatic function (blood flow and blood clotting). The research programme involved human and rat studies.

The risk of coronary heart disease is influenced by dietary factors such as type of fat, amount of fat and polyunsaturated/saturated (P/S) ratio. The effects of these different factors on the various plasma lipoproteins were still unclear. Previous studies by Professors O'Dea and Sinclair (an associated senior investigator) had shown that that very low-fat diets containing large quantities of fully lean red meat (500g/day) were associated with a similar reduction in total cholesterol levels in normal subjects as comparably low-fat vegetarian or fish-supplemented diets (O'Dea et al., 1986, and Sinclair et al., *Lipids*, 1987). However, this beneficial effect of very lean red meat was negated by the addition of beef fat to the diet. These results indicated that it was the fat and not the red meat per se that results in high levels of 'bad' cholesterol (hypercholesterolaemia).

Following the above-mentioned studies, in which subjects were re-fed with beef fat, the research investigators conducted (as part of the research programme associated with the

case study grant) another two series of studies with 22 healthy, weight-stable subjects, in which they assessed the effects of adding olive oil or safflower oil to the very low-fat diet supplemented with fully fat-trimmed lean beef. Each study lasted five weeks; in the first week, the subjects consumed their usual diet; in weeks 2 and 3, they consumed a very-low-fat diet (9%) containing 500g/day of fully fat-trimmed lean beef, and in weeks 4 and 5 they continued with the lean beef but added either safflower oil or olive oil in a stepwise manner (10% and 20% energy, respectively). In these studies, the total cholesterol fell during the low-fat dietary period. In contrast with the results with beef fat, when olive oil or safflower oil were added back in a stepwise manner to reach a fat level of 30% energy, the cholesterol levels did not rise. Somewhat unexpectedly, cholesterol levels did not fall further when the P/S ratio was increased from 0.5 on the very-low-fat diet to more than 3 on the safflower oil-supplemented diet. These results suggested that, in normal subjects, red meat is quite compatible with cholesterol lowering as long as the background diet is low in saturated fat.

A further two human studies were conducted (as part of the research programme associated with the case study grant) in subjects who had moderately high cholesterol to assess the effect of adding olive oil or safflower oil to the very-low-fat (10%) diet supplemented with fully fat-trimmed lean beef. In both studies, total cholesterol levels fell significantly after three weeks on the 10% fat diet, including 300g lean beef daily. This level remained lower than baseline when both olive and safflower oil were added to the diet in week 5 through week 7. The lowered total cholesterol was the result of a decrease in both low-density lipoprotein (LDL; bad) cholesterol and high-density lipoprotein (HDL; good) cholesterol fractions. There seemed to be no difference in the effects of olive oil and safflower oil. There was a tendency for levels of HDL and LDL cholesterol to increase towards baseline following the addition of oil in the final three weeks. There was a range of responses to the reintroduction of added oil to the low-fat diet in terms of LDL cholesterol levels. Four of the 19 subjects did not respond to the dietary manipulation, whereas, at the other extreme, two subjects responded with a 29% reduction in the LDL cholesterol fraction over the six-week dietary period. The mean reduction for all subjects was 9%.

These results suggested that lean beef can be included in cholesterol-lowering diets and that olive oil and safflower oil may be included in the diets of some people with elevated cholesterol levels. Studies reversing the order of the two dietary periods were undertaken to establish the effect of unsaturated fats compared with carbohydrate in the diets of those with moderate hypercholesterolaemia.

The rat studies involved male Sprague-Dawley rats, which were fed a butter-enriched diet (50% fat) for two weeks and then supplemented orally with either 90mg ethyl arachidonate or ethyl linoleate daily for two weeks. The data from these rat studies indicate that supplementation with small doses of preformed arachidonic acid was more effective at reversing the effects of prostanoid production and phospholipid fatty acid composition than supplementation with its precursor, linoleic acid, in rats fed butter-enriched diets.

As acknowledged by reviewers of the grant application, research investigators came to the project with a very good record of research in nutrition.

22.2.2 Understanding the broader research field

The research investigators suggested, at the time of the grant application, that there continued to be controversy surrounding the relationship between consumption of red meat and the risk of cardiovascular diseases. The consumption of meat had been shown in epidemiological studies to be associated with increased mortality from coronary heart disease and cancer (Snowdon, Phillips and Fraser, 1978, and Phillips et al., l978) in association with higher frequencies of the common risk factors for cardiovascular disease, high cholesterol and high blood pressure (Bursten et al., 1978, and Sachs et al., 1975).

Short-term dietary studies had produced conflicting data. On the one hand it had been shown that lean meat does not affect cholesterol concentrations adversely when added to the diet and can even be part of a cholesterol-lowering diet (Watts et al., 1988), whereas other work indicated that the addition of meat to the diet is associated with increased cholesterol concentrations (Sachs et al., 1981). It was suggested by Professors O'Dea and Sinclair that the source of these apparent contradictions could possibly be found in the amount of fat that is consumed together with beef (Bull et al., 1983). Earlier work by the research investigators, initially reported in an article that has been cited more than 180 times, showed that when Australian Aboriginal people revert temporarily to their traditional hunter–gatherer diet, which is rich in kangaroo (a very lean red meat), their lipid profile improves (O'Dea, 1984). In a subsequent examination of this question in Caucasian Australians, they had shown that low-fat diets supplemented with 500g kangaroo meat per day were as effective in lowering cholesterol concentrations as similarly low-fat vegetarian or fish-supplemented diets (Sinclair et al., *Lipids*, 1987). These results suggested that it is the fat that is usually consumed together with the red meat, rather than the red meat per se, that is responsible for its apparently detrimental effects on cardiovascular disease risk factors. One of the aims of the case study grant research therefore was to differentiate between the effects of consumption of lean beef and beef fat as risk factors for coronary heart disease.

22.2.3 The case study approach

The case study based on this research grant involved a combination of: a review of documentation for the grant, a one face-to-face interview with the PI on the project (Professor Kerin O'Dea), interviews with other members of the research team, a review of the PI's curriculum vitae and documentary analysis of key publications arising from it.

22.3 Stage 0 – topic/issue identification

As identified by Professor O'Dea at interview, the project idea was significantly influenced by her previous work in relation to diabetes with indigenous Australian people, who could eat a diet rich in their traditional lean red meats, such as kangaroo and other wild meats, but have very healthy lipid[1] profiles. The following examines how the research topic was identified and the factors that were crucial:

[1] A broad group of naturally occurring molecules that includes fats, waxes, sterols, fat-soluble vitamins (such as vitamins A, D, E and K), monoglycerides, diglycerides, phospholipids and others.

- earlier studies of indigenous Australians hunter–gatherer diets
- human and rat studies on lean meat and concerns that common beliefs at the time resulting from other studies may, in fact, have a negative impact
- interest of the meat industry.

22.3.1 Earlier studies of indigenous Australian hunter–gatherer diets

This research programme was originally stimulated by the research investigators' previous observations that when urbanised diabetic Aboriginal people reverted temporarily to their traditional hunter–gather diet, there were improvements not only in the metabolic abnormalities of diabetes (O'Dea, 1984) but also in several risk factors for cardiovascular disease, such as reductions in high cholesterol and blood pressure and prolongation in bleeding time. O'Dea said, 'The real precipitating factor was my work with indigenous [Australian] people who could have a diet high in red meat and have very healthy lipid profiles when they were on their traditional foods. So that was when I really started' (O'Dea interview, 2009).

These beneficial changes were found to occur despite the diet containing substantial quantities of animal foods such as kangaroo and despite marked increase in the proportion of arachidonic (omega-6 fatty) acid in plasma phospholipids (O'Dea and Sinclair, 1985). The diet was found to be low in fat (13% energy) despite being rich in red meat, because meats from wild animals are extremely lean (1–2% fat wet weight). It was also noted that much of the fat in wild meats is structural lipids (fats and triglycerides) and therefore rich in polyunsaturated fatty acids, including arachidonic acid (Naughton et al, 1987). These observations lead the research investigators to question certain widely accepted beliefs at the time relating to diet and heart disease, which included:

- whether red meat per se is a risk factor for hypercholesterolaemia or rather the saturated fat that is often consumed with it
- whether increased arachidonic (omega-6 fatty) acid in tissue phospholipids (a class of lipids and a major component of all cell membranes) is a risk factor for blood clotting within blood vessels (thrombosis).

O'Dea said, 'We were very interested in differentiating between meat per se and fat in terms of its adverse impact on health, because there was a lot of work which suggested that it was red meat that was the problem. I had been doing some work with indigenous people and I had got very interested in kangaroo meat and wild meats, lean meats. I had shown that when Aboriginal people went back and lived off the land, even when they were eating a lot of red kangaroo meat, their cholesterol was very low. They were very healthy. So my hypothesis was that it would be the same with beef' (O'Dea interview, 2009).

22.3.2 Findings of human and rat studies of lean meat counter to beliefs at the time

In relation to the first mentioned widely accepted belief at the time, published data from sources such as the Australian National Dietary Survey (Department of Community Services and Health, 1987) had identified meat and meat products as the largest contributor to the fat intake of Australians (31.9%), with diary foods another important

contributor to the total (21.4%). These data suggested that a reduction in the consumption of meat and dairy products would result in reduced saturated fat intake.

However, Professor O'Dea and her colleagues had completed a study that had shown that low-fat diets supplemented with large quantities (500g/day) of very lean red meat (kangaroo) were as effective in lowering cholesterol levels as similarly low-fat vegetarian or fish-supplemented diets (Sinclair et al., *Lipids*, 1987). They had also subsequently examined the effect of more popular red meat (beef) on cholesterol levels in a similar dietary protocol (Sinclair et al., 'The Lipid Content and Polyunsaturated Fatty Acid Concentration of the Lean Portion of Australian Beef and Lamb', *Food Technology in Australia*, 1987). The study was designed to differentiate between the effects of lean beef and beef fat as risk factors for occlusive vascular disease and to show that it is possible to have a diet containing large amounts of lean beef but that is still low in fat and as effective in lowering cholesterol levels as other low-fat diets. Furthermore, the low-fat lean beef diet only reduced LDL (bad) cholesterol, leaving HDL (good) cholesterol unchanged. However, these beneficial effects on cholesterol levels were found to be reversed when beef fat (dripping) was added back in to the diet. The study demonstrated that it is not the red meat per se that is the problem but rather the fat consumed with it. O'Dea said, 'We felt that a lot of women were avoiding red meat because they thought it was unhealthy and for many women it was an excellent source of iron. We actually thought it was an important nutrient' (O'Dea interview, 2009).

The aim of the research funded through the case study grant and other funding sources thus was to examine the effects on these parameters of adding back other fats (butter, safflower oil and olive oil). The four fats were selected for the following reasons (Keys et al., 1986, and Mattson and Grundy, 1985):

- dripping, because it is beef fat
- butter, because it represents a major dietary fat (dairy) and has a different composition to dripping (being more highly saturated)
- safflower oil, because it is a rich source of linoleic acid (a major component of polyunsaturated margarines and oils)
- olive oil, because it is rich in oleic acid and may protect against occlusive vascular disease.

The research also intended to explore the implications of raised arachidonic acid levels in plasma phospholipids.

At the time, it was also assumed that diets that raise levels of arachidonic acid in plasma lipids and increase platelet production of thromboxane A_2 (TXA_2) must be thrombogenic (promoting thrombosis). However, the research investigators' previous observations that the traditional hunter–gatherer diet consumed by Australian Aboriginal people for tens of thousands of years was associated with elevated levels of arachidonic acid suggested to them that it is most unlikely that raised arachidonic acid levels are harmful to health. For this reason, they were keen to develop a method to monitor activity of prostaglandin I_2 (PGI_2) in the human vasculature. They believed that measurement of the cold pressor response of

forearm blood flow (measured indirectly using venous occlusion plethysmography) provided such a tool.

The research investigators had already been able to demonstrate that diets that raise arachidonic acid levels in plasma phospholipids (low-fat diets rich in kangaroo meat or tropical fish) were associated with a marked, consistent reduction in the cold pressor response (ie the dietary fat had been shown to affect blood flow). They cautiously interpreted these data as indicating an association between raised plasma arachidonic acid level and increased PGI activity of the vasculature. These diets were also associated with increased TXA_2 production by whole blood and platelets in vitro (but they did not cause increased platelet aggregation) and provided a good illustration of the potential problems in using TXA_2 production in vivo as the main marker of thrombosis tendency.

In addition, the research investigators presented in the grant application that: 'Although saturated fats as a class of lipids have been shown to affect adversely both atherogenesis and thrombosis, individual fatty acids can affect the processes quite differently. For example, the most hypercholesterolaemic fatty acids are myristic (14:0) and palmitic (16:0), with stearic (18:0) considered to be almost neutral (Hegsted et al, 1965). In contrast, stearic acid is considered to be more thrombogenic than myristic or palmitic (Renaud, 1985). Oleic acid (18:1) appears to be as effective as linoleic acid (18:2) in lowering LDL cholesterol, with the added advantage of not causing reductions in HDL cholesterol (Mattson and Grundy, 1985), probably explaining why the olive oil-consuming populations of the Mediterranean are so protected from coronary heart disease (Keys et al, 1986). Furthermore, it appears that not only are the constituent fatty acids important in determining the atherogenic and/or thrombogenic potential of a particular fat, but also the position of those fatty acids on the glyceride backbone. Certain vegetable fats such as palm oil and cocoa butter have high contents of saturated fatty acids but are less hypercholesterolaemic than dairy fats and fats from ruminant meats such as beef and lamb. This may be related to a fundamental difference in the triglyceride structures of animal and vegetable fats. In vegetable fats, the fatty acid on the 2-position of the triacylglycerol is usually unsaturated, whereas in animal fats it is not (Mattson and Lutton, 1958)'.

In a preliminary examination of this question, the research investigators had found that diets rich in palm stearate (which is rich in saturated fatty acids) did not affect plasma or tissue fatty acid composition in rats, arterial PGI_2 or platelet TXA_2 production in contrast with the effects of the saturated animal fats (butter and dripping) (O'Dea et al., 1988). They were to investigate this further in dietary studies (associated with the case study grant) in rats by studying the effects of other saturated vegetable fats (coconut oil and cocoa butter) and randomised animal and vegetable fats on plasma phospholipid fatty acid composition, arterial PGI_2 production, platelet TXA_2 production and plasma lipid levels.

22.3.3 Interest of the meat industry

Professor O'Dea noted during interview the interest of the meat industry in the research of her team and the funding that enabled the associated investigations. This applied not only to the earlier research but also to a significant grant that was obtained from the Meat Research Corporation not long after the case study grant was awarded (O'Dea interview, 2009). O'Dea said, '[The meat industry] were being criticised, people were saying you have to reduce your fat intake and part of that was reducing your red meat intake. People still

say that and largely it is correct still. What I am saying is that it does not have to be like that. If you have lean meat you will be a lot better off' (O'Dea interview, 2009).

22.4 Interface A – project specification and selection

22.4.1 Aims of the study

The aims of the study as stated in the grant application were to:

- determine the effect of the addition of different dietary fats (butter, safflower oil and olive oil) on the cholesterol-lowering effect of low-fat diets rich in lean beef
- assess the use of low-fat diets rich in lean meat in the treatment of hypercholesterolaemia, focussing on the addition of particular fats (eg olive oil and/or safflower oil)
- further investigate the relationship between diet-induced increases in arachidonic acid content of plasma phospholipids and changes in haemostatic function, in particular forearm blood flow (eg to identify if the inhibition of the cold pressor response that occurs on low-fat, kangaroo- or barramundi-enriched diets is reversed by aspirin and the screening of a range of meats for their ability to increase plasma phospholipid arachidonic acid)
- investigate (using dietary studies in rats) why saturated fats of animal origin are more atherogenic (promoting build up of fatty materials such as cholesterol in the artery wall) than saturated fats of vegetable origin (eg comparing the effects of increasing levels of animal and vegetable saturated fats on lipid levels and haemostatic function – the process that causes bleeding to stop – and feeding randomised animal and vegetable saturated fats to investigate the importance of triglyceride structure in determining the atherogenic potential of a saturated fat).

A combination of human and rat studies were proposed to achieve the stated aims. Details for each of the studies are provided in Section 22.6 later in this case study. However, Professor O'Dea did note that in terms of all the avenues of research proposed in the grant application, there was more than what was actually funded by the case study grant. The files notes also indicated that the funding was for the project 'excluding the rat studies'. However, based on the interviews conducted for this case study, it is more than likely that the case study grant was the first of a much larger pool of funding used for the project and mostly attributed to the costs associated with the rat studies rather than the more expensive human studies. O'Dea said, 'That [rat study] would have been the study that we definitely could have done quickly and then would have certainly been supported by this grant. So we did prostacyclin production via arteries and platelet aggregation and we looked at fatty acid composition of the diet…we did publish a number of studies from that' (O'Dea interview, 2009).

22.4.2 Negotiation with potential users

From interviews, we understood that the main negotiation with potential users occurred with the meat industry, who were particularly interested in the research and ultimately funded the research programme following the award of the case study grant from the

National Heart Foundation of Australia. Professor O'Dea said, 'The meat industry was particularly interested in it. They could see real benefits from it. I was always a big advocate for leaner meat and I did a lot of work with the meat industry separate to this, trying to convince them and looking at the barriers to lean meat production in Australia. It is a public health issue. So I understood the meat industry pretty well by the end. We could produce lean meat. I still see that today. We could produce a lot leaner meat than what we do if we paid producers for saleable yield not for live weight of cattle and sheep. They get paid for everything the...fat and everything. It would be a very simple thing that we could do' (O'Dea interview, 2009).

22.4.3 Review of the grant application

All three reviewers of the grant application rated the application as 'good' (out of a scale of poor, moderate, good, outstanding and not able to judge). Two of the assessors of the grant application indicated that they recommended funding the application; however, one of those reviewers indicated that the applicants should receive funding for aims 1 and 2 stated in the application and left it to the other as to whether aims 3 and 4 be funded on the basis that he did not have sufficient expertise in the related areas to make a judgment and felt that aim 4 (in relation to the rat studies) was complementary to the other parts of the programme rather than being essential. The third reviewer did not recommend funding of the application.

The following statements reflect the positive views expressed by the reviewers (Grant –in-Aid Assessor Report, 1988):

- 'This is an imaginative and well written application which proposes to investigate the interaction of dietary lipids and plasma cholesterol, platelet function, vascular reactivity and phospholipid fatty acid composition. The applicants provide data that very lean meat does not impair the cholesterol-lowering effect of a low fat diet. It is argued that the adverse effect of red meat on plasma lipids is due, not surprisingly, to its saturated fat content. This study now proposes to examine the effects on plasma cholesterol of 'adding back' a range of fats to the lean meat diet. Another beneficial effect of lean meat is attributed to its arachidonic and linoleic acid content, providing substrates for the anti-aggregatory and vasodilator prostanoid PGI_2. An additional, and somewhat tangential proposal, aims to examine the effects of saturated animal and plant fats on atherogenic mechanisms in rats.'
- 'The budget appears reasonable. The applicants have a very good record of research in nutrition and despite the geographical separation of their resources should bring this project to a successful conclusion.'
- 'Aims 1 and 2, to investigate the effects of type and quantity of dietary fat on lipids and lipoproteins, is a continuation of work already supported by the National Heart Foundation of Australia. Progress and results to date have been very satisfactory and the future research plan seems satisfactory. I have no major criticism of this part of the application, although the need and time has come to study an increased number of subjects with hypercholesterolemia.'

The reviewer who did not recommend funding described the application overall as follows (Grant-in-Aid Assessor Report, 1988):

- 'This is a multifaceted proposal in the area of nutrition. It does not focus specifically on a single hypothesis nor examine issues in depth. It is a continuation of current work; some of it funded by NHF [National Heart Foundation of Australia].'

Along the same lines, each of the reviewers expressed the fact that the application included a number of studies in one, and one reviewer specifically acknowledged their difficulty in reviewing all components of the application (Grant-in-Aid Assessor Report, 1988):

- 'It is difficult for a referee to do justice to this application because it really covers three separate (but related) programs of research. The applicants would have been better served by submitting two or three separate applications, thus giving them access to a wider pool of referees and the opportunity of presenting separate budgets (with the chance of more generous funding).'

Each reviewer raised some queries and issues in regard to a lack of specific detail about some areas, the length of the study period (to reliably demonstrate the quantitative effects) and perceived gaps in the controls. For example, in relation to the rat studies (Grant-in-Aid Assessor Report, 1988):

- '[They] lack clarity of design and did not fit in well with the human studies and there were inherent dangers in extrapolating the findings in rat to the human situation – again no details are provided, particularly concerning platelet aggregation studies.'
- '[They] alone constitute a major project. It worries me that studies of platelet aggregation etc. which are technically simple to do but difficult to execute with precision and reliability are to be conducted by a multipurpose [research assistant] outside a coagulation laboratory.'
- 'They make a valid point about the varying positional specificity of glyceride fatty acids but are wrong to generalise that there is a clear distinction between plant and animal sourced glycerides (whereas palmitate does preferentially occupy position 2 in porcine adipose glycerides, it occupies position 1 in ruminant glycerides as in those of many plant oils).'

The reviewers indicated that at interview the applicants should be asked (Grant-in-Aid Assessor Report, 1988):

- whether there may be differential effects between subjects with low or elevated levels of LDL cholesterol
- whether the results in subjects with hypercholesterolemia have more clinical relevance
- if a sample size calculation has been performed to estimate how many subjects will be required to demonstrate a 10% differential in LDL cholesterol

- to predict the results of the low-fat diet with added fat interactions on plasma cholesterol and postulate mechanisms
- to provide data that dietary changes can alter the cold pressor response and give information about its within-subject variability
- to give details about prostaglandin analytical methods.

The report of the interview scored the application at 3.5 out of 5 and recommended funding with comments such as 'a good and interesting project' and 'a well presented project with potentially interesting results'. The report suggested a budget for the research assistant and maintenance costs for two years. The report also stated that, 'the rat study constitutes a separate component and should not be considered for funding at this stage'. It also indicated that 'the design was flawed by inadequate controls and lack of attention to methodological detail'.

At interview Professor O'Dea could not recall if the issues raised by the reviewers were considered and addressed and this could not be established through other interviews. Based on the publications attributed to the project, there seems to have been no significant adjustments from that proposed in the grant application. In addition, with major alternative funding coming into the research group at the time for the project, the full project, including the rat studies, proceeded.

22.5 Stage 1 – inputs to research

In this section we outline the key inputs to the grant research.

In summary, Professor O'Dea thought that all were important, with funding and collaborators possibly the most important. She said, 'You can't do anything without funding but you do need a lot of money. This is generally why these little grants in aid add a little bit extra to another core block of funding that you have got from somewhere else. We all like the little grants in aid but we can't pretend that they are delivering more than they really are' (O'Dea interview, 2009).

22.5.1 Facilitators

One of the facilitators for the research was funding, although it is worth noting again that the case study grant represented a small amount of the overall funding towards the project in the end.

The grant application was for an amount of Aus$181,661 over three years. This comprised of salary and on-costs for a graduate research assistant (starting at Aus$27,713 for year one), salary and on-costs for a technical assistant (starting at Aus$29,596) and maintenance costs (starting at Aus$9,900). The maintenance costs included lipid standards, gases for gas–liquid chromatography (GLC), capillary columns, chemicals, solvents, plates for thin-layer chromatography (TLC), lipoprotein lipid measurements, radioisotopes, antisera standards for prostanoid essays, purchase of 200 rats per year and their housing and diet, disposables of tubes and pipette tips, strain gauge for plethysmography and purchase of lean meat.

It was argued in the grant application that the project realistically required at least four full-time staff if both the human and animal dietary studies were to proceed at an entirely satisfactory rate: one person was needed to coordinate and conduct the human studies, one for the animal studies and two for the fatty acid analyses.

The latter measurements were said to be essential to the project but are extremely labour intensive. Each sample was to be subjected to lipid extraction and TLC (to separate phospholipids) before being methylated and separated into component fatty acids by capillary GLC. The two people for whom salaries were being requested had worked on the project since 1984: one was funded by the National Heart Foundation of Australia and the other from other sources. The graduate research assistant had worked on the research programme leading up to the case study grant for more than five years and was being funded by the National Heart Foundation of Australia until the end of 1988. She had been responsible for the running of the animal studies: care and preparation of rats, aortic PGI_2 production in vitro, radioimmunoassay (RIA) of 6-oxo-PGF_1, platelet aggregation and RIA of TXB_2. She had also taken over the measurements of haemostatic function in the human dietary studies (previously performed by staff in the Department of Haematology at the Repatriation General Hospital, Heidelberg, Germany). It was argued that it was not possible for the graduate research assistant to maintain both aspects of the work on her own, and for this reason the full-time salary for technical assistance was requested. The technical assistant had been assisting with the animal studies over the previous five years while being paid from other sources until the end of 1988. He had been responsible for the care of the rats, preparation of their diets, administration of any dietary supplements and assistance with all laboratory work and was developing expertise in fatty acid analyses. He was to assume responsibility for the animal studies in 1989, when it was envisaged that the graduate research assistant would be fully occupied with the clinical studies.

It was also noted in the grant application that substantial labour input was to be funded from other sources:

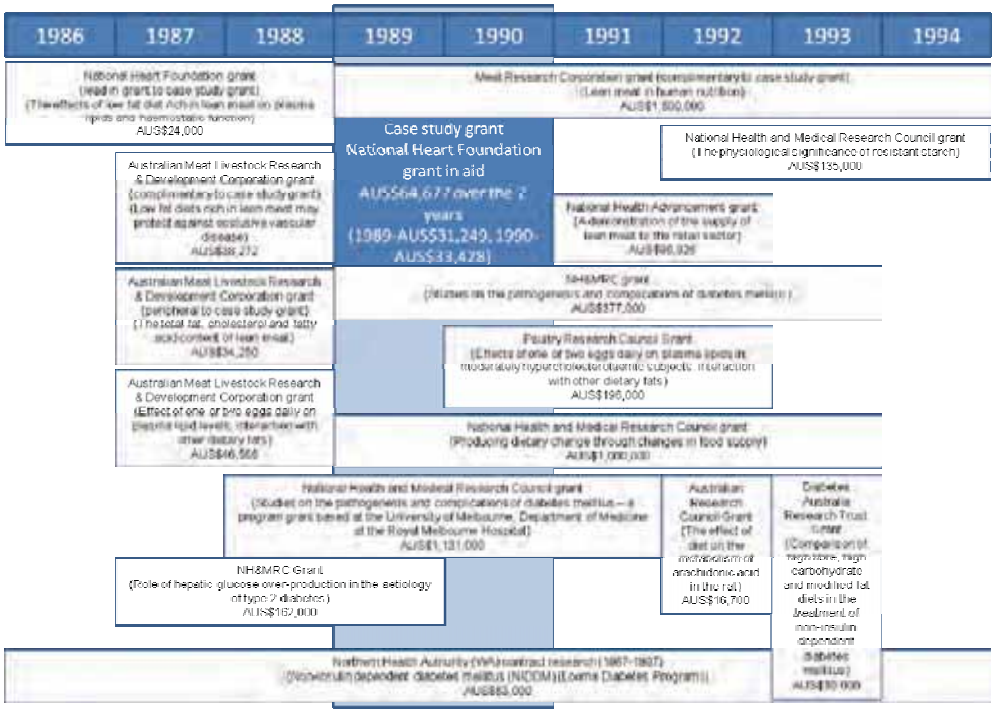
- fatty acid analyses (two full-time staff to handle samples from both human and animal studies)
- dietician (at least six days/month, supervising and counselling the participating individuals and calculating their food intakes (15–28 daily food records per subject))
- urinary metabolites of prostaglandins, which were to be conducted at the State Chemistry Laboratories (1–2 days per month for these analyses) until capacity to process the large number of urine samples generated by this and other projects was established at Deakin University.

Finally, the grant application presented that the maintenance expenses of Aus$9,900 did not represent the true costs of the study but rather a contribution to them. Major expenses not fully covered in the budget were to be incurred in the human dietary studies (purchase of meat and other expenses (at least Aus$5,000 per year) and of reagents for the prostanoid assays (Aus$5,000 per year)).

Letters on file indicate that the final grant awarded was Aus$64,677 for two years (Aus$31,249 for 1989 and Aus$33,248 for 1990). The grant was to cover the salary and on-costs for the graduate research assistant only (Aus$27,249 for 1989 and Aus$29,428 for

1990) and unspecified maintenance costs of Aus$4,000 for each of the two years. The file notes also indicated that the funding was for the project 'excluding the rat studies'. However, as noted earlier, it is more than likely that the case study grant contributed to the pool of funding used for the project and was mostly attributed by those interviewed to the costs associated with the rat studies rather than the more expensive human studies. Professor O'Dea said, 'The next year [1989] I got a big grant, when this [case study grant] was funded...[It was] a grant from the Meat Research Corporation so it is actually very difficult for me to separate the two. Meat Research Corporation as it was then gave us a grant funding in 1989 to 1994 of Aus$1.5 million so it was Aus$300,000 a year...it was a very big grant in those days. That was what funded most of it...The Heart Foundation's [National Heart Foundation of Australia] contribution was miniscule, let's be frank...we had money from the Poultry Meat Council as well...The meat corporation one was entirely human studies. So the rat ones we can attribute to the Heart Foundation [National Heart Foundation of Australia], that's important...I think in reality we would have [needed] some [more] support for the human dietary studies as it would have been much more expensive and that's why we needed this big grant from the Meat Research Corporation' (O'Dea interview, 2009).

Figure 22-1 Funding map for the grant; this shows major inputs to the PI's research activity around the period of the case study grant.



Although being acknowledged as appreciated, it has been difficult to obtain confirmation as to what extent the further funding of the lean meat and related research by the National Heart Foundation of Australia (through the case study grant) supported attraction of the other funding sources. Professor Sinclair indicated at interview that the case study grant helped lay the foundation for the research team to obtain a better understanding of 'what was going on in that area' and to apply for more research grants and publish more papers.

Although a representative of Meat and Livestock Australia (previously the Meat Research Corporation at the time of the case study grant) could not advise if the National Heart Foundation of Australia grant specifically influenced their later involvement and funding of the larger meat research programme, she could advise that the involvement of the National Heart Foundation of Australia would have certainly added credibility. She said, 'The fact that the National Heart Foundation [of Australia] would have supported it would have certainly added credibility and acceptance to the research findings, absolutely and no doubt...We wouldn't want to compromise them [National Heart Foundation of Australia] in any way but I guess by having their involvement it did help us make sure that the research design was robust and therefore that the findings would be applicable…You don't want to fund research that is only useful for marketing purposes. The more widely useful it is the more beneficial…[The] more people find that information useful to answer the questions that they have in their work, the more credible it is' (Representative of the Meat and Livestock Australia, 2009).

Interviews and literature for the case study also identified that there were important collaborations. The first was with the associated senior investigator for the project, Professor Sinclair (at the time of the grant application, his title was Dr Sinclair and he was a senior lecturer in the Department of Applied Biology and at the Royal Melbourne Institute of Technology). Other collaborations included Professor R.G. Larkins of the Royal Melbourne Hospital Department of Medicine, Dr David Hunt of the Royal Melbourne Hospital Department of Cardiology and Mr Xeno Cominos of the State Health Laboratories. There was also collaboration through the other funding sources with dietician practitioners and a research dietician at the Department of Medicine, Royal Melbourne Hospital, who was coordinating the dietary aspects of the clinical studies, including the close supervision of subjects during the studies and the calculation of dietary composition from records of weighed food intake.

22.5.2 Study recruits/samples

The study recruits and rat samples were, of course, important. Professor O'Dea explained at interview that with the total pool of funding it ensured that they were able to obtain the study recruits and rat samples they needed and the Deakin of University had the necessary animal house facilities (O'Dea interview, 2009). She said, 'We never had problems getting volunteers for these studies'.

22.5.3 Knowledge, expertise and techniques

As acknowledged by reviewers of the grant application, the research investigators came to the project with a very good record of research in nutrition and specifically in this area of study.

At the time of the grant application, Professor O'Dea was Professor of Human Nutrition and Director of the Deakin Institute of Human Nutrition at Deakin University in Victoria, Australia. Professor O'Dea's main role in the project was the overall management and quality assurance as the PI, although she did undertake aspects of the research. She said, 'I did a little bit of research. I was hands on with some of the clinical work, with the design of the diet...with some of the data analysis, quality assurance, quality control of the lab work, [and] all of those sorts of things. The more senior you get the harder it is to do

that but I was still close enough to being a hands on bench person myself at that stage. I have moved a bit further away from it now but the quality assurance is just so important. More because the buck stops with you and you have got to know that the data that's yours is correct' (O'Dea interview, 2009).

In addition to the research work, a major part of Professor O'Dea's role at Deakin University was the graduate coursework programme in nutrition and dietetics for dieticians, nutritionists and nutrition scientists. She was responsible for upgrading and completely redeveloping the dietetic training programme from a one-year graduate diploma of dietetics to a two year masters in nutrition and dietetics.

Prior to taking up the Chair of Human Nutrition at Deakin University in 1988, Professor O'Dea was a full-time researcher, although she regularly contributed lectures to undergraduate science and medical courses. She had a bachelor of science majoring in biochemistry and pharmacology and the equivalent of an honours year in pharmacology and doctoral studies in biochemistry and had been a National Health and Medical Research Council (NHMRC) research fellow at Baker Medical Research Institute and the University of Melbourne (Department of Medicine, Repatriation General Hospital) and an NHMRC senior research fellow at University of Melbourne, Department of Medicine, Royal Melbourne Hospital.

Professor O'Dea had produced peer-reviewed articles since 1969, conducted book reviews since 1977 and had presented at a number of international and national conferences. More specifically, Professors O'Dea and Sinclair had already produced a number of articles relating to their studies on lean meat and its impact on cardiovascular disease risk factors, largely driven by their work on indigenous Australians and related work on diabetes. This included:

- O'Dea, K., A.J. Sinclair, M. Niall and K. Traianedes, 'Lean Meat as Part of a Cholesterol-Lowering Diet', *Progress in Lipid Research*, Vol. 25, 1986, pp. 219–220.
- Sinclair, A.J. and K. O'Dea, 'The Lipid Content and Polyunsaturated Fatty Acid Concentration of the Lean Portion of Australian Beef and Lamb', *Food Technology in Australia*, Vol. 39, 1987, pp. 228–231.
- Sinclair, A.J. and K. O'Dea, 'The Lipid Levels and Fatty Acid Compositions of the Lean Portions of Pork, Chicken and Rabbit Meats', *Food Technology in Australia*, Vol. 39, 1987, pp. 232–233.
- Sinclair, A.J., K. O'Dea, G. Dunstan, P.D. Ireland and M. Niall, 'Effects on Plasma Lipids and Fatty Acid Composition of Very Low Fat Diets Enriched with Fish or Kangaroo Meat', *Lipids*, Vol. 22, 1987, pp. 523–529.

It is worth noting that this earlier work attracted considerable attention, with the last publication listed above being cited more than 70 times.

As a biochemist and specialist, Professor Sinclair was responsible for all of the fatty acids analyses, which involved the analysis of food and blood samples. Professor O'Dea added at interview that other people who worked on the project and in the laboratory had their

special roles and brought certain knowledge, expertise and resources. These are explained further below.

22.5.4 Space, equipment and personnel

Deakin University was the administering institution and the location where the project was primarily undertaken. The grant application indicated that all facilities of the Department of Human Nutrition at Deakin University were made available for the study, including laboratory space, animal house and equipment. Professor O'Dea said, '[Deakin University] provided the laboratory and all of that. They were very supportive…we had plenty of space…we were not cramped for space or anything like that. They were trying to build their research up at the time so it was actually quite a good place to be. They were very supportive of successful researchers. I was new there. That was my first year there [as Professor of Human Nutrition]' (O'Dea interview, 2009).

Laboratory space and equipment were also used by the investigators associated with the project based at Royal Melbourne Hospital, including Professor R.G. Larkins of the Department of Medicine and Dr David Hunt of the Department of Cardiology.

The Department of Applied Biology at the Royal Melbourne Institute of Technology (RMIT) provided access to three capillary gas chromatographs and conducted fatty acid analyses, while the State Chemistry Laboratories provided the gas chromatograph mass spectrometer for the measurement of urinary metabolites of prostacyclin and thromboxane. Mr Graeme Dunstan, a graduate research assistant in the Department of Applied Biology at RMIT, conducted the fatty acid analyses in the human dietary studies (plasma phospholipids) and animal studies (plasma and tissue phospholipids). Mr Dunstan was being supported full-time by a grant from the Australian Meat and Livestock Research and Development Corporation.

Mrs Merryn Steele, who had a bachelor of science with honours (BSc (Hons)) from the University of Melbourne, was the graduate research assistant whose salary was funded under the case study grant and whose primary role was to conduct the 'rat work'. As previously explained, Mrs Steel had worked on the project leading up to the case study grant for more than five years and had been funded by the National Heart Foundation of Australia. She had been responsible for running the animal studies and had also taken over the measurements of haemostatic function in the human dietary studies over the two years prior to the case study grant. Professor O'Dea stressed at interview that based on the amount that the case study grant represented, it essentially only contributed directly to the Mrs Steele's work on the project.

Also mentioned in the grant application was Mrs Helen D'Emden (a research dietician at the Department of Medicine, Royal Melbourne Hospital), who was coordinating (on the basis of six days per month) the dietary aspects of the human clinical studies, including close supervision from records of weighed food intake. Professor O'Dea also indicated at interview that she had some students who were dieticians in the laboratory working on the wider research programme funded by the other larger sources. They included Sally Morgan, in relation to work on the effect of adding sunflower or olive oil to very-low-fat diets rich in lean beef, and Kerry Sanders, who was completing a masters degree. The

dieticians supervised the clinical side of the study. Finally, a technician, Leanne Johnson, also performed a lot of the fatty acids work.

22.6 Stage 2 – research process

Professor O'Dea indicated at interview that the research process for the project overall (including those aspects funded by other sources) was successful. The following provides the research process that was used for each elements of the study.

22.6.1 Human studies – dietary fat and plasma cholesterol levels

The aim of these studies was to determine the effect in normal healthy subjects (volunteers) of three dietary fats (butter, safflower oil and olive oil) on the cholesterol-lowering effect of low-fat diets rich in lean beef, with particular emphasis on the LDL and HDL cholesterol fractions. The second aim was to assess the use of low-fat diets rich in lean meat in the treatment of hypercholesterolaemia and, based on the results of the studies in normal healthy subjects, to test the possibility of liberalising the fat intake of people with hypercholesterolaemia, focusing on the addition of particular fats (olive oil and safflower oil).

The diet contained 500g/day lean beef (low in fat but relatively rich in linoleic and arachidonic acids) and the subjects were healthy volunteers or hypercholesterolaemic but otherwise healthy volunteers recruited in collaboration with the Department of Medicine, Royal Melbourne Hospital.

Each study (10 subjects) lasted for five weeks. Energy intake remained constant and weight maintaining throughout. In the first week the subjects consumed their usual diet. In the second and third weeks they were to consume a low-fat diet that contained the equivalent of 500g/day for a person requiring 2,000 calories for weight maintenance. In order to maintain energy intake constant despite the very low-fat content and energy density of this diet, a carbohydrate supplement was provided, which was equivalent to 20% of the total energy intake. The fat content of the diet was increased in a stepwise fashion to 20% energy in week 4 and 30% energy in week 5 by substituting the particular fat (butter, safflower oil or olive oil) for half of the carbohydrate supplement in Week 4 and all of it in week 5. This was to ensure that there were no major changes in the diet over weeks 2–5 except for the substitution of fat for a refined carbohydrate. The subjects weighed and recorded all food and liquid consumed over the five-week period and dietary composition was calculated. Before and at weekly intervals throughout the study, cholesterol and triglyceride concentrations, plasma fatty acid composition, thromboxane production, forearm blood flow and blood pressure were measured after an overnight fast.

22.6.2 Human studies – diet-induced increases in plasma phospholipid arachidonic acid

The aim of these studies was to extend the research team's investigations on the relationship between diet-induced increases in the arachidonic acid content of plasma phospholipids and changes in the cold pressor response and its relation to prostacyclin-like activity of the vascular system.

The first experiment was to look at whether diet-induced changes in the cold pressor response could be reversed by aspirin. As noted earlier in this case study, the two diets that

have been shown to produce the most pronounced increases in arachidonic acid in plasma phospholipids were very-low-fat diets enriched with kangaroo meat or tropical fish. These diets contained 500g/day of the kangaroo meat or fish, as well as fruit, vegetables, cereals and non-fat dairy products. Ten healthy volunteers participated in each study, which lasted for three weeks. In week 1 they were to weigh and record their usual food and liquid intake to establish their weight-maintenance requirements. In weeks 2 and 3 they consumed the very-low-fat diet containing 500g/day kangaroo meat or barramundi and continued to weigh and record their food intake. Measurements were made after an overnight fast at the start and at weekly intervals in regard to body weight, lipoprotein lipids and plasma phospholipid fatty acid composition, forearm blood flow, the cold pressor response and 24-hour urine collection. Immediately after the measurements on day 21, the subjects were given two tablets of 325mg aspirin and asked to take a further two tablets 12 and 22 hours later. The last two measurements described above were repeated after an overnight fast on day 22.

The second set of experiments was to screen a range of lean meats for their ability to increase the level of arachidonic acid in the plasma phospholipids. Again, 10 healthy volunteers were used for each test diet. The diet was as described above, substituting other wild and domesticated lean meats (buffalo, venison, rabbit, skinless chicken and fully fat-trimmed pork) in place of kangaroo, ie 500g/day lean meat for two weeks as part of a low fat, weight-maintaining diet. The measurements described for the first experiment were undertaken with the volunteers at the start and at weekly intervals (three times in all).

22.6.3 **Rat studies**

The grant application argued that animal studies were an essential part of this research programme for two main reasons:

- They allow direct measurement of arterial prostacyclin production, 'which, for obvious reasons, cannot be done in humans' (O'Dea interview, 2008).
- They provide a cost-effective way of screening a large number of different dietary fats and pure fatty acids for their effects on haemostatic function and lipid levels. Human dietary studies are much more expensive and time-consuming.

Where possible, the dietary studies in rats paralleled the human clinical studies, in that as many common parameters as possible were measured. The aim of these studies was to investigate some of the factors influencing the different atherogenic potentials of saturated fats of animal and vegetable origin. As noted earlier, the research team had already conducted studies using two saturated animal fats (butter and dripping) and one saturated vegetable fat (palm stearate). In this research programme associated with the case study grant, they expanded the range of fats studied (mutton fat, chicken fat, cocoa butter, coconut oil and palm linoleate) and conducted studies with randomised fats (butter and palm stearate initially) to investigate the importance of triglyceride structure (position of saturated fatty acids on the triglyceride backbone) in determining the atherogenic potential of a saturated fat.

Male Sprague-Dawley rats weighing 100–120g were used in the studies. They were fed carefully prepared diets with increasing proportions of the fat source being studied (10%, 30% and 50% energy as fat) for two weeks. For the study of the effect of triglyceride

structure on the parameters being measured, randomised fats were prepared by inter-esterification and their structure determined. The rats were then fed other fats, as described above. At the end of this time, a number of measurements were made, using techniques that were previously established in the research team laboratory (Naughton et al., 1988, and O'Dea et al., 1988).

22.6.4 More on the techniques used

Professor O'Dea advised at interview that the techniques used for the research programme were not new but that some were new for the research team. She said, 'We used non-invasive methods to look at vascular reactivity so that they weren't new. We did not develop them but they were new for us and they were very good for how diet can affect vascular function quite quickly. So we did something called the cold pressure response, where you are actually looking at the blood flow in one arm just by looking at the volume of the arm really and you see how it changes when you put the other arm into a bucket of ice. What it shows is how your body responds, when you put your arm in a bucket of ice, it responds all over the body. That was a very nice technique' (O'Dea interview, 2009).

22.7 Stage 3 – primary outputs from research

22.7.1 Knowledge production

There were two articles identified by Professor O'Dea and three by bibliometric analysis as being directly attributable to the case study grant:

1. Steel, M.S., J.M. Naughton, G.W. Hopkins, A.J. Sinclair and K. O'Dea, 'Arachidonic Acid and Linoleic Acid Supplementation Increase Prostanoid Production in Rats Fed a Butter-Enriched Diet', *Prostaglandins Leukotrienes and Essential Fatty Acids*, Vol. 40, 1990, pp. 249–253.
2. O'Dea, K., K. Traianedes, K. Chisolm, H. Leyden and A.J. Sinclair, 'Cholesterol-Lowering Effect of a Low-Fat Diet Containing Lean Beef is Reversed by the Addition of Beef Fat', *American Journal of Clinical Nutrition*, Vol. 52, 1990, pp. 491–494.
3. Morgan, S.A., A.J. Sinclair and K. O'Dea, 'Low Fat Diets Rich in Lean Meat: The Effects of Addition of Safflower and Olive Oil, '*Proceedings of the Nutrition Society of Australia*, Vol. 15, 1990, pp. 33.

Table 22-1 illustrates the publication output attributed to the case study grant application and its impact, and Figure 22-2 shows the extent of knowledge diffusion.

Table 22-1 Publication output and impact of directly related publications

Number of journal articles:	3
Number of articles included in citation analysis:	3
Total number of citations (all papers):	50
Aggregate relative citation impact:	0.54 (Class II)
Self-citations:	24%

Distribution of publications across relative citation impact classes:

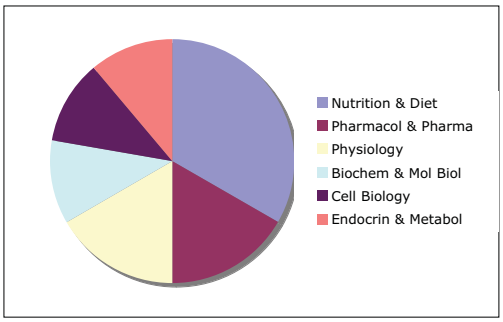
	Class I (uncited)	Class II (>0 and <0.8 citations)	Class III (0.8 to 1.2 citations)	Class IV (1.2 to 2.0 citations)	Class V (>2.0 citations)
Number of publications		2		1	
Proportion of total output		67%		33%	

Most highly cited publication[2]:	O'Dea, K., K. Traianedes, K. Chisolm, H. Leyden and A.J. Sinclair, 'Cholesterol-Lowering Effect of a Low-Fat Diet Containing Lean Beef is Reversed by the Addition of Beef Fat', *American Journal of Clinical Nutrition*, Vol. 52, 1990, pp. 491–494
Times cited:	39

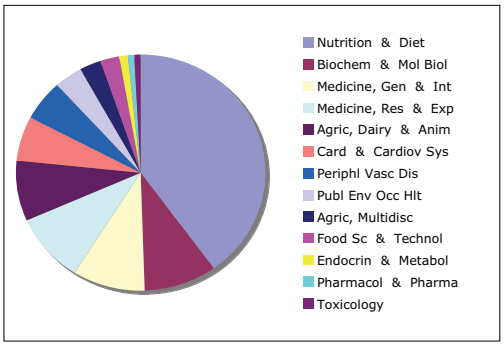
[2] Citation count extracted April 2009.

Figure 22-2 Knowledge diffusion: fields of research of output (a), fields of citing papers (b) and countries of citing papers (c)

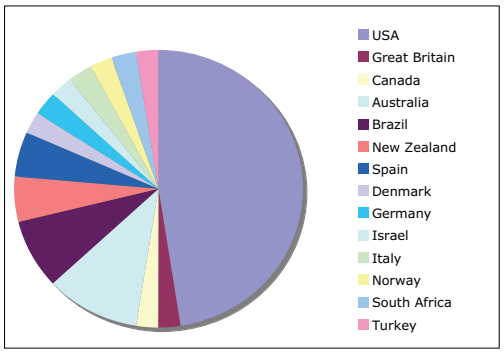
(a)



(b)



(c)



The first article by Steel et al. (1990) was cited eight times. As noted in the article, previous studies by the research team had shown that feeding a diet to rats in which 50% energy was derived from fat in the form of butter resulted in significant, dose-dependent reductions in aortic $PG1_2$, which were also accompanied by a dose-dependent reduction in the proportion of arachidonic acid but dose-dependent increases in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DIIA) in plasma and aortic (PL). The most significant findings of the study were that these effects could be reversed by supplementation with small doses of preformed arachidonic acid and, to a lesser extent, its precursor linoleic acid.

Professor O'Dea said, 'It gave a better understanding of the biology of how fatty acids and omega acids-3 and -6 would affect vascular function. That is what was really important about that. You can do studies in rats that you obviously can't do in humans and you can control the diets much better' (O'Dea interview, 2009).

The second article, by O'Dea et al. (1990), was cited 37 times. The most significant finding was the clear demonstration that it was consumption of the beef fat, not the beef itself, that was a dietary risk factor in raising cholesterol concentrations. The low-fat diet containing large amounts of lean beef with fat trimmed off was just as effective at lowering cholesterol concentrations as other low-fat diets tested under similar conditions. However, the beneficial effects on the cholesterol concentrations were negated by adding beef fat back in to the diet. The second significant observation was that the decreases in plasma cholesterol concentrations that occurred in response to the low-fat, lean-beef diet and their reversal by the addition of beef fat were due almost entirely to changes in concentrations of LDL cholesterol. Concentrations of HDL cholesterol did not vary significantly in response to these dietary manipulations. The results of this study indicated that it is very unlikely that the low-fat content per se was responsible for the reduction in HDL cholesterol that occurred with kangaroo meat in the research team's previous study. This was an important point to establish, because low-fat, high-carbohydrate diets had been widely reported to reduce both LDL and HDL cholesterol concentrations; however, the study indicated that this effect is due not to the high carbohydrate content but rather to the fat composition of the diet. In conclusion, the results of the study confirm the reports by Watts et al. (1988) and indicated that lean beef could form part of a cholesterol-lowering diet, as long as the saturated fatty acid content of the diet was kept low.

A meeting abstract was located in the grant files as part of a project update to the National Heart Foundation of Australia and linked to the project specification indicated in the grant application (Morgan et al., 1990). The meeting abstract was not included in the bibliometric analysis and was not identified as directly attributable to the case study grant by Professor O'Dea but as part of the wider project funded by the other larger sources. This study specifically looked to determine the effect of the addition of two common dietary fats – one polyunsaturated and one monounsaturated – on the cholesterol-lowering effect of low-fat diets rich in lean beef. It found that although neither mean body weight nor total energy intake changed significantly during the study, total cholesterol level reduced on the low-fat diet rich in lean beef. The effect was found to be significant at the end of week 3 ($p<0.05$). This level remained below the baseline value when both safflower and olive oil were added back in to the diet, in contrast with the effect of adding beef fat (O'Dea et al., 1990). The decrease in total cholesterol levels seemed, to the research team, to be due to a decrease in both the LDL cholesterol and HDL cholesterol fractions. Concentrations of HDL cholesterol increased again with the introduction of oil in weeks 4 and 5, whereas LDL cholesterol remained decreased. It was pointed out that diets similarly low in fats (fish- or kangaroo-supplemented low-fat diets) had been shown to produce a comparable reduction in the HDL cholesterol fraction (Sinclair et al., 1987) and that this decrease could be due to the extremely low-fat content of these diets. The article concluded that lean beef can be included in cholesterol-lowering diets as long as the saturated fat content of the diet is low and that increasing total fat by the addition of safflower or olive oil will not only maintain the beneficial effect of a diet low in saturated fats but will

improve palatability. Professor O'Dea said, 'The main impact [the research programme had] was [in relation to] diet and heart disease risk, no question about that. It is a very important area and very important for the National Heart Foundation [of Australia]' (O'Dea interview, 2009).

22.7.2 Benefits to future research and research use

Capacity building and career development

It has been difficult to separate the influence and impact of the larger meat research programme and the specific impact of the case study grant. Interviews with Professor O'Dea and others tend to suggest that the wider research programme proposed in the original case study grant application and largely funded in the end by the Aus$1.5 million grant from the Meat Research Corporation did have an impact on the research agenda and careers of the research team. The meat research programme was described in interviews as setting up a number of people and supporting them through their PhD degrees and on the path to their future careers. Professor O'Dea said, 'So that is where I think substantial long term funding is always preferable' (O'Dea interview, 2009).

Whereas, beyond contributing to the larger research programme, the smaller case study grant was thought by Professor O'Dea to have no or limited attributable impact beyond the direct funding towards the work of Mrs Steele, who had a family following the case study grant and her career since has been unclear. However, Professor Sinclair suggested that the National Heart Foundation of Australia funding provided, through the funding of Merryn Steel's position and related work for two years, an important component or foundation of the 'jigsaw or pyramid' that was built. Professor Sinclair said, 'With that grant it was two years of money and you had a person employed for two years and able to produce a few papers with Merryn Steele's name on it and others, and it laid the foundation for us to get a better understanding of what was going on in that area and able to apply for more research grants, which we got, and increased our understanding, so we published more papers. It led to about 10 years of research, so it was an effective injection of money. Lots of people got employed, and so we had a bit of an industry going on…a science industry that was helped by the [National] Heart Foundation [of Australia] money, and once we got some data we could apply to other people for money and we got lots of students that got degrees out of these areas and so on' (Sinclair interview, 2009).

Professor O'Dea advised that the impact of the specific case study grant on her career would have been limited due to a number of reasons. Firstly, it was a small grant – only one of numerous grants she has been awarded in her career – and the significantly larger Meat Research Corporation grant ended up having the major impact on the meat research programme. O'Dea also advised at interview that, after five years of funding from the meat industry, she made a conscious decision to go back to NHMRC funding and to her main area of research on diabetes and Aboriginal health. As indicated in her curriculum vitae, she is on numerous national committees, including NHMRC committees in regard to nutrition and health and Aboriginal health, and she believes her recognition is attributed to the whole body of her work and more specifically her work in the area of diabetes – the glycaemic index and Aboriginal health – rather than in relation to the meat research programme. O'Dea said, 'We asked the questions we were interested in but then after those five years of funding I did not go back to the meat industry…I actually went back

more to diabetes after that, which is my main area of research. I also do a lot of Aboriginal health. We are very interested now in different types of fat and their impact on energy balance, body fat distribution and therefore indirect decay of vascular risk. They are very big in many ways. There is a thread that goes through them' (O'Dea interview, 2008).

The impact in relation to Professor Sinclair was similar, with the whole body of work being important and the specific case study grant being valued but of minimal overall impact. However, Professor Sinclair did describe the case study grant as being helpful to his career, in that it was part of a foundation of research that lead to a further 10 years in the area. Sinclair said, 'I would say it was helpful for my career for sure' (Sinclair interview, 2009).

Targeting future research

Follow on from the case study grant, future studies investigated the role of decreased arachidonic acid and increased EPA and DIIA separately in order to establish the relative importance of these changes on prostanoid metabolism and platelet reactivity. After achieving as much as they thought they could around lipids, etc, from the wider meat research programme, the research team also undertook work on digestibility of meat in relation to bowel health. Professor O'Dea said, 'That was one of the other areas we moved into afterwards, because I am very interested still in the potential role of, say, red meat and colon cancer risks. If meat gets into the colon and digestive [system generally], it produces toxic by-products. So the way you cook the meat becomes very important. If it is tough and it is poorly digested then it will be negative, but if it is tender and very well digested there is no negative outcome. I think that kind of issue is very important' (O'Dea interview, 2008).

As part of, or leading from, the wider meat research programme, other publications have been attributed to the research team's meat research. They include:

- Morgan, S.A., A.J. Sinclair and K. O'Dea, 'Effect on Serum Lipids of Addition of Safflower Oil or Olive Oil to Very-Low-Fat Diets Rich in Lean Beef', *Journal of the American Dietetic Association*, Vol. 93, No. 6, June 1993, pp. 644–648, which analysed the influence of lean pork and veal consumption on the lipid profile of healthy subjects within the framework of a healthy diet comprising low levels of total fat, saturated fatty acids and cholesterol and found that lean pork and veal produces similar effects on the lipid profiles of healthy subjects and its consumption, as part of the saturated fat and cholesterol controlled diet, could therefore be included in food guidelines, both for normal and therapeutic diets.
- Morgan, S.A., K. O'Dea and A.J. Sinclair, 'A Low-Fat Diet Supplemented With Monounsaturated Fat Results in Less HDL-C Lowering than a Very-Low-Fat Diet', *Journal of the American Dietetic Association*, Feb 1997, Vol. 97, No. 2, pp. 151–156, which showed a drop in serum triglycerides (rather than the rise that is typically seen on high-carbohydrate diets) and concluded that 'there appear to be important cardiovascular benefits from choosing a plant-based diet over a convenience food based diet for meeting national dietary guidelines'.
- Sanders, K., L. Johnson, K. O'Dea and A.J. Sinclair, 'The Effect of Dietary Fat Level and Quality on Plasma Lipoprotein Lipids and Plasma Fatty Acids in

Normocholesterolemic Subjects', *Lipids*, Vol. 29, No. 2, February 1994, pp. 129–138.

- Sinclair, A.J., L. Johnson, K. O'Dea and R.T. Holman, 'Diets Rich in Lean Beef Increase Arachidonic Acid and Long-Chain Omega 3 Polyunsaturated Fatty Acid Levels in Plasma Phospholipids', *Lipids*, Vol. 29, No. 5, May 1994, pp. 337–343, which examined the effect of grilling and frying different cuts of fat-trimmed lean beef on the long-chain polyunsaturated fatty acids content and investigated the effect of including 500g lean beef daily (raw weight) for four weeks on the fatty acid content and composition of plasma PL in 33 healthy volunteers. This study was part of a larger trial investigating the effect of lean beef on plasma cholesterol levels.
- Steel, M.S., J.M. Naughton, G.W. Hopkins, A.J. Sinclair and K. O'Dea, 'Effects of Dietary Fats on Prostanoid Production and Aortic and Plasma Fatty Acid Composition in Rats', Lipids, Vol. 25, 1990, pp. 719–723, which investigated whether the effects of dietary arachidonic acid on eicosanoid production in the rat were correlated with arachidonic acid and EPA levels in platelets and aorta (eicosanoid-producing tissues).
- Watson, M.J., N.J. Mann, A.J. Sinclair and K. O'Dea, 'Impact of Outlet and Neighbourhood on the Fat Content of Untrimmed Retail Beef and Lamb Cuts Over a 12 Month Period in 1990–91', *Food Australia*, Vol. 44, 1992, pp. 511–514.
- Watson, M.J., N.J. Mann, A.J. Sinclair and K. O'Dea, 'Fat Content in Untrimmed Retail Beef and Lamb Cuts', *Food Australia*, Vol. 44, 1992, pp. 516–518.

In the end, the driving theme of Professor O'Dea's work has been indigenous health rather than specifically research relating to cardiovascular disease. O'Dea says, 'I am very interested in the traditional diet of Aboriginal people and hunter–gatherers. That is what drove this work and I guess I always go back to that model to see what I can learn...' (O'Dea interview, 2008).

The impact on further research in regard to Professor Sinclair seems to be more significant. Sinclair said, 'It led to about 10 years of research, so it was an effective injection of money...I continued that research after I stopped collaborating with Kerin O'Dea, so I got another grant and there would be additional papers and grants that flowed from that because we hadn't answered all the questions' (Sinclair interview, 2009). Sinclair provided the following publications on research that followed from the case study grant research:

- Eaton, S.B., S.B. Eaton 3rd, A.J. Sinclair, L. Cordain and N.J. Mann, 'Dietary Intake of Long-Chain Polyunsaturated Fatty Acids During the Paleolithic', *World Review of Nutrition and Dietetics*, Vol. 83, 1998, pp. 12–23.
- Hearnshaw, H., P.F. Arthur, W.R. Shorthose, A.J. Sinclair, D. Johnston and P.D. Stephenson, 'Evaluation of Angus, Charolais, and Hereford as Terminal Sires on Hereford and First-Cross Cows. III Meat Quality of Progeny', *Australian Journal of Agricultural Research*, Vol. 49, 1998, pp. 1009–1019.

- Kelly, F.D., A.J. Sinclair, N.J. Mann, A.H. Turner, L. Abedin and D. Li, 'A Stearic Acid-Rich Diet Improves Thrombogenic and Atherogenic Risk Factor Profiles in Healthy Males', *European Journal of Clinical Nutrition*, February 2001, Vol. 55, No. 2, pp. 88–96.
- Li, D., A. Ng, N.J. Mann and A.J. Sinclair, 'Contribution of Meat Fat to Dietary Arachidonic Acid', *Lipids*, April 1998, Vol. 33, No. 4, pp. 437–440.
- Li, D., A.J. Sinclair, N.J. Mann, A. Turner and M.J. Ball, 'Selected Micronutrient Intake and Status in Male with Differing Meat Intakes, Vegetarians and Vegans', *Asia Pacific Journal of Clinical Nutrition*, Vol. 9, 2000, pp. 18–23.
- Li, D., A. Sinclair, N. Mann, A. Turner, M. Ball, F. Kelly, L. Abedin and A. Wilson, 'The Association of Diet and Thrombotic Risk Factors in Healthy Male Vegetarians and Meat-Eaters', *European Journal of Clinical Nutrition*, Vol. 53, No. 8, August 1999, pp. 612–169.
- Li, D., H. Zhang, B.H. Hsu-Hage, M.L. Wahlqvist and A.J. Sinclair, 'The Influence of Fish, Meat and Polyunsaturated Fat Intakes on Platelet Phospholipid Polyunsaturated Fatty Acids in Male Melbourne Chinese and Caucasian', *European Journal of Clinical Nutrition*, Vol. 55, No. 12, December 2001, pp. 1036–1042.
- Mann, N.J., D. Li, A.J. Sinclair, N.P. Dudman, X.W. Guo, G.R. Elsworth, A.K. Wilson and F.D. Kelly, 'The Effect of Diet on Plasma Homocysteine Concentrations in Healthy Male Subjects', *European Journal of Clinical Nutrition*, Vol. 53, 1999, pp. 895–899.
- Mann, N.J., L.G. Johnson, G.E. Warrick and A.J. Sinclair, 'The Arachidonic Acid Content of the Australian Diet is Lower than Previously Estimated', *Journal of Nutrition*, Vol. 125, 1996, pp. 2528–2535.
- Mann, N., A. Sinclair, M. Pille, L. Johnson, G. Warrick, E. Reder and R. Lorenz, 'The Effect of Short-Term Diets Rich in Fish, Red Meat, or White Meat on Thromboxane and Prostacyclin Synthesis in Humans', *Lipids*, Vol. 32, No. 6, June 1997, pp. 635–644.
- Mansour, M.P., D. Li and A.J. Sinclair, 'The Occurrence of Trans-18:1 Isomers in Plasma Lipids Classes in Humans', *European Journal of Clinical Nutrition*, Vol. 55, 2001, pp. 59–64.
- Morgan, S.A., K. O'Dea and A.J. Sinclair, 'A Low-Fat Diet Supplemented With Monounsaturated Fat Results in Less HDL-C Lowering than a Very-Low-Fat Diet', *Journal of the American Dietetic Association*, Feb 1997, Vol. 97, No. 2, pp. 151–156.
- Ponnampalam, E.N., A.J. Sinclair, A.R. Egan, S.J. Blakeley and B.J. Leury, 'Effect of Diets Containing n-3 Fatty Acids on Muscle Long-Chain n-3 Fatty Acid Content in Lambs Fed Low- and Medium-Quality Roughage Diets', *Journal of Animal Science*, March 2001, Vol. 79, No. 3, pp. 698–706.

- Ponnampalam, E.N., A.J. Sinclair, A.R. Egan, S.J. Blakeley, D. Li and B.J. Leury, 'Effect of Dietary Modification of Muscle Long-Chain n-3 Fatty Acid on Plasma Insulin and Lipid Metabolites, Carcass Traits, and Fat Deposition in Lambs', Journal of Animal Science, Vol. 79, No. 4, April 2001, pp. 895–903.
- Ponnampalam, E., A. Sinclair, A. Egan, G. Ferrier and B. Leury, 'Dietary Manipulation of Muscle Long Chain Omega-3 and Omega-6 Fatty Acids and Sensory Properties of Lamb Meat', *Meat Science*, Vol. 60, 2002, pp. 125–132.
- Ponnampalam, E.N., A.J. Sinclair, B.J. Hosking and A.R. Egan, 'Effect of Dietary Lipid Type on Muscle Fatty Acid Composition, Carcass Leanness and Meat Toughness in Lambs', *Journal of Animal Science*, Vol. 80, 2002, pp. 628–636.
- Ponnampalam, E.N., G.R. Trout, A.J. Sinclair, A.R. Egan and B.J. Leury, 'Comparison of the Colour Stability and Lipid Oxidative Stability of Fresh and Vacuum Packaged Lamb Muscle Containing Elevated Omega-3 and Omega-6 Fatty Acid Levels From Dietary Manipulation', *Meat Science*, Vol. 58, 2001, pp. 151–161.
- Sanigorski A.J., A.J. Sinclair and T. Hamazaki, 'Arachidonic acid supplementation causes an increased thromboxane to prostacyclin ratio even in the presence of n-3 PUFA', *Lipids* Vol. 31, 1996, pp. 729-736.
- Sinclair, A.J. and N.J. Mann, 'Short-Term Diets Rich in Arachidonic Acid Influence Plasma Phospholipid PUFA Levels and Prostacyclin and Thromboxane Production in Humans', *Journal of Nutrition*, Vol. 126, 1996, pp. 1110S–1114S.
- Sinclair, A.J., N.J. Mann and J. Kelly, 'Kangaroo Meat for Human Consumption', *Proceedings of the Nutrition Society of Australia*, Vol. 21, 1997, pp. 2–57.

22.8 Interface B – dissemination

The key dissemination vehicles for the research findings relating to the case study grant were publications as identified in primary outputs under knowledge production. With the larger meat research programme as a whole, and as part of Professors O'Dea and Sinclair's other research work, the dissemination channels also included conferences and frequent addresses to professional and community groups, such as diabetes educators, medical practitioners, Aboriginal health workers and field staff, primary producers and lay groups interested in preventive health, nutrition and Aboriginal health. It also included regular interviews on radio and television discussing nutrition, diabetes, heart disease and Aboriginal health.

Professor O'Dea said, 'Publication was probably the most important. We got into some good journals – the *American Journal of Clinical Nutrition*, for example…the top journal for nutrition. Speaking at conferences…I spoke at a conference which I think was around the same time when I was applying for this grant. It was a conference looking at the links between agriculture and human nutrition. This is when I first put forward the hypothesis that red meat could be good, and of course the farmers loved it. I had a lot to do with the

meat industry because I used the model of Aboriginal diets and said that we could learn from that…In terms of agricultural techniques, I did a lot of talking to farmers about how they could change their product. I did not get anything published out of that but still it was very important' (O'Dea interview, 2009).

Professor Sinclair said, 'Every year there would have been conferences that we presented at with this data, and the Nutrition Society of Australia [conference] would be a major place we would have presented at every year in 10 or 15 years in this area' (Sinclair interview, 2009). Some of the related conferences include:

- 17th annual conference of the Nutrition Society of Australia, Perth, Western Australia, December 1992 (Mann et al., 1992, and Gallagher, Rutishauser and O'Dea, 1992).
- Australian Nutrition Foundation Forum, Melbourne, 26 March, 1991. *'Kangaroos for food?'*.
- Eastern Australian Saleyard Executive, National Saleyard Convention, Wagga Wagga, New South Wales, 19–20 March 1991, 'Red meat and risk of heart disease – differentiating between lean and fat meat'.

The case study grant did not provide any funding for the dissemination activities, such as the conferences.

22.9 Stage 4 – secondary outputs

22.9.1 Policy/product impacts

No patent licences resulted from the case study grant research. In addition, although it seems that the case study grant research and more significantly the larger meat research programme overall contributed to the body of evidence on lean meat and cardiovascular disease that has been adopted in terms of nutrition guidelines and policies, there have been limited attributions that could be identified in relation to the case study research publications. Professor O'Dea also confirmed that although she talked about lean meat being a key source of such things as iron and high-quality protein and the need to avoid saturated fat as part of her work, she and the research team did not produce any formal guidelines or policies.

In addition to other research published by Professors O'Dea and Sinclair, an article by the group (O'Dea et al., 1990) was cited in the report 'The Role of Red Meat in Healthy Australian Diets', which was published as a supplement to *Nutrition & Dietetics* in September 2007 (Caterson et al., 2001). The report was written by 16 leading Australian public health and nutrition experts and was peer reviewed by an independent expert editorial committee convened by Meat and Livestock Australia. The report's conclusions concurred with nutrition statements from the National Heart Foundation of Australia and had the support of the Dietitians Association of Australia as a useful summary of the contribution of red meat to healthy eating. In line with the case study grant research, the report concluded, among other things, that:

- trimmed of fat, red meat is generally lean and contains low levels of saturated and trans fats and cholesterol
- after fish, red meat makes the second highest contribution of omega-3 oils to the Australian diet and is an important natural resource of long chain omega-3 oils, which are essential to the healthy functioning of the nervous system and important to heart health
- lean red meat can be included in the diet of people with or at risk of heart disease and can assist in the lowering of blood pressure.

Other published research by Professors O'Dea and Sinclair in this area – both prior to and following on from the case study grant research – are cited in Australia's National Health and Medical Research Council dietary guidelines. Some examples of the research indicated as following from the case study grant research by O'Dea and Sinclair and cited in the guidelines include:

- Li, D., A.J. Sinclair, N.J. Mann, A. Turner and M.J. Ball, 'Selected Micronutrient Intake and Status in Male with Differing Meat Intakes, Vegetarians and Vegans', *Asia Pacific Journal of Clinical Nutrition*, Vol. 9, 2000, pp. 18–23.
- Mann, N.J., D. Li, A.J. Sinclair, N.P. Dudman, X.W. Guo, G.R. Elsworth, A.K. Wilson and F.D. Kelly, 'The Effect of Diet on Plasma Homocysteine Concentrations in Healthy Male Subjects', *European Journal of Clinical Nutrition*, Vol. 53, 1999, pp. 895–899.
- Sinclair, A.J., N.J. Mann and J. Kelly, 'Kangaroo Meat for Human Consumption', *Proceedings of the Nutrition Society of Australia*, Vol. 21, 1997, pp. 52–57.
- Sinclair, A.J. et al., 'Estimation of the Long Chain n-3 Fatty Acid Status of Australians', *Proceedings of the Nutrition Society of Australia*, Vol. 22, 1998, p. 196.
- Watson, M.J., N.J. Mann, A.J. Sinclair and K. O'Dea, 'Impact of Outlet and Neighbourhood on the Fat Content of Untrimmed Retail Beef and Lamb Cuts Over a 12 Month Period in 1990–91', *Food Australia*, Vol. 44, 1992, pp. 511–514.

Professor O'Dea explained at interview that she also organised a large conference in 1990 of the different stakeholders in the meat industry and the government ministers of health and agriculture. She said, 'I actually ran a big conference on this where I got all the players in the meat industry together in 1990 [and] the Ministers of Health and Agriculture. It was about barriers to lean red meat production in Australia. I have recently been contacted by somebody in the meat industry again to ask if I still had a copy of that report, which I did send to him. We did identify what you could do. In reading it again I thought, I should activate this again as it really is pricing signals. I think in public health you cannot just leave things to the market. As we are finding in the world at the moment that the market won't drive things in a healthy way, it might drive them in a short-term profitable way. I got extremely interested in what drove the meat industry and all the different players. I actually had a really natural alliance with farmers because they can produce

whatever they are paid to produce. At the moment they are not paid to produce a healthy product' (O'Dea interview, 2009).

Professor O'Dea suggested that change has and can occur in the production of lean meat. She provided the example that when England entered into the European Community and the bottom fell out of the New Zealand lamb market, within 18 months they had changed and were producing lean lamb to get back into the market.

Investigations did indicate that the research influenced the meat industry in Australia. Professor O'Dea said, 'It certainly influenced the meat industry, no question about that. They used the work to promote their view that red meat is good. They always had lovely pictures of lean red meat when they did that, which was fine by me' (O'Dea interview, 2009). Professor Sinclair said, 'This work really underpinned the entire campaign by the Australian Red Meat Industry from about that period [of the case study grant] until even today, where they talk about lean red meat being a source of omega-3 fatty acids, so it had a major impact and if you look up Meat and Livestock Australia, that is the organisation now, [they] talk a lot about omega-3 fatty acids and…90% of the work that they were relying on came from…Kerin O'Dea's and my work, so it underpinned a very important public health area and we couldn't have predicted that from the money but that is the nature of the research' (Sinclair interview, 2009).

Discussions with a representative of Meat and Livestock Australia confirmed the influence of the research and that it provided practical steps that could be pursued to address concerns that consumers and health professionals might have about red meat and cholesterol, etc. It showed the importance of trimmed 'lean' meat and fatty acids in meat and that lean meat can be as part of a cholesterol-lowering diet. A representative of Meat and Livestock Australia said, '[It was used, along with other research from around the world] with marketing that was targeting consumers but also healthcare professionals. Probably more towards healthcare professionals, as it is quite complex stuff. I guess it more contributed to policymaking and dieticians. Policymaking in terms of what sort of recommendations: instead of having a dietary recommendation that said you must cut out red meat, it was more about if you choose lean meat. Mostly epidemiological studies showed links that were with heart disease. Then [with this research] you could say, well it might be because the meat was fatty, because lots of times you can't really tease out those reasons, but at least in clinical trials, when you remove the fat, there is not a problem. That was really a more balanced approach. It allowed the development based on evidence rather than no evidence. I guess that was the first thing. The other thing was that it could be translated into practical tips for dieticians that they could actually say to their patients or clients. I guess it was really good guidance to not only help in recommendations but also to interpret the information as it arrived especially from epidemiology [studies]' (Representative of Meat and Livestock Australia interview, 2009).

Discussion with the Meat and Livestock Australia representative also confirmed that, at the time of the case study grant research, the meat industry in Australia was relying on European and American data. As a result, the case study research and the larger meat research programme helped to understand where the problems were in Australia…that most of the fat was external and could be trimmed, whereas in the United States and Europe there was more marbling of fat in the meat. In addition, in terms of the different

types of fat, it also created a greater understanding with meat component having more monosaturated fat and therefore less of a concern. A representative of Meat and Livestock Australia said, 'At that time everybody was into fat, and the dietary goal was 30 percent less fat, which public health people thought was a simpler message to give to people, and that really did spurn a whole generation of low-fat products. Even though we had the evidence that it was about type of fat, whilst that was useful, it wasn't until later on when that became more accepted' (Representative of Meat and Livestock Australia, 2009).

22.10 Stage 5 – adoption by practice and public

Interviews indicate that when the research team published work in the *American Journal of Clinical Nutrition* regarding low-fat diets rich in lean meat and then adding beef fat back (O'Dea et al., 1990), it was not only used by the meat industry but others were also very interested in the data, including dieticians, nutritionists, other practitioners and people generally who had been very negative about red meat. Professor O'Dea said, 'They would say to me prior to that, "Well, you know, kangaroo is different". I would say, "Well, it is better", but actually the principles are exactly the same. I think from that point of view it was very valuable…particularly to be able to say to people, "Well you can eat red meat and it can be part of a cholesterol-lowering diet, but it has got to be lean and the rest of the fat in the diet has got to be lean"' (O'Dea interview, 2009).

Statistics and industry analysis also show that although people are eating less red meat than 20 years ago, changes in the processing and butchering practices have combined to produce red meat today with significantly lower separable fat. Nutrient analyses in 2002 showed the separable fat on raw retail samples was up to 38% less than in comparable cuts in 1983. Further trimming by consumers prior to consumption is also increasing, and 84% of consumers reported removing some or all fat prior to consumption in 2007. The National Nutrition Survey in 1995 showed that red meat contributed less than 10% of dietary saturated fat intake in Australia and continues to make an important contribution to intake of iron, zinc, omega-3 fatty acids and vitamin B_{12}, as well as encouraging consumption of vegetables. A representative of Meat and Livestock Australia said, 'It has definitely had an impact. It just helped clarify that it wasn't the meat; it was the fact that you needed to get rid of that fat. It helped inform the type of changes that had to take place in production, processing and butchering practices, retail and at the consumer level...and they have occurred' (Representative of Meat and Livestock Australia, 2009).

The fact that lean meat is now produced, widely retailed, promoted and contained in many policies, guidelines and health promotion for healthy diets certainly indicates the change in attitudes since the original research and the likely impact that the body of research in this area and the associated health promotion has had nationally and internationally. The information from Meat and Livestock Australia in relation to their extensive use of the research results over the years in public and health education campaigns and the references in key guidelines and reports indicates that an important part of the impact can be attributed back to the larger meat research programme undertaken by Professor O'Dea and her colleagues. However, based on interviews and the scale of the specific case study grant research, it is reasonable to suggest that it would have played only a very small but (based on some views expressed) potentially valuable part.

An important limiting factor in the impact of improving the use of lean meat seems to be affordability and socioeconomic status. Professor O'Dea said, 'That is one of the sad things in a way, as poor people eat poor-quality meat due to it being cheap, and you will maximise the amount of calories per dollar. If you can afford it, you can get very high-quality meat. Kangaroo is still probably the best value for money in terms of good-quality lean meat' (O'Dea interview, 2009).

22.11 Stage 6 – final outcomes

Certainly there are health benefits of lean meat as part of a healthy diet, although it is hard to correlate this health message with an actual impact on community behaviour when considering health trends and outcomes in relation to obesity, diabetes and other key indicators. There has also been an economic benefit for the meat industry in the development of a higher value market for lean meat. However, in both cases, it is difficult to attribute these benefits directly to the larger meat research programme and particularly to the small case study grant research beyond contributing a piece of the larger picture.

The case for the contribution of the outputs of the case study grant research and the larger red meat research is strongest with the success and changes to the red meat industry in Australia, which has grown significantly in value, being worth Aus$14.5 billion, and is a key export earner for the country. This is based on information from the Meat and Livestock Australia itself and the exponential return on investment for the National Heart Foundation of Australia, as reflected in the following comments by Professor Sinclair: 'I think that, for a very small investment by the National Heart Foundation [of Australia], in hindsight it had an amazing impact on the ability of an industry to respond [to a serious threat to its future]...and we were continuing to publish until 2001 in this area, and then I started work with guys in agriculture…these were people changing agricultural practice and so on. We were showing the red meat industry that they didn't have a product that was necessarily fatty, it was lean, and you could lower your cholesterol rather than raise it, so the impact was really unbelievable that someone could measure it, and I think around the time that we were starting to get money from these people. They had a very defensive attitude to meat and health, and we came along and said, 'Hey guys, it isn't all bad news, and if you give us some money, we will show you why'. So we got [the money] from the meat and livestock [industry], and so on, over a period of time, and I think that was a very good investment from them too, and National Heart Foundation [of Australia] money would have been a component of the jigsaw or pyramid that was built. If you could go back 20 years you could see the difference of advertising for meat…it used to be, 'Feed the man meat', and it was all fatty, and the whole industry has changed towards lean meat, the preparation of quick meals with lean meat and how it is low in fat and rich in nutrients including omega-3 fatty acids' (Sinclair interview, 2009).

22.12 Summary of case study impacts

The payback framework describes the process from research to several categories of impact. The categories allow us to compare across different case studies. Table 22-2 shows, in

point form and by impact category, some of the impacts, described more fully above, that have emerged from this grant.

Table 22-2 Payback

Payback category	Impacts from case study
Knowledge production	• Two peer-reviewed publications (cited 45 times combined)

Research targeting and capacity building	• As part of the wider meat research programme undertaken by the research team:
	  • Training of PhD students and dieticians
	  • Funding of the graduate research assistant to enable continued work on the research programme

Informing policy and product development	• Difficult to directly attribute inclusion of lean meat in health diet policies and guidelines to case study grant research beyond saying it was part of larger meat research programme and body of wider research on this matter and it directly challenged negative perceptions about meat at the time • Influenced meat industry marketing and practices towards lean meat
Health and health sector benefits	• Generated interest in data among dieticians, nutritionists, other practitioners and people generally, who had been very negative about red meat. Although cited in key reports (with the larger meat research cited in national nutritional guidelines), it is unclear how definitely and directly the research programme impacted on nutritional policies and guidelines, etc
Broader social and economic benefits	• Health benefits of lean meat as part of healthy diet • Also an economic benefit for meat industry in development of higher value market for lean meat • Although information from the meat and livestock industry suggests that the research did contribute in some way to economic benefit and that published research from the larger research programme and grant research contributed to key guidelines, in both cases it is difficult to attribute benefits directly and conclusively to the larger meat research programme, let alone to the small case study project

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CHAPTER 23 Cell–cell interactions in the disposition of natriuretic peptides in the bovine chromaffin cells

23.1 Overview of case study grant

The grant of interest to this case study, titled 'Cell–Cell Interactions in the Disposition of Natriuretic Peptides in the Bovine Chromaffin Cells', was funded from 1991 to 1993 by the Heart and Stroke Foundation of Canada (HSFC). The work conducted for this grant was intended to document the mechanisms involved in the secretion of cardiac natriuretic peptides within bovine chromaffin cells and was led by the principal investigator (PI), Dr Ong, and a co-applicant, Dr De Léan. This research was carried out at the Clinical Research Institute of Montreal (CRIM), a non-profit organisation devoted to understanding the causes and mechanisms of diseases in order to find diagnostic tools and means of prevention and treatment. Ong's expertise in identifying peptides and extracting them from tissues and De Léan's expertise in binding techniques were complementary and their collaboration was fruitful. Through this grant, the research team, which also included various graduate students, revealed for the first time the presence of a new type of natriuretic peptide, known as C-type natriuretic peptide, in bovine chromaffin cells. They also found that natriuretic peptides inhibit nicotinic stimulation of catecholamine secretion from bovine chromaffin cells. Team members described this work as high-quality research, and although it did not translate into a change in practice or a product, it did build the knowledge base.

23.2 Introduction to case study

In the early 1980s, people thought that the heart was a mechanical pump and that its only function was for systemic circulation. The discovery of natriuretic peptides,[1] peptides originating in the heart, triggered an enormous amount of research activity throughout Canada, the United States and Japan. It was found that these peptides work on the vessels

[1] It is now known that natriuretic peptides comprise a family of three structurally related molecules: atrial natriuretic peptide (ANP; also known as atrial natriuretic factor (ANF)), brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP).

to decrease blood pressure. The existence of these peptides suggested that, in addition to being a mechanical pump, the heart is also an endocrine organ.

In the 1980s, researchers knew that natriuretic peptides were closely related peptides of varying chain lengths and were secreted by the heart muscle cells. By the late 1980s it had been shown that the carboxy-terminal, 28-amino-acid peptide $ANF^{99–126}$ was involved in circulation in both animals and humans (Cernacek et al., 1988). Its involvement was through its effects on the release of pressor- and volume-conserving hormones, namely vasopressin, renin and aldosterone (Atlas and Maack, 1987). The circulating form of $ANF^{99–126}$ results from the proteolytic processing of its precursor $ANF^{1–126}$ (also called pro-ANF), which is the unique storage form of ANF found in the secretory granules of the atria. Pro-ANF is mainly synthesised in the cardiac atria, which is considered the major site of *ANF* gene expression (Zisfein et al., 1986). For a better understanding of the processing of this precursor, the primary structures of ANF precursors of various species, including rats, mouse, man, dog, rabbit, pig and cows, were determined from the nucleotide sequences of the cloned complementary DNA (cDNA) that encodes these precursors (Matsuo et al., 1987).

Although the cardiac atria are considered the major source of ANF production, evidence of other sites of ANF production in the lung, pituitary gland and hypothalamus raised the hypothesis that this peptide could act at a local level in an autocrine or paracrine manner to modulate functions relevant to fluid electrolyte balance and maintenance of blood pressure (Gardener et al., 1986 and 1987). Following this hypothesis, Dr Ong's research team worked to complete various projects investigating the existence of natriuretic peptides in the bovine adrenal medulla through the late 1980s. In 1987, Dr Ong's team had characterised the co-existence of both precursors and mature forms of ANF in the bovine adrenal medulla (Ong et al., 1987). The team then documented the secretion and biosynthesis of ANF by adrenal chromaffin cells[2] in culture (Nguyen, Ong and DeLean 1988) and discovered another member of the natriuretic peptide family, which they named aldosterone secretion inhibitory factory (ASIF) (Nguyen et al., *Endocrinology*, 1989), which is a polypeptide that is equipotent to ANF in the inhibition of aldosterone secretion by the zona glomerulosa cells and its precursor within chromaffin cells, confirming the neuropeptidic character of ASIF within chromaffin cells (Nguyen et al., *Molecular Endocrinology*, 1989).

Consistent with Ong's work, an analogous ANP called brain natriuretic peptide (BNP)[3] was isolated from porcine brain and identified as a variant form of ANF (Sudoh et al., 1988). Two years later, the same Japanese team identified the third member of the natriuretic peptide family, CNP (Sudoh et al., 1990).

With these findings in mind, the team hypothesised that the existence of both natriuretic peptides (ASIF and ANF) within the same chromaffin cells raises the significance of their relative roles in the paracrine regulation (ie amine or peptide production) of adrenal steroidogenesis. In the grant titled 'Cell–Cell Interactions in the Disposition of Natriuretic

[2] Chromaffin cells are neuroendocrine cells found in the medulla of the adrenal gland (a suprarenal gland – ie that is located above the kidneys) and in other ganglia of the sympathetic nervous system and thus form neuronal tissue.

[3] Aldosterone secretion inhibitory factor (ASIF) is the elongated form of BNP consisting of 26 amino acid residues.

Peptides in the Bovine Chromaffin Cells', which was funded by the Heart and Stroke Foundation of Quebec (HSFQ) from 1991 to 1993, the team proposed to document the mechanisms involved in the secretion of ASIF and ANF, which are structurally different but display the same pharmacological properties, from chromaffin cells. The team believed that understanding the mechanisms of natriuretic peptide secretion would allow new insights into the local regulation of mineralocorticoid[4] secretion and therefore sodium and water retention in the body, thus affecting the electrolyte levels. The implications of this research were important not only from a cardiovascular view but also for understanding how the nervous system processes peptides and neural peptides. Studying the importance of peptides in the overall physiology and how they can be used as targets for new therapeutics was a new and exciting avenue of research at that time.

The PI on this project was Dr Huy Ong, who completed a doctor of philosophy (PhD) degree in pharmacology from the Faculty of Medicine at University of Montréal in 1979, after completing a master of science (MSc) degree in the Department of Pharmacology. From 1980 to 1985, Ong was an assistant professor in the Faculty of Pharmacy at the University of Montreal; he became an associate professor in 1985 and then became a professor in 1991. From 1986 to 1992, Dr Ong was also a senior researcher in the Molecular Pharmacology Laboratory at the CRIM.

23.2.1 The case study approach

The findings presented in this case study are based on a combination of three face-to-face interviews with Dr Ong (the PI), Dr De Léan (a co-applicant) and Dr Babinski (a member of the research team); a review of the PI's curriculum vitae; a review of relevant and available administrative documents from the HSFC; documentary analysis of the scientific literature; and bibliometric analysis. We also spoke with one of Dr Ong's former students, Dr Meloche.

It is also important to note for this case study that the PI had recently moved offices and was without administrative support at the time of data collection.

23.3 Stage 0 – topic/issue identification

Two key factors led the PI to identify and pursue this research project. These were:

- previous research
- collaboration.

These two factors are elaborated on below.

23.3.1 Previous research

Dr Ong explained that his work in the area of angiotensin II led to his research of natriuretic peptides. Thus, in the early 1990s, Ong's focus was on the activity of natriuretic peptides in the adrenal gland. The adrenals are divided primarily into two parts: the

[4] Mineralocorticoids are a class of steroid hormones characterised by their similarity to aldosterone and their influence on salt and water balance.

adrenal medulla, which synthesises all of the catecholamines, and the adrenal cortex, which synthesises all of the corticosteroids, including the mineralocorticoids in the cortex and the glucocorticoids in the fasiculata. The adrenal is the biggest ganglia, and so Ong focused his work here for two reasons. Firstly, his team had uncovered at that time that natriuretic peptides were expressed and synthesised not only in the heart but also in the periphery. His team was the first at that time to discover the presence of those peptides in the adrenal gland. Secondly, one of the effects of natriuretic peptides is on the regulation of the secretion of another hormone, aldosterone, which is a mineralocorticoid. Natriuretic peptides inhibit the activity of aldosterone, thereby reducing reabsorption of sodium at the kidney level, as well as reabsorption of water, thus decreasing blood pressure. Ong thought that this regulation of mineralocorticoid secretion was perhaps the most important biological activity of the natriuretic peptide, and this is where his interests were.

As previously discussed, various discoveries through prior research projects led the team to study the disposition of natriuretic peptides in the bovine chromaffin cell. While studying the biosynthesis of ANF by adrenal chromaffin cells, the team had demonstrated the co-existence of $ANF^{99–126}$ and its precursor $ANF^{1–126}$ within the bovine chromaffin granules, confirming the maturation process of atrial peptides in the adrenal medulla (Ong et al., 1987). This was the first biochemical evidence that $ANF^{99–126}$ and its precursor $ANF^{1–126}$ are co-secreted by chromaffin cells, suggesting that the maturation process might occur in the chromaffin granules. It was observed that chromaffin cells could interact with the zona glomerulosa cells, which are cortextual, to regulate the secretion of mineralocorticoids and glucocorticoids. This process is called paracrine regulation. Paracrine regulation is not a result of systemic circulation but is due to the interaction between cells. Ong was interested in the cell–cell interaction between the chromaffin cells and the zona glomerulosa cells of the adrenal cortex.

23.3.2 **Collaboration**

Dr De Léan, a co-applicant on the grant, arrived at the CRIM in 1982, following his postdoctoral training at Duke University. At the time, he had recently established his laboratory, bringing expertise in binding studies to the institute. De Léan was part of a group grant on hypertension, directed by Jaques Genest, the founder of CRIM. The group grant included five PIs and more than 30 scientists. It was on this team that De Léan had started his work on natriuretic peptide receptors. Eventually De Léan wanted a smaller group with molecular biologists. At the end of the group grant, he formed another group with four researchers, including biochemists, biologists, protein chemists and physiologists. They collectively applied for a five-year grant.

Dr Ong had a lot of expertise in terms of identifying peptides and extracting them from tissues; however, he had wanted to gain additional expertise in binding studies for his own personal growth. Ong asked to work in Dr De Léan's laboratory for a period of three months. De Léan and Ong agreed to partner in order to get a broader approach in the field of natriuretic peptides, which De Léan had been researching since their discovery in 1983.

Dr De Léan claims the idea for the grant of interest to this case study was his, influenced by his previous work. Ong had the expertise in terms of methods, and he set up the

experiments and retrieved the peptides from the cultured chromaffin cells. Ong and De Léan devised several techniques prior to this grant application, in which they had tried to document the interaction between the two regions of the adrenal gland. De Léan encouraged Ong to submit a grant application for this project so they could continue work on a larger scale.

Their three-month term turned into 12 years of collaboration, during which Drs Ong and De Léan continued to exchange ideas and skills. Ong learned De Léan's binding technique and De Léan learned from Ong's expertise and ideas. This long-standing co-location and collaboration was fruitful for many years, until De Léan was nominated as Head of the Department of Pharmacology at the University of Montreal.

23.4 Interface A – project specification, selection and process

The idea for this grant originated from De Léan via his previous research. At the time the team was focused on trying to characterise these peptides: how they were synthesised, how the peptides are then processed and how they are released into the general circulation. The existence of both ANF and ASIF within the same chromaffin cells raised questions regarding their relative roles in paracrine regulation of adrenal steroidogenesis. It had also been reported that an increase in secretion of immunoactive ANF by cultured rat cardiomyocytes is induced by a cross talk of bovine aortic endothelial cells secreting endothelin (Lew and Baertschi, 1989). The team wondered if the same cellular mechanisms were involved in the secretory processes of neuropeptides within the chromaffin cells.

Through the grant titled 'Cell–Cell Interactions in the Disposition of Natriuretic Peptides in the Bovine Chromaffin Cells', the team proposed to:

1. document differential regulation in the biosynthesis and secretion of both ANF and ASIF within chromaffin cells using various stimuli such as protein kinase A and C activators, as well as nicotinic and depolarising agents; it was then believed that BNP acts as a neuromodulator in the regulation of electrolytic balance, contrasting with the systemic role of ANF, so a differential regulation of the disposition of both peptides in the neuronal-like chromaffin cells model was suspected
2. document the cellular mechanisms involved in the secretion of ANF and ASIF as neuropeptides by the chromaffin cells; the cell–cell interactions between bovine adrenal medullary endothelial cells and chromaffin cells in the regulation of natriuretic peptides and enkephalins as a neuropeptide marker in co-culture system were to be evaluated.

The team proposed to study the mechanisms that regulate the biosynthesis, processing and secretion of ANF and ASIF using cultured adrenomedullary chromaffin cells. The influences of potassium, nicotine and endothelin on secretion were to be monitored. This research involved various steps, including chromaffin cell culture and extraction, radioimmunoassay/radioreceptor assay/high-performance liquid chromatography (HPLC) of chromaffin cell extracts for the biosynthesis and secretion studies of both ASIF and

ANF. Parallel measurements of enkephalin and enkephalin-related peptides in the system were thought to provide a good point of comparison to study the natriuretic system.

The aim of this project was to understand the role of natriuretic peptides in the adrenal medulla as potential local modulators of mineralocorticoid production by the adrenal cortex, which was thought to play a key role in electrolyte balance.

The rationale for this project was that it had become obvious to researchers that natriuretic peptides were involved in the regulation of homeostatic balance of body fluid and blood pressure; however, the respective role of each peptide was not clearly defined. This research project was meant to define the respective roles of ANF and BNP through their biosynthesis and secretion, as well as local mechanisms involved in the regulation of their secretion.

Dr Ong wrote the majority of the grant application, although Dr Babinski, a graduate student at the time, was involved in writing a part of the grant as part of his learning process. Babinski claims this 'was a tremendous experience' and that he felt lucky to have had this experience, which was not common among his peers (Babinski interview, 2008). Babinski recalled that there were some modifications to the hypotheses and methods through the course of the project. Overall the grant application received a high score and the review committee commented that 'this [was] a very good proposal coming from two productive and competent investigators...the productivity of these applicants is very high' (Scientific Review Committee Report, 1990). The review committee felt that some savings could have been made in the budget.

This project used a neuronal model of cultured bovine adrenomedullary cells, which was originally created by Livett (1984). The culture facility was well mastered in Drs Ong and De Léan's laboratory and the protocols were well designed. The review committee commented that these experiments were well facilitated by the availability of the necessary materials and reagents (ie the chromaffin cells). Ong also said that the team could not have conducted this study or much of their previous work, such as discovering BNP in the chromaffin cell, without this model.

23.5 Stage 1 – inputs to research

23.5.1 Funding

The grant of interest for this case study was titled 'Cell–Cell Interactions in the Disposition of Natriuretic Peptides in the Bovine Chromaffin Cells'. It was funded by the HSFC, after the first attempt to secure funding, from June 1991 to July 1993 with a total amount of Can$96,436. Dr Ong recalled that they did not receive the amount of money requested, but we were not able to confirm how much funding was provided. He explained that when a grant is applied for to the HSFC, a review is done nationally, although the amount of funding depends on the provincial foundation and thus the funds raised within each province. The amount of money available within Quebec to HSFC applicants is often lower than that in other provinces. The amounts requested were as shown in Table 23-1, where materials and supplies included cell-culture studies and chromatographic studies, as well as chemicals, disposables and travel expenses.

Table 23-1 Funding

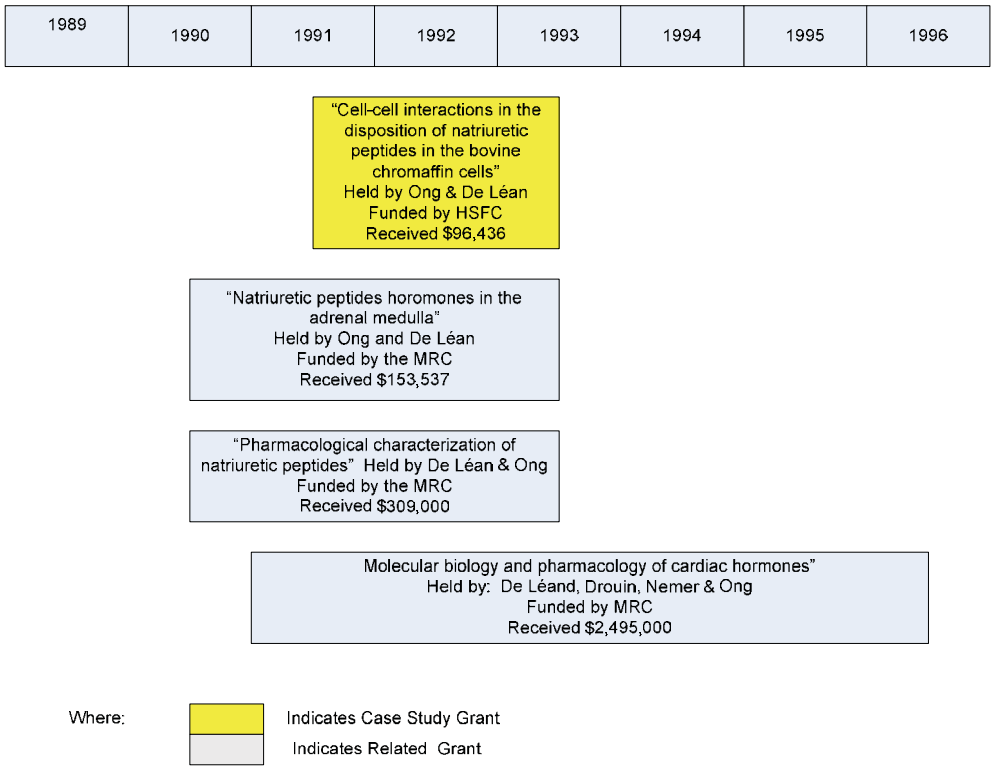
Summary	1991–1992 (Can$)	1992–1993 (Can$)
Technician (salary and benefits)	15,164	15,922
Equipment	–	–
Experimental animals (252 hamsters)	–	–
Materials and supplies	28,500	28,500
Other	2,000	2,000
Total	**47,786**	**48,650**

The Medical Research Council (MRC) funded a project titled 'Natriuretic Peptide Hormones in the Adrenal Medulla' from July 1990 to June 1993 with an amount of Can$153,537. Drs Ong and De Léan had originally requested Can$103,274 per year, but the MRC granted them about half of that. The MRC funding was mentioned in the application to HSFC, with acknowledgement that there was overlap between these two grants. Ong and De Léan said that the support of the HSFC was absolutely necessary for the achievement of the research work proposed. This additional financial support was critical in allowing the team to devote more time to culturing the chromaffin cells, extracting peptides and identifying natriuretic peptides from those cells. The money from the HSFC was also crucial in allowing the team to commit more focus to cardiovascular disease rather than different aspects of cell biology.

Drs Ong and De Léan and two others, Jacques Drouin and Mona Nemer, were members of a team grant supported by the MRC from 1990 to 1996 with a total amount of Can$2,495,000. De Léan claimed that the work of this larger grant corresponded to the topic supported by the HSFC grant.

Figure 23-1 shows related funding within the laboratories of Drs Ong and De Léan over the span of the case study grant (highlighted in yellow), plus two years before and after.

Figure 23-1 Related funding held by Drs Ong and De Léan between 1989 and 1996



All interviewees agreed that this research would not likely have happened without either the HSFC or MRC funding. That said, Dr Babinski described Dr Ong as a very strategic thinker (a point that he believes has been demonstrated through Ong's career). Babinski recalled a time when the MRC significantly reduced the team's funding and Ong 'saw that coming. He was able to refocus the research and attract other funding to maintain necessary levels of funding to stay on top of his research'. Babinski recalled that the team was well funded; however, both Ong and De Léan said they did not have enough money.

Other potential sources of funding the team could have applied to include the Natural Sciences and Engineering Research Council of Canada, although Dr De Léan said that the team would not have been able to easily find other funding because no other organisation clearly supported cardiovascular research at the time. The MRC was the major source of biomedical funding, and the HSFC was the team's second greatest source of funding. However, the financial budget of the Heart and Stroke Foundation of Quebec (HSFQ) is dependent on the funds collected in the province, as are the budgets of all of the provincial chapters of the HSFC. Due to poor donor rates in Quebec, grants from the HSFQ were, and still are, notoriously lower in Quebec than in the other provinces. Despite the challenges of funding, De Léan said that about 40 percent of all Canadian cardiovascular research work is done in Quebec.

Dr Ong claimed to have had support from industry, although amounts are unknown for this time period. He said that it would have been hard to get through some years of scarcer funding without access to 'soft' money.

23.5.2 Facilities

This work was done in the Laboratory of Molecular Pharmacology at the CRIM, which was one of the leading institutes working on natriuretic peptides in the early 1990s (Babinski interview, 2008). Dr De Léan said that the CRIM is 'one of the outstanding institutes in Canada'. Dr Ong claimed that the CRIM was doing some of the most important biomedical research in Montréal at the time. Molecular biologists were discovering and microsequencing new peptides rather than mass producing them. Much of this sequencing was done at the institute, and so expertise in sequencing existed at the CRIM.

Dr Ong also explained that the CRIM had lots of core facilities and provided good support to its researchers. The CRIM housed some of the more sophisticated instrumentation and had qualified personnel on staff who helped each other (Ong interview, 2008). Although researchers at the CRIM are required to get their own grants and personal equipment, the institute provides common equipment and services as well. Dr De Léan said that availability of equipment was essential for this project because of the technical aspects it involved. The grants from both the HSFC and MRC allowed the team to acquire some new chromatography equipment (De Léan interview, 2008).

Dr Babinski, who also worked at the CRIM, recalled that the facilities were 'very, very good'. He said he felt fortunate to work there because it was a leading institution that made good associations between the clinical and biomedical aspects that he believes were an important component of the training he received.

23.5.3 Collaborations

The team did not have a lot of external collaboration, although team members stated that internal collaboration within CRIM and between its laboratories was good.

As mentioned earlier, Dr Ong came to work in the laboratory of Dr De Léan, an expert in binding studies, to further his own professional development. Ong had intended to join De Léan's laboratory for six months, yet he found himself in a complementary collaboration that led him to work within De Léan's laboratory for 12 years. Throughout this time, Ong remained a professor in the Faculty of Pharmacy at the University of Montreal, where he maintained his own laboratory, although much of his research was conducted in De Léan's laboratory. De Léan said the interaction was quite productive and together they produced many publications. He called it 'a true collaboration' and said that 'working together with Ong was a facilitator, because we had complementary technical expertise. We were both excited by this idea.'

23.5.4 Research team

The research team consisted of Drs Ong and De Léan as the senior researchers, various graduate students and occasionally a postdoctoral fellow. In his interview, Babinksi who was a graduate student in the laboratory at the time, recalled that 'Ong and De Léan were both experts in their own aspects but were very complementary and that made the strength in the laboratory at that time. De Léan had a longstanding and strongly recognised expertise in molecular pharmacology, specifically receptor pharmacology. He did binding studies and characterised infinities; certainly we were one of [the] top labs in the field' (Babinski interview, 2008). Dr Babinski continued by saying, 'one of the great things in

the lab…was the very strong molecular basis and [expertise in] molecular pharmacology. The part of the lab where I [worked] was very focused on the peptides but there was a big aspect of the lab focused on the actual receptors. So [they] had the broad picture of how these peptides would be working from a synthesis and processing perspective but also from the pharmacology perspective on the receptor. It was very complementary'. De Léan referred to Ong as his 'equivalent collaborator because of his larger expertise in chromatography'. De Léan thought he had more expertise in biology and biochemistry at the time. All interviewees confirmed that the team held the right expertise and functioned well together.

Dr Babinski mentioned that he pursued work with Dr Ong, who he thought was serious, helpful and knowledgeable. He said he was more attracted to this project because of who Dr Ong was than the actual topic of research itself. Dr Babinski said he wanted to be trained by Ong and continued to have several summer studentships in his laboratory.

23.5.5 **Research environment**

In the mid-1980s, the research environment in the area of natriuretic peptides was very competitive domestically and at the international level. The field was brand new and so research at the CRIM was comparable with research elsewhere in the world. Researchers in this field were publishing similar findings at the same time. There was little disagreement about the results of this research but much pressure to publish quickly. Researchers began to feel that presenting at a meeting was dangerous unless a publication was already submitted and well advanced in the acceptance process, otherwise someone else would 'steal it'. De Léan claimed that the patent for using natriuretic peptides clinically was obtained by a Japanese team, although it was a Canadian discovery. He said the original work was done in Ontario by de Bold,[5] who conducted the key pioneering experiments that led to the discovery of the natriuretic peptide. De Léan had 30 people working with him when he heard about de Bold's work and they became competitors. De Léan's team was ultimately faster than de Bold's and he was the first to discover and publish the structure. In addition to domestic competition and rivalry with the Japanese, the United States was also a player in the area of natriuretic peptides.

Compounding the issue of competitiveness with the Japanese group was the fact that the Japanese researchers could publish in a non-peer reviewed journal while Dr Ong's team felt pressure to publish in high-impact journals in order to maintain their level of funding. Ong recalled publishing a paper within 48 hours, over a weekend, when a colleague reported back from a meeting in Japan that he had seen very similar work being presented. In that case, Ong and his team published their work just three weeks before their Japanese colleagues.

Dr De Léan said that any new field that is extremely active will experience unfair or harsh competition. Thus this type of environment was not unique for any researcher working in a 'hot' field. Dr Ong described this type of environment as demanding but exciting.

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[5] Dr A.J. de Bold is currently Professor of Pathology and Physiology at the University of Ottawa and Director of the Cardiovascular Endocrinology Laboratory at the University of Ottawa Heart Institute. De Bold also takes credit for the discovery of ANF, as observed in the following quote: 'Dr de Bold's research developed from his discovery of the endocrine function of the heart and the cardiac hormone ANF' (University of Ottowa, 2007).

23.6 Stage 3 – primary outputs from research

In the field of natriuretic peptides, Canada was recognised globally as one of the leaders. In the late 1980s and early 1990s, groundbreaking work was being conducted in the laboratory of Drs Ong and De Léan through a successful research programme that was developing new information (Babinski interview, 2008).

Within the parameters of the grant titled 'Cell–Cell Interactions in the Disposition of Natriuretic Peptides in the Bovine Chromaffin Cells', the team was trying to understand peptides and chromaffin cells in terms of neuroscience, not limiting their work to the cardiovascular system, which yielded two major findings. Firstly, the team revealed for the first time the presence of CNP in bovine chromaffin cells (it was already known to exist in the brain). Secondly, the team found that natriuretic peptides inhibit nicotinic stimulation of catecholamine secretion from bovine chromaffin cells. Team members described this work as high-quality research, and although it did not translate into a change in practice or a product, it did build the knowledge base that is currently used.

23.6.1 Knowledge production

Dr Ong identified the following papers as originating from the line of research supported by the HSFC grant. However, it is difficult to draw direct attribution between the grant and these papers, largely due to the other funding and research ongoing in the laboratory at the time, which contributed to the overall research programme focussing on natriuretic peptides within the laboratory.

1. Babinski, K., R.M. Féthière, A. De Léan and H. Ong, 'C-Type Natriuretic Peptide in Bovine Chromaffin Cells, *FEBS Letters*, Vol. 313, No. 3, 1992, pp. 300–302.
2. Babinski, K., P. Haddad, D. Vallerand, N. McNicoll, A. De Léan and H. Ong, 'Natriuretic Peptides Inhibit Nicotine-Induced Whole-Cell Currents and Catecholamine Secretion in Bovine Chromaffin Cells: Evidence for the Involvement of the Atrial Natriuretic Factor R_2 Receptors', *Journal of Neurochemistry*, Vol. 64, No. 3, 1995, pp. 1080–1087.
3. Bodart, V., W.E. Rainey, A. Fournier, H. Ong and A. De Léan, 'The H295R Human Adrenocortical Cell Line Contains Functional Atrial Natriuretic Peptide Receptors That Inhibit Aldosterone Biosynthesis', *Molecular and Cellular Endocrinology*, Vol. 118, 1996, pp. 137–144.

The first article reports on regulation of the biosynthesis and secretion of CNP in cultured bovine chromaffin cells (Babinski et al., 1992). The combined treatment with protein kinase A and C activators induced a six-fold increase in intracellular levels of $CNP^{1–103}$. The team found that upon stimulation by nicotine or depolarising agents, the biosynthesised $CNP^{1–103}$ was co-released with its mature forms, confirming the neuropeptidic character of CNP. However, no further maturation process was observed under stimulatory conditions, which contrasts with findings for both ANF and ASIF. The biosynthesis profile of the mature form of CNP was found to be similar to ANF, with a 3–4-fold increase in intracellular levels promoted by protein kinase A and C activators. The team concluded that CNP may have a role in catecholamine secretion but that further work was required to investigate this role.

The second paper explains the team's results when chromaffin cells were treated with either ANF or CNP (Babinski et al., 1995). Via secretion experiments, the team demonstrated that both CNP and C-ANF[6] are equally effective in reducing nicotine-evoked catecholamine secretion by cultured chromaffin cells, raising the possibility that this effect of CNP is predominantly mediated by a specific ANF receptor. The team believed the response to be specific to nicotinic agonists, because neither histamine- nor potassium chloride-induced secretions were affected by natriuretic peptides. The team reported: 1) the presence of ANF receptor subtypes in bovine chromaffin cells, 2) inhibition by natriuretic peptides of nicotinic whole-cell currents, as well as nicotine-induced catecholamine secretion, 3) the possible mediation of these effects by a class of ANF receptors and 4) the specificity of this inhibition to nicotinic agonists. The team concluded that because bovine chromaffin cells release ANF, BNP and CNP together with catecholamines, all three peptides could exert negative feedback regulation of catecholamine secretion in an autocrine manner by interacting with a non-discriminating ANF receptor subtype.

In the third paper, the team demonstrated the presence of fully functional ANF receptors in the recently characterised angiotensin II-responsive adrenocortical carcinoma cell line H295R (Bodart et al., 1996). Photoaffinity labelling of membrane preparations revealed a protein, which was further identified by immunodetection with a specific antibody directed to the human ANP-specific receptor natriuretic peptide receptor A (NPRA). The team concluded through various stimulation experiments that the human H295R cell line contains NPRA receptors positively coupled to the particulate guanylate cyclase that antagonises angiotensin II stimulation of aldosterone secretion.

Bibliometric analysis was conducted on four publications identified by the PI as related to the case study research. The fourth publication is not mentioned above as it predates the specific case study grant, but it was an important publication in this programme of research. Table 23-2 shows the results of the publication output and impact.

[6] C-ANF is another form of the atrial natriuretic peptide. It is not as active as ANP, which binds to another receptor.

Table 23-2 Publication output and impact of directly related publications

Number of journal articles:	4
Number of articles included in citation analysis[7]:	3
Total number of citations (all papers):	48
Aggregate relative citation impact:	0.45 (Class II)
Self-citations:	17%

Distribution of publications across relative citation impact classes:

	Class I (uncited)	Class II (>0 and <0.8 citations)	Class III (0.8 to 1.2 citations)	Class IV (1.2 to 2.0 citations)	Class V (>2.0 citations)
Number of publications		3			
Proportion of total output		100%			

Most highly cited publication[8]:	Nguyen, T.T., H. Ong and A. De Léan , 'Secretion and Biosynthesis of Atrial Natriuretic Factor by Cultured Adrenal Chromaffin Cells', *FEBS Letters*, Vol. 231, No. 2, 1988, pp. 393–396
Times cited:	25

23.6.2 Dissemination

The team worked to disseminate their findings via publications, poster sessions and meetings. Meetings were said to be important to researchers for sharing ideas and getting immediate feedback and discussion. The team presented largely at cardiovascular meetings domestically and in the United States but also attended one meeting in Scotland, another in Israel and various symposia (such as the International Peptide Symposium) on this topic. Dr Ong was a keynote speaker at some of these forums, although specific details were not obtained. Interviewees also said that an important method of dissemination was discussions in the halls and telephone conversations with colleagues.

The team did not conduct tours for the public as it was not allowed within the policies of the research institute. The CRIM did host colleagues from around the world, who could visit the laboratory, but these types of tours were restricted to academics.

Dr De Léan said that this project played a huge part in the existing stock of international knowledge on natriuretic peptides. Ong also said that he and his group were well known at the international level. At the end of the 1980s, this was a very active field, because it was new and because cardiac natriuretic peptides are fundamental to how the heart, kidney and blood pressure are regulated. It took many years for researchers to understand the physiology of the natriuretic peptides.

Both Dr Ong and Dr De Léan reflected that the high degree of competition they experienced in the late 1980s and early 1990s is normal for such an active area of research and has a positive side. It triggers more energy, as people have to work faster, be more active and publish faster to get recognised. The downside to such intense competition was

[7] During production it emerged that there had been confusion over whether three or four articles were directly related.

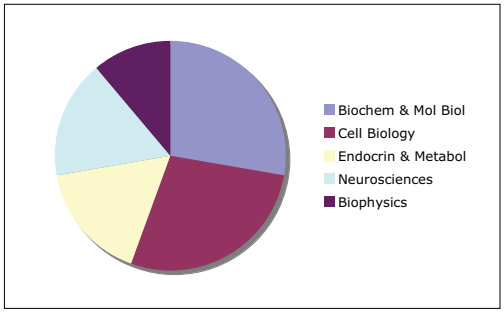
[8] Citation count extracted April 2009.

that, at times, the team felt that they had to cut some corners in order to be the first to publish an idea or else they would have to accept publication in lower ranked non-peer reviewed journals, which have a faster publication process given the lack of peer review.

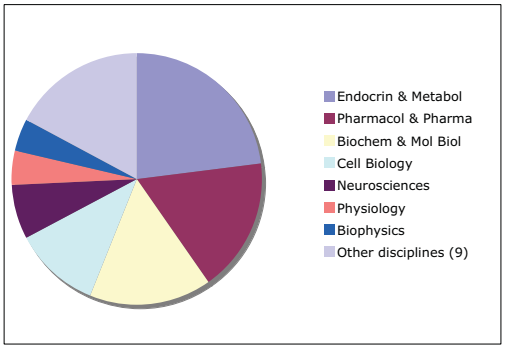
The bibliometric analysis also investigated knowledge diffusion. Dr Ong and his team publish in various areas or fields of research, including biochemistry and molecular biology, cell biology, endocrinology and metabolism, neurosciences and biophysics. Their work is most commonly cited by those working in endocrinology and metabolism, pharmacology, and biochemistry and molecular biology in the United States.

Figure 23-2 Knowledge diffusion: fields of research of output (a), fields of citing papers (b) and countries of citing papers (c)

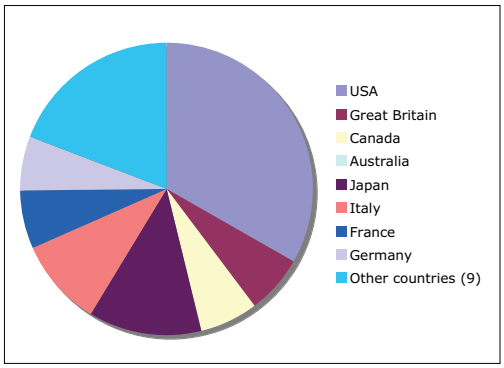
(a)



(b)



(c)



23.6.3 Capacity building and career development

Overall, Dr Ong said that this project, and the ongoing research programme in the early 1990s, helped to attract and recruit bright students, facilitated new collaborations (such as those with researchers in France, as well as at the University of Sherbrooke, Quebec) and indirectly helped the team secure more funds.

Dr Babinski trained with Drs De Léan and Ong from 1987 to 1994 throughout his MSc and PhD degrees. Babinski claimed to have benefitted tremendously from this research and the HSFC funding, as well as his own funding, which, collectively with other sources of grant funds, assisted the whole research programme in the laboratory at the time. He said that it was important to be associated with reputable scientists, as this has an impact on future funding for research later on. Dr. Babinski is now the Chief Executive Officer of the Centre of Excellence in Personalized Medicine (CEPMed). Cofounded by the Montreal Heart Institute and Génome Québec with funding from the Centres of Excellence for Commercialization and Research (CECR) programme, CEPMed focuses on diagnostic tests, medical treatments and tools to support drug development. Dr Babinski is also the Vice President of Research at Painceptor, a biotech company focusing on innovative treatment of pain. He said, 'we were fortunate to have our focused, brilliant PIs who were focused on their role of training us to further our careers. Students in our lab were not just a pair of hands to further their careers – this happens too often. I think too often students leave with diplomas but were not formed or shaped at a broader sense of thinking, beyond the techniques…The training environment within the lab was excellent'. Ong was very open to his students' questions. Babinski said Ong was especially inquisitive and taught him not to reject immediately any unexpected results but rather to consider unusual results. He said that this training helped him to think outside the box and always give things sufficient thought, which has been the main preoccupation throughout his career. It is especially applicable in an industrial setting for the development of new therapeutics.

Drs Ong and Babinski had a lot of discussions not only about the science but also about the involvement of researchers in the healthcare system. Babinski thus developed skills in understanding projects and extracting practical applications. Ong's research always had a clinical objective and he would ask his students to translate their research into application. Babinski said that these teachings were very useful and that, as a result, he learned how to think, approach a problem and understand the objectives. This training provided experience in asking about results, which has helped Babinski write grant proposals and move forward in his career. Babinski says he can certainly make the links between where he started and where he is now professionally. His current research at the Montreal Heart Institute encompasses his overall approach to scientific thinking, especially in his ability to relate research to practice.

Another fellow, Dr Nguyen, participated on this project and obtained his qualifications. Dr Ong did not mention names of other students who were involved, as he did not have the specific details within easy access. He did claim that he has always included students in his research projects and has trained various graduate-level students. No undergraduate students were involved in this research. As Ong remained a professor at the University of Montreal, this research fed into the training of all of his students. Ong claimed that the skills he developed were beneficial to his subsequent students.

Dr De Léan continued to have a successful research career in the field of natriuretic peptides, focussing on their molecular mechanisms and receptors. He has recently retired as a professor and planned, at the time of writing, on closing his laboratory in a year. Although the work completed via this funding was of good quality and complete, it did not become a mainstream area of research. De Léan said the work from the grant titled 'Cell–Cell Interactions in the Disposition of Natriuretic Peptides in the Bovine Chromaffin Cells' did not influence his subsequent research. By the mid 1990s, the team realised they had looked at the various aspects of cardiac natriuretic peptides, which is a very specific topic, and then stopped. They felt that they had answered the important questions and then moved on to explore new things within the bigger field.

Dr Ong said he left the natriuretic peptide area of research in 1995–1996 due, in part, to a change in the funding environment. At the time, he chose to pursue his interests in how neuropeptides impacted on the whole neurophysiological system. He continues to use his previous experience in the cardiovascular field to study the cardiovascular and antiatherosclerotic activities of growth hormones and secretagogues.

23.6.4 Benefits to future research and research use

The findings from this project did have an impact outside the field. The adrenal medulla, the central part of the adrenal gland that produces catecholamines and peptides, is located above the kidney. The team's work attracted attention from people who wanted to understand how the adrenal medulla was working, and Ong's work helped others learn about natriuretic peptides, their presence and their involvement in the adrenal medulla.

As mentioned in the previous section, the team began to collaborate with researchers in France and at the University of Sherbrooke. The group in France, led by H. Vaudry at the Laboratoire de Bioactivation des Peptides, Institut Jacques Monod, Université de Paris, worked to identify natriuretic peptides in frogs. The frog model proved interesting to study because the adrenals operate in two parts: the adrenal medulla expresses mineralocorticoids and the cortex expresses catecholamines. These two systems are mixed in the frog model, which make it interesting for the study of cell–cell interactions. Colleagues from the University of Sherbrooke collaborated with Ong's group, using the radioimmunoassay in their studies (eg Brochu, Lehoux and Picard, 1997) and publishing receptor studies together (eg McNicoll et al., 1992).

23.7 Stage 4 – secondary outputs

As previously mentioned, Dr Ong and his team identified a novel peptide in the bovine chromaffin cell (a neuronal tissue), which they called ASIF. This same peptide was later found in the porcupine brain by Japanese researchers, who called it the BNP. It has been accepted through subsequent studies involving other research groups, clinicians and cardiologists that BNP, which is found in the blood plasma, is a biomarker of myocardial function or heart failure. As the heart fails during myocardial infarction (MI) due to injury, it triggers much greater production of BNP, and thus the higher the level of BNP, the higher the intensity of MI. Clinical guidelines now cite this subsequent research and advise that BNP should be used as the standard biomarker to treat and monitor MI. For example, in 2007, the American College of Cardiology and the American Heart Association updated

their 'Guidelines for the Management of Patients with Unstable Angina/Non-ST-Elevation Myocardial Infarction' published in 2002 (Braunwald et al., 2002) and identified, for the first time, BNP as a biomarker test that can be considered to supplement global risk assessment in patients with acute coronary syndrome (Anderson et al., *Journal of the American College of Cardiology* and *Circulation*, 2007). Ong claimed that using BNP as a biomarker is now standard practice in cardiology.

The radioreceptor assay used in these experiments was created by Dr Ong. The innovative assay has since been used by other researchers and was picked up by Amersham, one of the biggest providers of radioactive compounds across Canada. The kits sold by Amersham are based on an article in *Clinical Chemistry* (Ong, De Léan and Gagnon, 1988).

The results from this research grant did not directly inform policy, drug or device development. Although not directly related, industry has pursued drugs that may be used in heart failure by building on the knowledge created by this project. The team's initial hypothesis was that ANF could act at a local level in an autocrine or paracrine manner to modulate functions relevant to fluid electrolyte balance and maintenance of blood pressure. It has been confirmed that the global action of natriuretic peptides is to try to relieve the heart by getting water out through the kidney and lowering blood pressure. The heart will pump less liquid and will more easily eject blood as a result, because the blood pressure is lower. Natriuretic peptides thus act as one of the only counter-regulatory systems against all the other systems that tend to maintain high blood pressure. The idea for the treatment is to add extra natriuretic peptides when the heart is suffering from MI, for example, to try to relieve the load and let the heart repair. Recently the drug industry marketed a specific form of natriuretic peptides for the treatment of heart failure called nesiritide. In Japan, a form of this drug has been approved for number of years. It was also accepted in the United States by the Food and Drug Administration in 2001, although it is not approved for use in Canada (Burnett et al., 2008).

23.8 Stage 5 – adoption by practice and the public

The monitoring of BNP to determine the severity of MI is now common practice.

23.9 Stage 6 – final outcomes

This research has had no direct final outcomes on society through improved health in the population, spin-off companies or the sale of pharmacological products. This research was indirectly fundamental to informing practice of the use of BNP as a biomarker of MI. The health gains or cost savings realised by using BNP to measure the intensity of MI is unknown.

23.10 Payback

The payback framework describes the process from research to several categories of impact. The categories allow us to compare across different case studies. Table 23-3 shows, in

point form and by impact category, some of the impacts, described more fully above, that have emerged from this grant.

Table 23-3 Payback

Payback category	Impacts from case study
Knowledge production	• Three publications directly related to HSFC grant • Team members attended and disseminated information at various domestic and international meetings
Research targeting and capacity building	• Positive experiences by graduate students within laboratory • PhDs obtained by Drs Babinski and Nguyen • PI learned new techniques and subsequently shared them with his students • New collaborations created with colleagues in France and Sherbrooke, Quebec
Informing policy and product development	• Radioimmunoassay created by Dr Ong previously and used in this study are now produced by Amersham • Subsequent work building on the case study grant was cited in clinical guidelines • Indirectly affected the development of a new therapeutic drug for heart failure
Health and health sector benefits	• Subsequent research building on this grant found that natriuretic peptides can be used as biomarkers to indicate the severity of MI
Broader social and economic benefits	• Radioammunoassay kits sold by Amersham

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CHAPTER 24 Is hepatic synthesis and secretion of lecithin-cholesterol acyltransferase (LCAT) linked to lipoprotein production by the liver and intestine?

24.1 Overview of case study grant

The main symptom of atherosclerosis is the formation of cholesterol-rich plaques on the walls of blood vessels. These plaques can reduce blood flow and, if they break away from the blood vessel wall, can lead to blockages that cause heart attacks and strokes. However, the body has a mechanism for transporting cholesterol away from the tissues and to the liver where it can be broken d006Fwn. It is transported in the blood bound to high-density lipoproteins (HDL), which is why high HDL levels are considered to be a sign of reduced risk of heart disease. The enzyme lecithin–cholesterol acyltransferase plays an important role in this process by helping cholesterol to combine with HDL so it can be transported away from the tissues. This case study is concerned with a grant studying factors that affect how lecithin–cholesterol acyltransferase is produced and secreted into the blood.

This grant was a two-year extension to a grant previously awarded by the British Heart Foundation (BHF) to look at the secretion of lecithin–cholesterol acyltransferase and its dependence on lipoprotein production. As well as its role in atherosclerosis, lecithin–cholesterol acyltransferase is important in other less common conditions such as congenital lecithin–cholesterol acyltransferase deficiency and liver disease. The work was conducted by a doctor of philosophy (PhD) student, under the supervision of Professor James Owen at the Royal Free Medical School in London. The value of the grant was £49,267, following on from an initial grant of £68,381. This extension allowed the work to be finished and the student to complete her PhD. Otherwise it is not possible to separate the outcomes and impacts of this grant from the first two years of the award.

This study investigated the relationship between lipoprotein and lecithin–cholesterol acyltransferase secretion in the liver. The liver was known to secrete both, but their interdependence was unknown. Secretion was investigated by studying levels of lecithin–

cholesterol acyltransferase messenger RNA (mRNA)[1], while in parallel measuring levels of mRNA for apoprotein B-100 (apoB-100) and apoA-I, which are major proteins of very low-density lipoprotein (VLDL)[2] and HDL, respectively.

The key outputs from this grant were in the area of future research targeting. The grant brought new techniques in molecular biology to the laboratory, which was a significant change in research methods. These techniques were used in further work on lecithin–cholesterol acyltransferase, following directly from this initial research, but also in the broader work in the laboratory across the spectrum of their research interests. Other outputs, including knowledge production, were limited, as most of the time and money made available through this grant were spent on learning and developing techniques that were then used in later studies to produce more significant numbers of publications.

24.2 Introduction to case study

24.2.1 Background

Scientific background

Cholesterol (and other fats) cannot be transported in the blood alone because they are not soluble in water – oil and water do not mix. In order for them to be transported to and from the tissues, they are joined with protein molecules to form lipoprotein particles. Lipoproteins shield the cholesterol from the surrounding aqueous environment of the blood during transport. Lipoproteins come in five different forms in the body, usually categorised by density[3]. The two most important forms for transport of cholesterol in the blood are low-density lipoprotein (LDL) and HDL: LDL transports cholesterol to the tissues of the body for use in cell walls and other cellular functions, while HDL transports cholesterol from the tissues to the liver for excretion in a process called reverse cholesterol transport. As HDL-bound cholesterol is being removed from the tissues – including the arteries – it is associated with lower risk of high blood pressure, heart disease and other related conditions. Conversely, high levels of LDL-bound cholesterol are associated with increased health risks.

Lecithin–cholesterol acyltransferase (LCAT) is an enzyme involved in the reverse cholesterol transport process, in which cholesterol from peripheral tissues, such as the walls

[1] Similar to DNA, mRNA contains genetic information that is involved in the production of proteins. It is unique to the corresponding protein and therefore can be used as an indicator for that protein.

[2] VLDL is another form of lipoprotein that is converted to low-density lipoprotein (LDL) in the blood. LDL is discussed more in the section titled 'Scientific background'.

[3] For reference, the five forms of lipoprotein, by density, are as follows: 1) chylomicrons, which transport lipids from the intestines to the liver, skeletal muscle and adipose tissue; 2) VLDL, which transport (newly synthesised) lipids from the liver to adipose tissue; they are converted to LDL via intermediate-density lipoproteins (IDL), in the bloodstream; 3) IDL, which are intermediate between VLDL and LDL; they are usually a minor fraction in the blood; 4) LDL, which transport cholesterol from the liver to cells of the body; and 5) high-density lipoproteins (HDL), which collect cholesterol from the body's tissues and bring it back to the liver.

of arteries, is transferred to the liver for excretion. The role of LCAT is to convert free cholesterol to cholesterol ester, which is then taken up into the core of a lipoprotein particle making HDL. As LCAT is involved in this reverse transport process, it is thought that enhanced levels of LCAT may remove excess cholesterol from arterial walls and hence reduce the risk of atherosclerosis, a condition that can lead to heart attacks and is implicated in a range of cardiovascular diseases. Atherosclerosis is the build up of plaques on the walls of arteries and involves the interaction of cholesterol with the surrounding tissue and cells in the blood. If the plaques grow large enough, they can obstruct the flow of blood through the arteries. Plaques can also fracture, releasing chunks of plaque into the bloodstream, which can block blood vessels, leading to acute conditions such as heart attacks. Plaque build up is complex and involves a range of different molecules.

There was significant interest in the study of lipids and their role in atherosclerosis at the time this grant was conducted. Although a number of risk factors had been identified for atherosclerosis, only blood cholesterol (HDL, LDL and other lipoprotein-bound forms) were thought to be a prerequisite for the progression of the disease. This had led to considerable interest in the role of cholesterol transport in the progression of the disease. There was a good understanding of the removal of lipoproteins as a result of previous work by Brown and Goldstein on the LDL receptor (Brown and Goldstein, 1984; Goldstein and Brown, 1984; Brown and Goldstein, 1985; and Goldstein and Brown, 1985) and Mahley on apoE[4] and its hepatic receptor (Mahley, 1982). However, less was known about the production of secondary (lipid-bound) lipoproteins and how this process was regulated. As LCAT was known to have an important role in the conversion of HDL, there was interest in further understanding its structure and physiology. At that time, work had taken place to investigate the structure of LCAT (Tata et al., 1987; McLean et al., *Proceedings of the National Academy of Sciences of the United States of America*, 1986; and McLean et al., *Nucleic Acids Research*, 1986) and its enzymology (Jauhiainen et al., 1987). However, little was known about its physiology.

There was also some interest in LCAT as a potential therapeutic target for the mobilisation of excess cholesterol deposited in the arterial wall. There were a number of cases of patients with familial LCAT deficiency who presented with symptoms including corneal glaucoma, anaemia and cardiovascular diseases and often had myocardial infarction at a young age, which suggested they had problems breaking down plaques. There was interest in whether an infusion of pure LCAT would improve the clinical condition of patients with familial LCAT deficiency. Furthermore, there was also a broader interest in LCAT and its potential to counteract cholesterol deposition for all those with atherosclerosis. In either case, sufficient quantities of pure LCAT would be required, so an increased understanding of LCAT secretion and possible mechanisms for production outside the body would be important in any such therapeutic approach.

As LCAT was known to be involved in the reverse transport of cholesterol and phospholipids, there was also interest in whether this enzyme was secreted by the intestine as well as the liver. Excess lipids and cholesterol are present in the intestine in the form of

[4] ApoE is an apolipoprotein involved in a number of biological processes and with a particular significance in lipoprotein metabolism. It is now known to play a significant role in atherosclerosis.

chylomicrons, another form of lipoprotein that contains dietary fats and transports them from the intestine to other organs and tissues. Chylomicrons are known to exchange components with HDL in the blood, so it was thought the intestine could also be a site of LCAT production. Therefore, LCAT secretion and its relation to lipids were also studied in the duodenum.

PI's background

The PI for this work was Professor James Owen. Trained as a biochemist to first-degree level, he started his career with a PhD on lipids, before travelling to Brazil to take up his first postdoctoral post at the Universidade Federal de Pernambuco in Recife. There he worked on schistosomiasis, a disease that causes liver damage and is caught from parasitic worms – usually through contaminated water. Part of this work included looking at LCAT, which is secreted by the liver, with secretion reduced when the liver is damaged. Upon his return to the United Kingdom (UK), Owen wanted to continue working on lipids and lipoproteins. His first position was an interdisciplinary research fellowship with the Wellcome Trust, in which he worked largely in a clinical department of the Royal Free Hospital School of Medicine (RFHSM) while officially a member of the Department of Biochemistry. He has since remained at the RFHSM, which is now part of University College of London (UCL) Medical School, working on a range of topics in lipids and lipoproteins. His most significant work concerns the connection between apoE and nitric oxide, and he now primarily works on gene therapy to treat dyslipoproteinaemias.

24.2.2 The case study approach

An interview was conducted with Professor Owen at the UCL Medical School, Royal Free Campus. with subsequent questions by phone and email as necessary. Owen provided a list of research grants for the period and relevant conferences attended, as well as identifying papers relevant to the case study from a list acquired through bibliometric analysis. Proposal forms, for both the original grant and the extension to that grant investigated in this case study, were studied. This was combined with desk-based research, investigations of the literature in the field, including previous and subsequent publications by Owen and others in the field, and citation tracking of the publications resulting directly from this work.

Unfortunately, it was not possible to conduct further interviews with people involved with this work. This is partly because there were so few other people involved, reflecting the small scale of the grant and the novel nature of the work involved for the laboratory at that time. The work was conducted by a PhD student. She has since left academia, and neither the PI nor the medical school have current contact details, so, despite best efforts, she could not be consulted. There were no other members of the laboratory working on this grant or related work at that time, as the techniques involved were all new to the laboratory. The PhD student learned the new molecular biology techniques for the grant at the Charing Cross Sunley Research Centre. Contact was made by email with the Director of this molecular biology laboratory; however, he did not have any specific involvement in this work. The PhD student at the Centre responsible for teaching new techniques to the principle researcher on the case study grant was also contacted by email; however, she had no direct involvement in the project and could only tell us the techniques she had taught at that time.

24.3 Stage 0 – topic identification

As described previously, there was interest at the time in LCAT and its role in reverse cholesterol transport because of its implications for atherosclerosis. Little work had been done to study the physiology of LCAT, and LCAT production had potential implications for treatment of LCAT deficiency and even atherosclerosis. Along with this broader scientific interest, two key factors led to this grant application:

- knowledge and experience of the PI
- link with the Director of the molecular biology laboratory at the Charing Cross Sunley Research Centre and interest in molecular biology.

Overall, the project selection and focus was completely driven by the PI, as the PhD student who was to conduct the work was not recruited until the initial grant was awarded.

24.3.1 Knowledge and experience of the PI

Professor Owen had worked extensively on LCAT before and had significant expertise in lipids and lipoproteins. At the time of application for the grant, he had done a range of work on LCAT, which served as a basis for this study. In collaboration with the Director of the molecular biology laboratory at the Charing Cross Sunley Research Centre, he had developed and used a probe to detect LCAT mRNA in human liver RNA and then developed antibodies to detect LCAT in plasma or secreted by the hepatoma cell line HepG2. These approaches formed the basis of initial studies conducted in this grant; however, the molecular biology work had previously been done in the Sunley Research Centre's laboratory and this was the first time such work had been conducted on site at the RFHSM.

24.3.2 Link with Charing Cross Sunley Research Centre and interest in molecular biology

The grant, and the preliminary work, stemmed from an interest in the burgeoning field of molecular biology. At that time, very few people had molecular biology skills, and most of them tended to work in specific molecular biology laboratories. Professor Owen had established a link with the Director of the molecular biology laboratory at the Charing Cross Sunley Research Centre Trust, where the preliminary work had been conducted as described above. Owen was able to spend a few months in this laboratory learning molecular biology techniques, which he was keen to bring back to his laboratory. This grant, and its predecessor, provided an opportunity to develop these skills working on a molecule in which the laboratory had significant expertise while addressing a pertinent scientific question.

24.4 Interface A – project specification and selection

This grant was the renewal of a previous two-year grant awarded by the BHF. It provided an additional two years to work on this project, which would allow the student to complete her PhD. Professor Owen had originally applied for three years of funding, but this had been turned down and he was only awarded a two-year grant. He suggested that this is because the project was the laboratory's first move into molecular biology, so they were untested in this field and the grant was, to some extent, speculative. He also

concluded, however, that the end result was beneficial for them, as they received four years funding in total, rather than the three for which they originally applied.

The grant was awarded following a second proposal reporting details of the work conducted in the first two years of work and a description of planned work in the second phase of the grant. Professor Owen commented that the review of prior work was notable in this case, as end-of-grant reports were often not required at that time. This was a special case, however, as the grant was to be extended, so justification for continued funding was required.

Professor Owen cannot remember receiving any feedback on this proposal from the BHF. This may have been partly as this was a renewal of a previously received grant; however, no feedback was received on the initial grant, and few BHF grants received by the group in this period were accompanied by significant reviewer feedback.

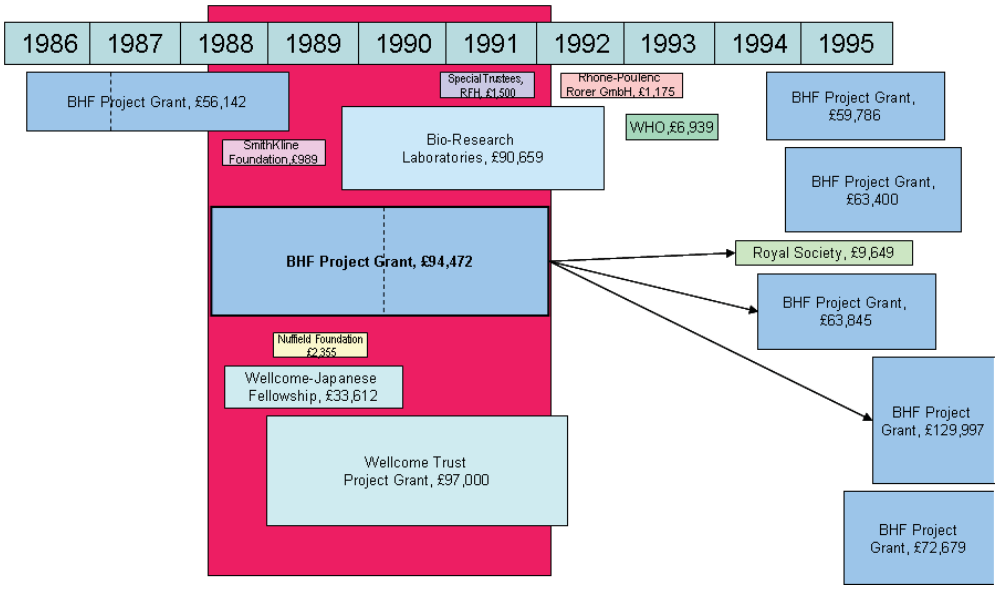
Professor Owen had submitted a larger proposal for £120,000 to the Wellcome Trust prior to his initial application to the BHF to do this work, but it had been turned down. This proposal would have included more work on liver disease, although the work conducted did necessarily include significant reference to liver disease and function. Professor Owen felt that it was easier to obtain funding from the BHF than the Wellcome Trust, and the work proposed was relevant as LCAT has implications for heart disease and atherosclerosis. The proposal was adjusted to emphasise this link in the BHF application.

A map of funding for the group for the period 1986–1995 is shown in Figure 24-1, which details all project grants and extended fellowships. In addition, funding was received for equipment and two vacation scholarships, as outlined in Figure 24-1, and an MRC Training Fellowship worth an estimated £90,000 was awarded to the group but was not taken up. Figure 24-1 shows that during this grant, the group received additional funding from the Wellcome Trust and Bio Research Laboratories (a pharmaceutical company, based in Milan, that is now part of Knoll Pharmaceuticals). However, none of this other funding was for work on LCAT or using molecular biology techniques, so it is easy to establish the outcomes that resulted from the BHF grant. Indeed, Professor Owen confirms that all of the consumables and equipment required for the work were covered by the money supplied in this grant.

Table 24-1 Funding for short-term scholarships and equipment grants received by the laboratory between 1986 and 1995

Funding source	Amount	Date awarded	Duration
Wellcome Trust Vacation Scholarship	£664	July 1988	2 months
Wellcome Trust Vacation Scholarship	£1,250	July 1994	2 months
University of London Central Research Fund	£1,790	March 1989	Not applicable; equipment grant

Figure 24-1 Funding streams commenced between 1986 and 1995. Three grants related directly to work conducted in this grant, two of which were from the BHF



24.5 Stage 1 – inputs to research

The key inputs to this research were two-fold. Firstly, the collaboration with Charing Cross Sunley Research Centre provided the knowledge surrounding techniques required to perform the work. Secondly, the BHF grant supported the work conducted, including the purchase of all necessary equipment.

24.5.1 Knowledge and expertise

The work was primarily conducted by a PhD student who had no previous research experience relevant to this field. The primary source of practical research expertise was the collaboration with the Director of the molecular biology laboratory at Charing Cross Sunley Research Centre Trust. Professor Owen had significant knowledge of the field of research and of LCAT in particular and provided overall guidance and support for the work. He was not involved in any direct laboratory work related to this grant.

24.5.2 Collaborators

As outlined above, the primary source of practical research expertise was the collaboration with the Director at Charing Cross Sunley Research Centre Trust. The PhD student was able to spend time training there, where she was mentored by another PhD student who provided practical guidance.

24.5.3 Techniques

Molecular biology techniques were crucial for this work, and these were provided through the collaboration with Charing Cross Sunley Research Centre Trust, as described above. Experience working with lipids and lipoproteins were also present in Professor Owen's laboratory, as this was their key research focus at the time.

24.5.4 Equipment, infrastructure and space

A range of new equipment for molecular biology work was required, and this was all purchased using money from this particular grant and its predecessor. These purchases are listed upfront in the grant application as costs of the work, and the PI confirms that this grant was used to cover the cost of these purchases. The work was conducted at the RFHSM, which provided laboratory space and other infrastructural support.

24.5.5 Consumables

Consumables not readily available at the RFHSM were covered by the grant.

24.5.6 Research samples

The grant proposed that key experiments conducted on HepG2 cells be replicated using monolayers of primary culture of human hepatocytes – ie cells that had recently been isolated from patients/animals. This is because HepG2 cells, which had been maintained in culture for many generations, were known to show differences in the lipoprotein particles secreted compared with normal hepatocytes. Hepatocytes were sourced from a researcher in Leiden, with the permission of the 'Rotterdam, Leiden and Rijswijk Liver Club'. However, as the primary cell lines were only sparsely available, experiments were to be conducted with HegG2 cells first and then only the most pertinent repeated with the primary cells. Samples of liver and duodenum were available through the RFHSM.

24.5.7 Financial

The grant covered all of the equipment and necessary consumables for the work, as described above. The grant also covered the funding to support the student in her PhD studies.

24.5.8 Reputation

Reputation was of limited importance as an input to the research. In fact, the PI had no reputation in the field of molecular biology, as this was a new research area for the laboratory. However, the RFHSM does have a strong reputation in cardiovascular research in general, which may have influenced funders' decision-making.

24.5.9 Time

The extension of the grant for an additional two years was significant, in that it allowed completion of the experiments and the student's PhD.

24.6 Stage 2 – research process

Due to the novel nature of this work for the laboratory, a number of changes to the planned approach were required during the course of the research. This required the PhD student to build on the standard approaches learnt from the collaboration with the Charing Cross Sunley Research Centre.

The key approach used in this study was to vary the levels of lipoprotein secreted by cultured human hepatoma cells and experimental animals and to assess whether any changes in the mRNA levels of one or more apolipoproteins are accompanied by a change in levels of LCAT mRNA. These levels could be altered in a variety of ways depending on

the cells in question, usually by introducing factors known to promote production of one or more of these molecules into the cell-culture medium. Generally, mRNA levels of LCAT, apoA-I and apoB-100 in the cell-culture medium or liver for animal experiments were measured using standard techniques[5]. If a change in LCAT was seen, additional apolipoprotein probes were used to assess the specificity of this effect.

All of this experimental work was conducted by the PhD student. Initially, techniques including cell culture, RNA extractions and northern blot analysis were learnt at the Charing Cross Sunley Research Centre, and experiments were then conducted at the RFHSM. Professor Owen was not directly involved in the laboratory work but took a supervisory role.

Work did not proceed precisely as planned, and various adjustments to methods were made during the work. In fact, a significant proportion of the initial grant was used in establishing effective techniques to conduct the research. This is partly because the approaches used were so novel for the laboratory, which had previously been a lipid, lipoprotein and membrane laboratory but had not had any previous experience in molecular biology. Although standard approaches were learnt from outside the laboratory, these had to be adapted and tailored to the research in question and often required building of experience – these approaches could not just be learned from a textbook but required technical proficiency. For example, in preliminary work before the grant, a complementary DNA (cDNA) probe had been developed to screen for LCAT mRNA amongst total RNA using a standard technique known as slot-blot hybridisation. However, this probe was found not to be sensitive enough to use in animal studies and did not provide enough sensitivity to draw conclusions about whether LCAT was secreted in the duodenum. Considerable time and effort was therefore spent during the first two-year stage of these grants in developing a new more-sensitive cRNA probe that could be used to detect lower levels of LCAT production.

Similarly, original experiments had planned to use HepG2 cells for the majority of cell-culture studies. However, it was found that HepG2 cells secrete only trace amounts of VLDL, instead secreting almost entirely in the HDL and LDL density ranges. This is a substantial difference from human liver in organ culture, which is known to secrete significant amounts of VLDL. A number of other hepatoma cell lines therefore had to be investigated to check for secretion of LCAT and the lipid-rich VLDL in order to find an appropriate alternative candidate cell.

24.7 Stage 3 – primary outputs from research

The most significant primary output of this work was the introduction of new molecular biology techniques to the laboratory. This had significant implications for future work that was much more fully published – both in direct follow-on studies looking at LCAT and broader work in a range of areas. Few publications were produced from this grant, as so much of the time and effort was spent in developing and establishing the new techniques.

[5] Northern blot, or, in cell culture, slot-blot hybridisation.

The grant may also have played a role in maintaining links to laboratories in northeast Brazil and building research capacity there.

24.7.1 Knowledge

This project was not very productive in terms of literature. This is partly because of the time required to establish new techniques in the laboratory and partly because the PhD student concentrated on writing up her PhD thesis, leaving little time for additional publications. However, two publications were produced as a direct result of a grant: a letter to the *Lancet* (Bingle et al., 1991), which has been cited twice, and an abstract presented at a hepatology conference (Imai et al., 1990), which has yet to be cited.

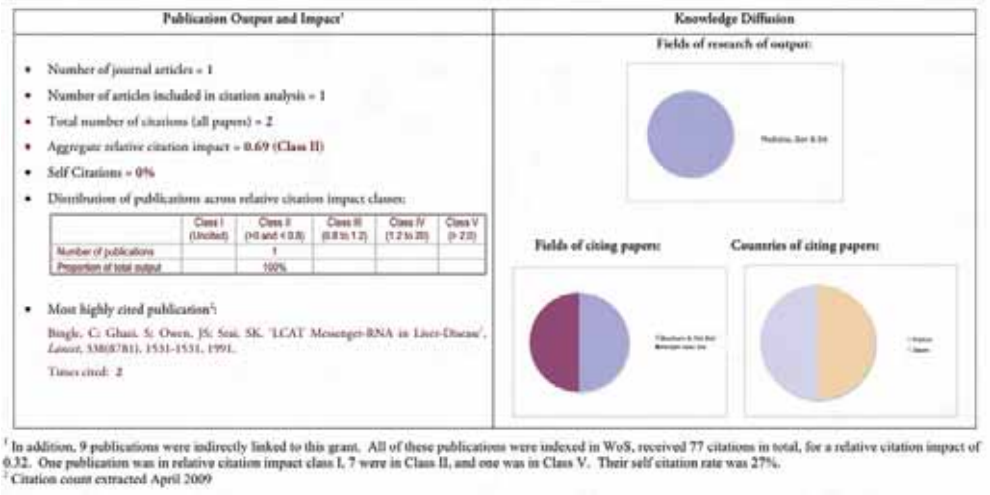
The letter outlined experiments showing marked differences in plasma LCAT activity between healthy subjects and patients with histologically confirmed cirrhotic liver disease (Bingle et al., 1991). It proposed that measurement of LCAT be incorporated into models assessing the best timing for liver transplantation.

The conference abstract outlined experiments showing that a cDNA probe can be used to detect LCAT mRNA in a number of hepatic cell lines but that LCAT catalytic activity can only be detected in the culture media of one cell type used, ie HepG2 (Imai et al., 1990). It described further experiments looking at the effect of hepatotoxic drugs on levels of LCAT secretion. These results suggest that HepG2 cells are a good model for LCAT processing and transport and for assessing the impact of hepatotoxic drugs.

Despite the limited number of publications, a number of approaches developed in this work were important for later studies and built the in-house knowledge of experimental approaches over and above the learning of standard experimental techniques. For example, a new cRNA (ribo) probe for LCAT mRNA, which allows more sensitive detection of the presence of LCAT than the previously available cDNA probe, was developed. Although not published, this technique was a new development that was used in later studies. Therefore, as well as bringing molecular biology techniques in-house as described below, work in this study developed these approaches for use with molecules of interest to the laboratory, such as LCAT and apolipoproteins.

Figure 24-2 shows the results of bibliometric analysis for the case study grant.

Figure 24-2 Publication output and impact of directly related publications



[1] In addition, 9 publications were indirectly linked to this grant. All of these publications were indexed in WoS, received 77 citations in total, for a relative citation impact of 0.32. One publication was in relative citation impact class I, 7 were in Class II, and one was in Class V. Their self citation rate was 27%.
[2] Citation count extracted April 2009

24.7.2 Benefits to future research and research use

Targeting of future research

The key outputs from this grant were in the area of capacity building. This was the group's first foray into molecular biology and the grant funded the purchase of the necessary equipment as well as demonstrating the laboratory's ability to do this kind of work. Molecular biology approaches were not trivial at this time as kits were not yet available to conduct these kinds of experiments and significant expertise and technical proficiency were required. By bringing these skills in-house, through the links with the Sunley Research Centre laboratory, this work paved the way for a large number of future grants looking at a range of topics that relied on molecular biology techniques. This includes the work on apoE that formed the basis of the laboratory's research output for more than a decade. In this sense, this grant was crucial in opening up a new avenue of research and paving the way for studies stemming through to the current work on gene therapy. A number of studies also followed directly from the work on LCAT. In fact, the proposal for the second round of this grant discusses the potential of the work to open up new avenues of research. Professor Owen wrote: '[It is] important for our future work (over the next 20 years!) that the present application for a two-year extension is approved. Firstly, because I am anxious that we should capitalise on our hard-earned proficiency by completing the full research programme and publishing the results. Secondly, because I would like to expand our work on LCAT into other areas, which would also rely heavily on molecular biology skills'.

Two potential studies outlined in the proposal would follow on from this work into the further study of the molecular biology of lipoprotein metabolism. The first of these was the possibility of transfection of the LCAT gene into animal cells[6] for production of significant quantities of the enzyme. This work did indeed take place using a one-year grant of £9,649 from the Royal Society (see Figure 24-1). The second proposed follow-on study was to express LCAT in insect cells using a baculovirus. This work was also later conducted successfully, producing biologically active normal and mutant forms of LCAT protein

[6] Chinese hamster ovary (CHO) cells were used.

(Chawla and Owen, 1995) as part of a £129,977 grant from the BHF starting in September 1995 (see Figure 24-1). In the grant extension proposal, Professor Owen explained: 'I anticipate being able to obtain financial support for these long-term projects from other grant awarding bodies, such as the MRC and Wellcome Trust, provided that our current experiments can be completed and fully published. Clearly, this will depend on the success of the present application!'

In total, 11 of Professor Owen's subsequent publications can be seen as following indirectly from the work done in this grant. These papers were published between 1990 and 2001 and have been cited a total of 74 times. Furthermore, in terms of the techniques and equipment introduced, this grant probably had a significant impact on the majority of the group's work for the following decade.

This grant was also part of a shift in research focus for Professor Owen and the group. Work had previously focused on the liver, which is where LCAT is secreted, but this grant, and others around this time, denoted a shift in emphasis, recognising the impact of molecules such as LCAT in other systems throughout the body. This trend has continued throughout Owen's research career, his focus being a set of molecules including LCAT, apoE and apoA-1. His work has investigated the implications of these molecules for a range of different processes from this starting point of liver disease through a diverse range of fields including cardiovascular disease and neuroscience (Tagalakis et al., 2005).

It is also worth noting that this was the only group specialising in work on LCAT in the UK at that time and that they investigated any patients with LCAT deficiency that became apparent during this period, looking at the family history and genetic origin of the condition (Owen et al., 1996; Wiebusch et al., 1995; and Winder et al., 1999).

This study was the starting point for a series of studies from Professor Owen's laboratory looking at LCAT secretion and methods for producing LCAT outside the body. These studies have significant implications for the potential therapeutic use of LCAT, be that in the case of LCAT deficiency or liver disease or to reduce atherosclerotic lesions. LCAT has still not been used therapeutically for any of these conditions. However, there has been some progress towards this eventuality.

Work is ongoing at the Royal Free Hospital to construct a bioartificial liver of encapsulated HepG2 cells for use while patients with liver disease are awaiting a transplant. Professor Owen is involved in this project, and a recombinant cell that efficiently secretes LCAT will be a key component of this bioartificial liver.

LCAT was known to be a possible therapeutic target for atherosclerosis when this research was conducted, but it is still not used as a treatment. Recent research has been published by a group based in Belgium investigating whether increased apoA-I and LCAT transfer induces cholesterol unloading in complex atherosclerotic lesions in a fat-fed rabbit (Van Craeyveld et al., 2009). They found that both apoA-1 and LCAT together and LCAT alone inhibited the development of atherosclerosis. This is the first preclinical trial of LCAT as a treatment for atherosclerosis. It is difficult to judge the extent to which this research was directly influenced by the work conducted in the grant considered in this case study; however, work following from the grant has had some impact on this study. As stated previously, two papers cite the letter published in the *Lancet* as a direct output of

this study. One of these two papers (Murata et al., 1996) is cited in this recent publication on the impact of LCAT on atherosclerotic lesions. It is therefore likely that the grant was a small part of the ground work towards this later study.

Capacity building and career development

This grant also allowed the PhD student to continue the work started in the first stage of the grant, enabling her to complete her PhD. However, the student subsequently left academia, and Professor Owen no longer has contact details that would enable us to establish what impact this PhD had on her future career path.

The grant also helped maintain links with the laboratory in Recife, Brazil, where Professor Owen had worked during his first postdoctoral position. He had studied LCAT there, and work on LCAT formed a significant part of the research focus of that laboratory. Over subsequent years, a number of PhD students and postdoctoral researchers came from Recife to work with Owen and subsequently set up research laboratories in this area of northeast Brazil, where there is no strong research base. Therefore, by continuing work with LCAT, these links were strengthened and several of the new techniques brought into the RFHSM laboratory through this project were then also transferred to laboratories in Brazil. Work conducted in this grant thus had a small indirect impact on research capacity in a number of biochemistry laboratories in Brazil.

24.8 Interface B – dissemination

As this is basic research, the main target for dissemination of the results was other researchers. This was achieved by attendance at conferences. One published conference abstract is available resulting directly from this work as outlined above in Section 24.5. However, Professor Owen also discussed this work as part of a general discussion of the work of the laboratory at the following conferences:

- International Symposium on Biotechnology and Dyslipoproteinaemias, Milan, Italy, 1989
- 12th European Lipoprotein Club Meeting, Tützing, West Germany, 1989
- Xth International Symposium on Drugs Affecting Lipid Metabolism, Houston, United States, 1989.

Another route of dissemination was through Professor Owen's teaching activities. As a biochemist working in a medical school, which at that time was not a part of a larger university, teaching opportunities were somewhat limited. However, he was involved in some biochemistry teaching at RFHSM and also with the intercalated bachelor of science course. Teaching on these courses encompassed several aspects of lipoprotein metabolism, including LCAT and reverse cholesterol transport.

24.9 Stage 4 – secondary outputs

Overall, despite the possible downstream impacts of this research in terms of a potential treatment for LCAT deficiency and even atherosclerosis, it is clear that this grant did not

have any direct impact on policy or drug or device development. There has also been no clear impact of follow-on research in any of these areas, although this may occur in the future. It is too soon to tell whether LCAT can and will be put to use as a therapeutic tool and, even if this does take place, it will be difficult to tell how significant an impact this grant had on that work, as the research was at such a basic level.

The work did, however, have some impact on the way in which medicine is taught at RFHSM. A molecular medicine course was also introduced at RFHSM in the early 1990s, and Professor Owen was involved in developing and teaching this course, which would also have included discussion of LCAT and molecular biology approaches. This would have built partly on his research, including some of the work conducted using this grant.

24.10 Stage 5 – adoption by practice and the public

The publication 'LCAT mRNA in Liver Disease' (Bingle et al., 1991) suggested, as a result of this research, that LCAT should be used as a test for liver function and an indicator for liver transplant decisions. However, these results have yet to impact upon clinical practice.

As noted above, the group were considered the experts on LCAT deficiency in the UK and investigated any patients with suspected LCAT deficiency during this period (Owen et al., 1996, and Wiebusch et al., 1995). As part of this, they would have been able to confirm clinical diagnosis and, in that form, the increased understanding of LCAT impacted upon the diagnosis and hence treatment of this small group of patients.

24.11 Stage 6 – final outcomes

This work has yet to have a wider impact upon society.

24.12 Payback

The payback framework describes the process from research to several categories of impact. The categories allow us to compare across different case studies. Table 24-2 shows, in point form and by impact category, some of the impacts, described more fully above, that have emerged from this grant.

Table 24-2 Payback

Payback category	Impacts from case study
Knowledge production	• Two publications – a letter and a conference abstract – were produced, which have been cited twice • A new cRNA probe for LCAT was developed, and the use of molecular biology techniques with LCAT and apolipoproteins was established
Research targeting and capacity building	• Molecular biology techniques were introduced to the laboratory – a significant change in research direction • Equipment for molecular biology work was purchased solely from the money provided by this grant • Samira Ghazi completed her PhD • The research conducted here was an early input to a stream of research that may yet result in a therapeutic use for LCAT – either for LCAT deficiency or, more broadly, for atherosclerosis
Informing policy and product development	• None
Health and health sector benefits	• None
Broader social and economic benefits	• None

24.13 References

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CHAPTER 25 Modulation of nitric oxide biosynthesis by polyamines

25.1 Introduction to the research project

25.1.1 Overview

This grant investigated the link between two varieties of signalling molecules: nitric oxide,[1] a molecule that was known to be involved in the regulation of blood pressure, and polyamines, molecules that had a wide range of biological functions, including regulating the cell cycle, but were known to be closely related.[2] Blood pressure is a key factor in heart disease and is influenced by a number of factors, one of which is blood vessel 'tone'. The walls of arteries are composed of smooth muscle cells – the innermost layer being referred to as the endothelium[3] – and contraction of these muscle cells squeezes the arteries, consequently raising blood pressure. The amount by which the muscles cells are contracted is referred to as the 'tone' of the arteries.

In the early 1980s nitric oxide was identified as a key signalling molecule controlling blood vessel tone. In the early 1990s it was still unclear how the level of nitric oxide was regulated, although the pathway by which nitric oxide was produced was reasonably well understood. This grant investigated a possible link between nitric oxide and polyamines – another family of signalling molecules that were known to be produced from the same precursor.

The grant focused on exploring the biochemistry of nitric oxide production. It investigated whether a link could be established between the uptake, or generation, of polyamines by blood vessel cells and the production of nitric oxide. The demonstration of such a link

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[1] Nitric oxide has important cellular signalling functions in a range of systems. Besides its role in relaxation of the blood vessel wall, it is also an important agent in the immune system: macrophages release it to kill invading bacteria. Finally, it is a key neurotransmitter.

[2] Polyamines are a class of compounds found in all living cells; they are essential for cell growth and differentiation (Morgan, 1994).

[3] The endothelium is a thin layer of cells that lines the interior of blood vessel walls and forms an interface between circulating blood and the blood vessel wall. It is of major importance in vascular homeostasis because of its regulated secretion or surface expression of modulators of a wide variety of processes, ranging from coagulation and fibrinolysis to cell permeability (Bogle et al., 1994).

would, it was hoped, lead to further research to understand the therapeutic potential of polyamines in controlling production of nitric oxide. The project was based in the Department of Vascular Biology at King's College London and was conducted between 1993 and 1995.

25.1.2 Understanding the broader research field

A series of discoveries in the late 1970s and early to mid 1980s had revealed the importance of endothelium in controlling blood vessel tone. Endothelium-derived relaxing factor (EDRF) was identified as the key agent of control in 1980 (Furchgott and Zawadzki, 1980), but its precise nature and the way in which it was produced was for some time unclear. The landmark discovery by Salvador Moncada and his group that EDRF was in fact nitric oxide (Palmer, Ferrige and Moncada, 1987) meant that research on this molecule was a 'hot topic' in the cardiovascular field by the late 1980s. A lot of effort was devoted to uncovering the various aspects of the pathway by which it was produced and broken down.

Just as there had been great interest in the therapeutic potential of prostacyclin (like nitric oxide, this is a blood vessel-relaxing agent, but it was far better understood at the time), so researchers were intrigued by the possibilities that nitric oxide might offer. How could its properties be harnessed? Were there effective ways of controlling its production? Could this be used to control blood pressure and therefore limit some of the more damaging effects of cardiovascular diseases and disorders?

It was in this wider context that research on the grant in question was undertaken. It looked specifically at the role that polyamines might have in controlling nitric oxide metabolism within blood vessel endothelial cells. The focus on polyamines was unusual in the context of research being conducted elsewhere at the time, but both nitric oxide and the most common endothelial cell polyamines were known to be derived from the same precursor molecule – L-arginine.

25.1.3 The case study approach

The case study based on this research grant involved a combination of: two face-to-face interviews with senior investigators on the project; a review of the curriculum vitae of the principal investigator (PI); and documentary analysis of key citing papers, publications and conference abstracts arising from it. Unfortunately, the absence of administrative documents for the grant – neither the relevant funding body nor King's College London retain records going back this far – meant that a fuller analysis of the circumstances surrounding the topic identification was not possible.

25.2 Stage 0 – topic/issue identification

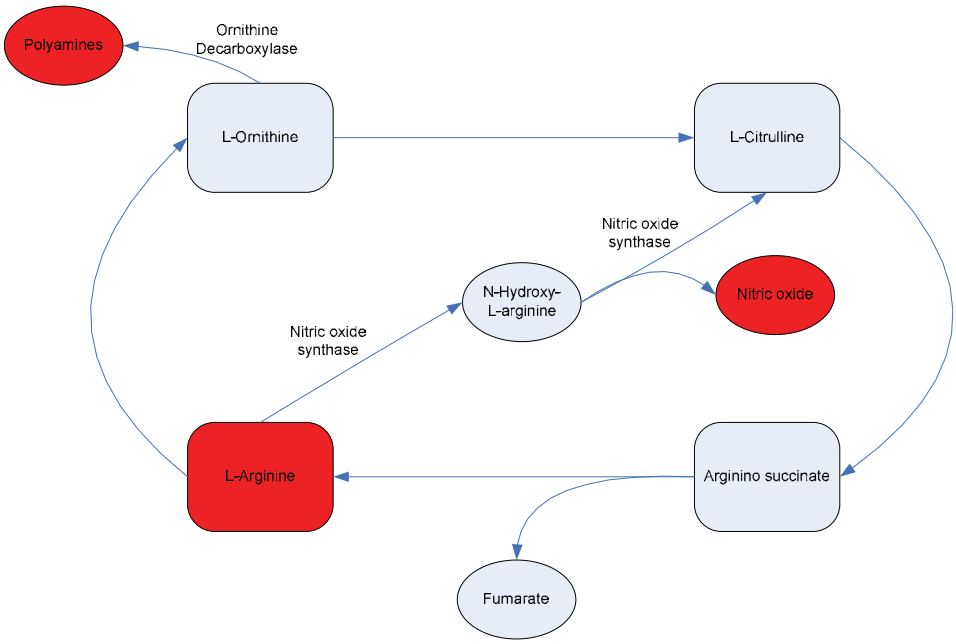
The idea for the project arose from discussions bridging the diverse interests of a number of individuals working in close proximity at the host institution. It was submitted as a proposal in open competition, and, after an initial rejection by the Medical Research Council (MRC), won funding from the British Heart Foundation (BHF). In this section, we examine how the research topic was identified and show that three factors were crucial:

- timing – the state of the wider research field in the early 1990s in light of advances in the wider field of cardiovascular research at this time
- prior research paths of the investigators
- career stage of one of the co-applicants.

25.2.1 Timing

Timing and the state of the research field in the early 1990s were crucial factors in the problem formulation for the grant application. The PI emphasised the importance of the discovery, at the end of the 1980s, that nitric oxide was synthesised from L-arginine and oxygen (Palmer, Ashton and Moncada, 1988). This immediately suggested several potential research avenues investigating various aspects of the pathway of which nitric oxide synthesis was part, which is illustrated in Figure 25-1 below. Many of these hinged on linkages with the synthesis or breakdown of other compounds, some of which the PI had investigated previously. For example, there was the suggestion of a potential link with intracellular polyamine metabolism, in that polyamines are also synthesised – via a series of intermediary steps – from L-arginine, as illustrated in Figure 25-1. In the context of the wider cardiovascular research field, the investigators saw an opportunity, as, to the best of their knowledge, no one else was investigating this potential link at the time.

Figure 25-1 An outline of the biological pathways competing for L-arginine in mammalian cells



25.2.2 Prior research paths of the investigators – a burgeoning stream in related fields

At first glance, links between the research interests of the key investigators in this project are not immediately obvious. The PI, Professor Jeremy Pearson, had established his reputation in endothelial cell biology and small vessel microcirculation at the MRC Clinical Research Centre in Harrow, North London. He had published widely on the properties of the vascular endothelium, including a number of review articles (Pearson,

1991, and Kaul, Blake and Pearson, 1991). The secondary investigator, on the other hand, had begun his career as a laboratory technician in cell pathology and perinatal biology, only later developing a strong research interest in polyamines. This was a field in which he was well recognised nationally and internationally by the mid to late 1980s. He had published quite extensively in his own right on the effects of polyamines on endothelial cells (Morgan, 1987; Morgan, Larvin and Pearson, 1989; and Morgan, 1992) but had not explicitly made a link with nitric oxide metabolism.

After moving to take up a chair in vascular biology at the host institution in September 1991, the PI increasingly focused his research on the pathway by which nitric oxide is generated from L-arginine. This focus stemmed from two major grants won in 1989, while he was still at the MRC Clinical Research Centre. One was a doctor of philosophy (PhD) studentship and the other a BHF award to a postdoctoral fellow working in his laboratory, titled 'Regulation of L-Arginine Transport and EDRF Biosynthesis in Cultured Vascular Endothelial Cells'. A series of publications around this topic with various members of his team followed in the early 1990s. Some of these were concerned with nitric oxide release within cells (Bogle et al., 1991; Bogle et al., *Biochemistry Journal*, 1992, and Bogle et al., *British Journal of Pharmacology*, 1992). Others looked directly at polyamine transport into, and interaction with, endothelial cells without ever specifically making the link between with nitric oxide synthesis (Bogle et al., 1990, and Morgan, Coade, and Pearson, 1990).

By the early 1990s, research work conducted within the group was beginning to strongly suggest the possibility of a link between nitric oxide and polyamines. The two blocks of funding won in 1989 provided important foundations for this. In the key paper published as a result of these grants in the *American Journal of Physiology* in 1994, the investigators noted that extracellular polyamines stimulate the influx of extracellular calcium ions into umbilical vein endothelium cells, and that 'this increase in intracellular free Ca^{2+} concentration, *which may be dependent on polyamine transport*, is sufficient to stimulate the release of potent vasoactive substances such as the endothelium-derived relaxing factor nitric oxide' (Bogle et al., 1994, emphasis added).

25.2.3 Career stage of one of the co-applicants

The desire to preserve an established working relationship was a key factor in the topic identification for this grant. The PI and secondary investigator had developed a close rapport at the MRC Clinical Research Centre at Harrow. The secondary investigator had frequently provided research support to the PI, and they had co-authored publications on a number of occasions (Morgan, Larvin and Pearson, 1989; and Bogle et al., 1990). Keen to bring the secondary investigator's experience and methodological rigour to his new team, the PI saw in the grant application an opportunity to extend this working partnership. The secondary investigator had already been involved in research on a previous grant after arriving at the host institution in early 1992.

This was a particularly important consideration, as the secondary investigator – whose position had been supported by the MRC – was then beyond official retirement age.[4]

[4] At the time the research on this grant was conducted, the retirement age for MRC-supported researchers was 60 years, which contrasts with 65 years for university staff.

Winning the grant would mean that he could continue to work – on an area of potentially great significance – until his full retirement at 65 years. On this basis, the PI and secondary investigator submitted the grant as co-applicants.

25.3 Interface A – project specification and selection

The career stage of the secondary investigator was a key motivating factor underlying the grant application. There is no evidence to suggest significant contributions by external researchers in the project specification. Although both the PI and secondary investigator had important links with other researchers in the United Kingdom (UK) and internationally (Nicolas Seiler and Heather Wallace in the field of polyamine research alone), there is no suggestion that they contributed in a definitive way to the proposal formulation.

Unfortunately, the original grant application has not been preserved, so it is difficult to corroborate what form the original project specification took. An initial application for funding made to the MRC was unsuccessful despite being awarded an 'alpha' for its scientific content. The applicants then reformulated the proposal to make it more specific to cardiovascular disease before submitting it to the BHF. They were duly awarded funding.

It was not common practice at this time for applicants to receive feedback on their applications, and the PI had no recollection that any advice was received from the BHF. Furthermore, no record of how the application was scored by peer reviewers remains; it is therefore difficult to be certain how well it was received by the funding body.

25.4 Stage 1 – inputs to research

In the sections that follow, we outline how each of these elements contributed to the research undertaken on this grant.

25.4.1 Facilitators

The key facilitator for the research was the grant from the BHF. This totalled £74,685, but there was important additional – though informal – financial support from the Japanese pharmaceutical firm that part-funded the PI's chair. Moreover, as the grant map below demonstrates, there was significant overlap between this grant and at least two others held by the PI at around the same time, which may have contributed in important ways to the research carried out at this time.

In reputational terms, both the investigators brought significant capital to the project. The PI was a recently elected professor, while the secondary investigator had an international reputation in the close-knit polyamine research community.

25.4.2 Knowledge and expertise

The PI and secondary investigator provided the key knowledge and expertise inputs to the research. Indeed, the PI emphasised the central importance of the secondary investigator in this context, in view of his expertise in polyamine research – exemplified by the publication

of an edited book on polyamine protocols in 1998 (Morgan, 1998). The two senior investigators were supported by a research staff of 10–12, including four or five postdoctoral fellows, a technician and three or four PhD students. The postdoctoral fellows included one who was supported by the BHF, one who received support from the Wellcome Trust and another recruited specifically by the PI for ongoing research in the laboratory. Their direct contribution to the research was limited, however, with the exception of one who appeared as a co-author on a number of the outputs from the grant. Finally, there was a regular flow of undergraduate students through the laboratory on three-month research placements. Supervised by the secondary investigator, they provided important support during the grant.

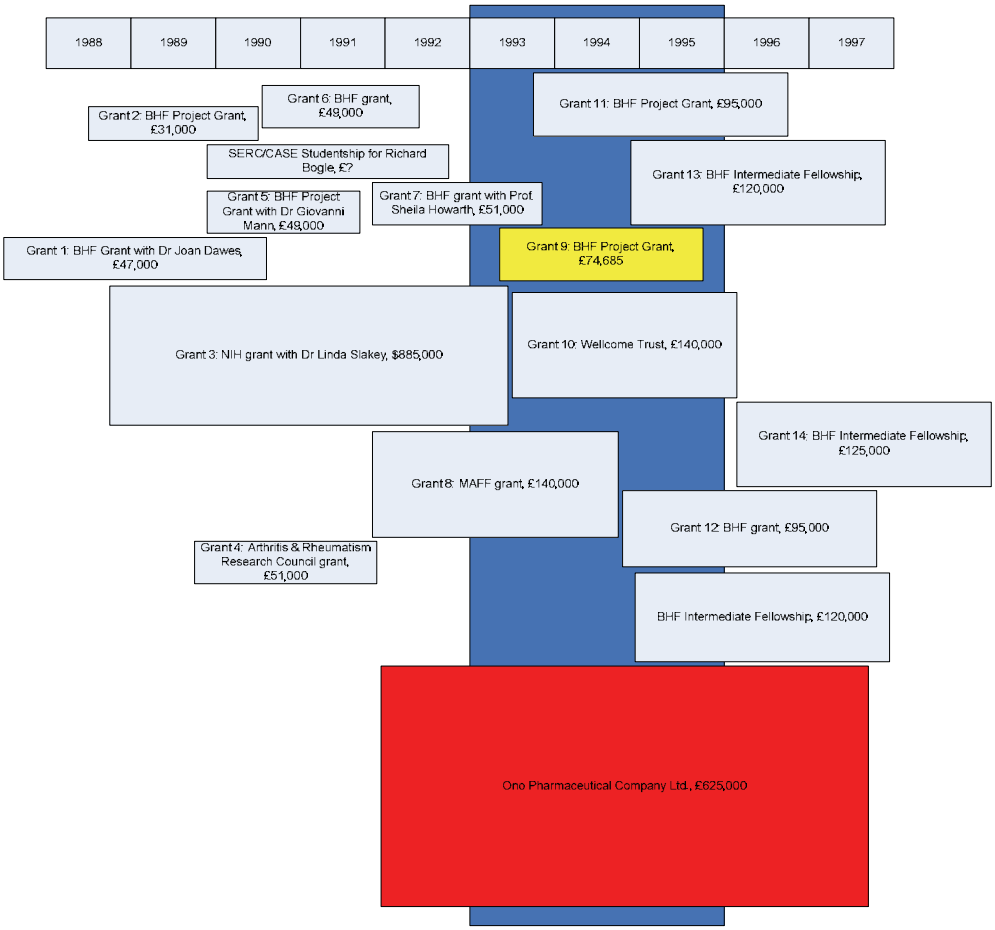
25.4.3 Techniques, consumables and space

No new techniques were developed, as the grant built on endothelial cell culture techniques used in the PI's earlier BHF grants. The secondary investigator asserted that some minor adjustments were made to the experimental protocol to improve the accuracy of measurements derived from endothelial cells in culture, noting that there are significant problems with ensuring that results derived from such fragile experimental media are not simply artefacts produced by the preparation method.

Reagents and samples to support the research were costly, and much of the grant was spent on consumables.

A final potential constraint – space – proved less of an issue for the research team than it had previously, as the PI's laboratory was refurbished by the host institution shortly before research on the grant commenced.

Figure 25-2 Funding map for the grant; this shows major inputs to the PI's laboratory over the period from 1988 to 1997



25.5 Stage 2 – research process

Although funding for this grant was secured from 1993 to 1995, our research suggests that a substantial amount of the work was in fact conducted before money was received. For instance, although one PhD student in the PI's laboratory had actually left by the time funding was awarded, research that he conducted with the PI and the secondary investigator provided a good deal of the groundwork and some of the preliminary data for the grant in question. The research on the grant consisted of extensive data collection from human umbilical endothelium cells in culture, looking at polyamine uptake and conversion, and the production of intracellular nitric oxide.

In the PI's view, the crucial aspect of the research process was the opportunity the grant afforded for the secondary investigator to support and train other researchers in the laboratory. The secondary investigator's background as a laboratory technician and manager meant that he brought both meticulousness and considerable expertise to the research process at a time when fewer and fewer people followed his particular career path.

His involvement provided an unusual opportunity for junior researchers to learn new methods.

25.6 Stage 3 – primary outputs from research

25.6.1 Knowledge production

The investigators sought specifically to test whether a link exists between polyamine transport and synthesis and nitric oxide metabolism in vascular endothelial cells. They found no clear evidence for such a link, although some of the data they produced were at least suggestive. Moreover, the papers that were claimed for this grant at interview with the PI are notable in that a number were submitted before the grant actually started. Bogle et al. (1994), which was claimed as the key paper from this grant, was actually originally submitted in late 1992, although it was not finally accepted until the end of 1993, and seems likely to have brought together research findings from previous work. Morgan and Baydoun (1994) and Morgan (1994), which were also cited by the PI as key outputs, were published in 1994 and seemed to summarise research that was undertaken before the grant commenced or represented the first stages of the research. Morgan (1994) is further complicated by the fact that it is a review article, summarising the author's knowledge of polyamine research built up over the length of his career.

This observation has a number of implications with respect to attribution. Firstly, it implies that the investigators may attribute papers that do not, at first glance, seem directly related to the grant. Secondly, it suggests that grant funding may be used flexibly by research laboratories to support both the work for which it was originally intended and new speculative avenues of research. Indeed, PIs might apply for specific funding support for these avenues if they seem promising. From this perspective, the delivery of money by a research funding organisation need not necessarily correspond exactly to the timeframe during which that funding is actually used.

We identified two peer-reviewed publications arising specifically from this grant (Morgan and Baydoun, 1994, and Baydoun and Morgan, *British Journal of Pharmacology*, 1998). These are shown in Figure 25-4 alongside other key events relating to the grant. Morgan and Baydoun (1994) was a statement of early findings emerging from the grant and a one-page contribution to *Biochemical Society Transactions*. It outlined a series of experiments demonstrating that polyamine transport into two endothelial cell types depends on the intracellular concentration of L-arginine and that this compound is the main precursor for polyamine synthesis within cells.

On the other hand, Baydoun and Morgan (*British Journal of Pharmacology*, 1996) reviewed possible links between changes in the activity of ornithine decarboxylase (the enzyme that generates polyamines) and that of nitric oxide synthase (which generates nitric oxide). It suggested that the expression of nitric oxide synthase can indeed be modulated by endogenous polyamines, apparently confirming the original hypothesis of the grant.

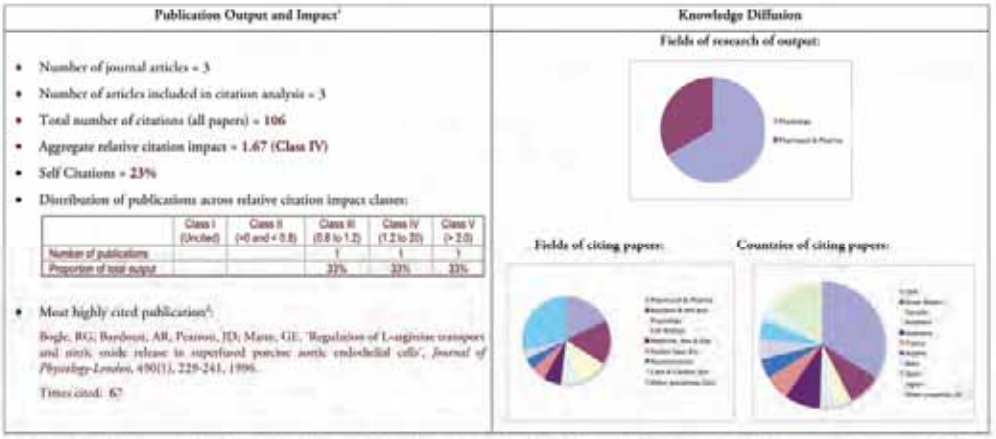
The secondary investigator highlighted the importance of early-stage knowledge production not captured by publications as a key knowledge production output from this grant. We address this in greater depth in Section 25.7, but it is arguable that the most

important outputs from the grant were a series of abstracts, conference and seminar presentations bringing the raw data – a considerable amount of which was produced by the research – to a wider audience. This included at least six abstracts published in a range of specialist journals, of which the most important in terms of its scope in summarising the research findings appeared in the *Journal of Vascular Research* (Morgan and Baydoun, 1996). This abstract outlined findings that suggested that changes in the pool of free polyamines in a particular cell type (macrophages) might affect the activity of the enzyme responsible for generating nitric oxide from L-arginine.

The secondary investigator and a co-researcher in the laboratory also pulled together some of the key findings for a short chapter in a book titled *The Biology of Nitric Oxide*, which was published in 1998 (Baydoun and Morgan, *The Biology of Nitric Oxide*, 1998). Again, however, there are problems of attribution here, because the overlap between grants during this period means that it is difficult to be clear which provided the most significant input to the research that these publications summarise.

Figure 25-3 shows the results of bibliometric analysis for the case study grant.

Figure 25-3 Publication output and impact of directly related publications



[1] In addition,1 publication was indirectly linked to this grant. This publication was indexed in WoS, recceved 23 citations in total, for a relative citation impact of 0.72. It was in relative citation impact class II and had a self citation rate of 4%.
[2] Citation count extracted April 2009

25.6.2 Benefits to future research and research use

Capacity building

A key benefit of the grant was to extend the research career of the secondary investigator, who would otherwise have had to retire. We have highlighted the importance of the secondary investigator's contribution to tacit knowledge transfer and training within the research group through the grant and the value the PI attached to it in terms management of the laboratory on a day-to-day basis and the space that it afforded him to produce papers and engage in other projects. Unfortunately, it is extremely difficult to capture outputs arising from this, as no PhD students graduated during the period of the grant, and it is extremely difficult to 'measure' tacit knowledge transfer. One leading researcher, supported by a Science and Engineering Research Council (SERC)/Collaborative Awards in Science and Engineering (CASE) studentship in the early 1990s, left the laboratory in early 1993 to pursue clinical training and had only limited input into the research on this

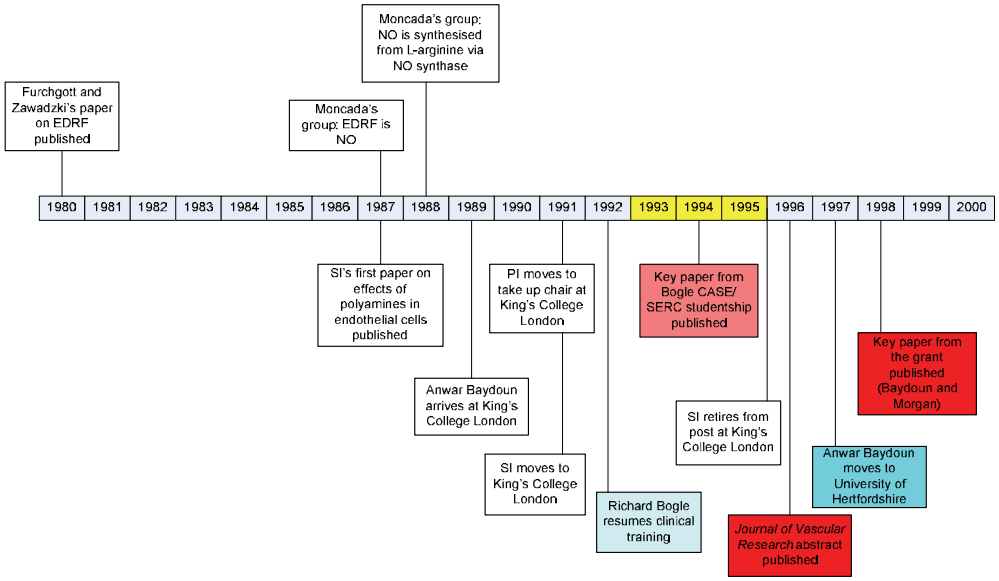
grant (although it is important to bear in mind that at least some of the research relating to this grant may have been undertaken before it started).

From the perspectives of other researchers involved in this research, the impact of the grant was low. The PI did not believe that it had contributed significantly to his own career advancement, which, overall, has been substantial: he is currently an associate medical director of the BHF and was, for a time, Director of the Centre for Cardiovascular Biology and Medicine at King's College London. Although his research interest in endothelial cell biology remained strong after this grant ended, he moved away from direct research on polyamines. Later grants that he received from the BHF reflected this, focusing more closely on arginine transport and various forms of signal transduction in endothelial cells. The postdoctoral fellow most directly involved in the research subsequently moved to take up a position at the University of Hertfordshire, where he is now a professor, but it is unclear how far this particular grant contributed to establishing his research career.

Future research

The impact of this grant on future research is unclear. Following the retirement of the secondary investigator, the link between polyamine transport, intracellular calcium ion concentration and nitric oxide synthesis was not pursued further by researchers in the laboratory. Moreover, although the topic of nitric oxide biosynthesis and competition for L-arginine utilisation remains popular, the main focus of this research is not directly within cardiovascular research (Boucher, Moali and Tenu, 1999). Finally, the contribution of the outputs from this particular grant to the sub-field of polyamine research seems to have been limited, although the secondary investigator continued to publish in this area well after his retirement.

Figure 25-4 Timeline illustrating key events relating to the grant. Key developments in the wider research field are shown above the line; grant-specific events below the line. Clear knowledge outputs from the grant are shown in red; clear training outputs in turquoise. Where the impact of the grant on particular outputs is debated, a lighter shade has been used. The years coloured yellow correspond to those in for which money on the grant was supplied



The contribution of this grant to developing new research processes was limited, as the primary focus was to investigate a possible link between polyamine transport and synthesis and nitric oxide metabolism. The research largely built on protocols and methods developed previously by the secondary investigator and PI. However, as the grant progressed, the research team perfected a method for running a large number of assays simultaneously on 96-well microtitre plates, generating large volumes of data in the process. This was an important development, as the fragility of human umbilical endothelial cells in culture had long been a problem.

25.7 Interface B – dissemination

The secondary investigator was, for much of the period in question, head of a European Union (EU) COST action on polyamine research.[5] COST Actions are EU-supported funding schemes designed to help build the capacity of subject area networks of researchers across member states. In this position, the secondary investigator presented a large amount of data from research conducted in the PI's laboratory, some of which was brought together in the COST Action handbook published at the end of his term.

25.8 Stage 4 – secondary outputs

The results of this grant do not seem to have informed policy development, drug development, device development or decisions to take drugs taken off the market.

25.9 Stage 5 – adoption by practice and the public

The results of the research, and any policies and drug development informed by them, do not seem to have been taken up by the health service, doctors, public health officials, etc.

25.10 Stage 6 – final outcomes

The findings of this grant do not seem to have had any demonstrable effect on society more broadly – eg through improved health in the population, spin-off companies employing people, sale of products by the pharmaceutical industry, etc.

[5] The EU COST initiative is one of the longest-running trans-European instruments for fostering cooperation among scientists and researchers working across the continent. It currently has 34 member countries and encourages cooperative activity in a number of areas through targeted funding. In particular, it invites proposals for the establishment of COST Actions, which normally last for four years and cover everything from basic to pre-competitive research, as well as that identified to be in the public interest

25.11 Summary of case study impacts

The payback framework describes the process from research to several categories of impact. The categories allow us to compare across different case studies. Table 25-1 shows, in point form and by impact category, some of the impacts, described more fully above, that have emerged from this grant.

Table 25-1 Payback

Payback category	Impacts from case study
Knowledge production	• Two full peer-reviewed academic papers • Six abstracts • One published book chapter
Research targeting and capacity building	• Tacit knowledge transfer from the secondary investigator to a postdoctoral fellow in the laboratory (although the major contribution arguably derived from a grant that was awarded shortly afterwards) • Tacit knowledge transfer from the secondary investigator to PhD students in the laboratory • Refinement of cell-culture methods using 96-well microtitre plates to produce large datasets in short periods of time
Informing policy and product development	• None
Health and health sector benefits	• None
Broader social and economic benefits	• None

25.12 References

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CHAPTER 26 Antiarrhythmic drug receptor

26.1 Overview of case study grant

The grant of interest to this case study, titled 'Antiarrhythmic Drug Receptor' was funded by the Medical Research Council of Canada (MRC) for three years for a total value of Can$178,824. This grant was a renewal of a previous grant and funding started in July 1991 and ran to July 1994. The research was led by Dr Robert Sheldon and conducted at the University of Calgary. The focus of the grant was a continuation of work to define the properties of a drug receptor for Class 1 antiarrhythmic drugs. The specific objective was to understand how Class 1 drugs bind to and block cardiac sodium channels. At the time the detailed mode of action for this class of drugs was unknown.

Around the time of this grant, there were a number of randomised clinical trials ongoing to study the effectiveness of antiarrhythmic drugs for the suppression of asymptomatic ventricular arrhythmias and the prevention of sudden cardiac death. One of the most notable and highest profile was the Cardiac Arrhythmia Suppression Trial (CAST), which began in 1986. In 1989, results from the CAST I trial suggested that two of the three compounds being studied (encainide and flecainide) increased the risk of sudden cardiac death by nearly fourfold. Informed by the data, the safety board recommended that the trial should continue to assess the effectiveness of the third drug (moricizine), as no significant differences in terms of mortality were observed compared with placebo. In 1992 the results of CAST II were published, showing again an increased risk of death. The CAST results were extrapolated to other antiarrhythmic drugs, which led to changes in regulatory guidelines and a dramatic decrease in drug development by the pharmaceutical industry (Pratt and Moyé, 1995). Dr Sheldon indicated that, after the 1992 results emerged, the research they were doing changed focus as there seemed to be little clinical relevance to the proposed research.

26.2 Introduction to case study

This grant investigated how Class I antiarrhythmic drugs[1] bind to and block the cardiac sodium channel[2]. Normal electrical conduction in the heart allows the impulses generated by the sinoatrial node (SA node) of the heart to move to and stimulate the myocardium (cardiac muscle). The myocardium contracts after stimulation. Ordered stimulation of the myocardium allows efficient contraction of the heart, thereby allowing blood to be pumped throughout the body. Cardiac arrhythmia is a term encompassing a large and varied group of conditions in which there is abnormal electrical activity in the heart. The heart beat may be too fast or too slow and may be regular or irregular.

There are four classes of antiarrhythmic drugs according to the Vaughan Williams classification system (Vaughan, 1970). Each Class has a different mechanism or target of action:

- Class I agents interfere with the sodium (Na^+) channel.
- Class II agents are anti-sympathetic nervous system agents. Most agents in this class are beta blockers.
- Class III agents affect potassium (K^+) efflux.
- Class IV agents block calcium channels and therefore the SA and AV nodes.

There are several different agents in each of the four classes. Class I agents are called membrane-stabilising agents. They are named as such because they decrease the excitogenicity of the plasma membrane. Class I agents are further subdivided into three groups: Ia, Ib and Ic. This subgrouping is based on the agent's effect on the sodium channel and its effect on the cardiac action potential. Class Ia agents lengthens the action potential, Class Ib agents shorten it and Class Ic agents do not significantly affect the length of the action potential.

In 1989, it was known that Class I antiarrythmic drugs blocked the cardiac sodium channel, which reduced the initial depolarisation of the action potential and slowed impulse propagation (Sheldon, 1989). Despite use of these drugs at the time, the mechanism of action involved in blocking the cardiac sodium channel was not completely understood. The antiarrythmic effect of Class I drugs was believed to result from their interaction with a receptor[3] associated with the cardiac sodium channel (Sheldon, Hill and Duff, 1989).

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[1] Antiarrhythmic agents are a group of pharmaceuticals that are used to suppress fast rhythms of the heart (cardiac arrhythmias), such as atrial fibrillation, atrial flutter, ventricular tachycardia and ventricular fibrillation (Vaughan, 1970). Class I agents interfere with the sodium (Na+) channel.

[2] Sodium channels are integral membrane proteins that form ion channels, conducting sodium ions through a cell's plasma membrane (Kandel et al., 2000, and Hillel, 2001). They are classified according to the trigger that opens the channel for such ions, ie either a voltage change (voltage-gated sodium channels) or binding of a substance (a ligand) to the channel (ligand-gated sodium channels).

[3] A receptor is a specifically shaped pocket in a protein that accepts other molecules, much like a lock and key.

A majority of cardiac arrests result from rapid or chaotic activity of the heart, others are due to extreme slowing of the heart. These events are called life-threatening arrhythmias and are responsible for sudden death (Pratt and Moyé, 1995).

The late 1980s and early 1990s, when this proposal was written, was an era of discovery and development of new antiarrhythmic drugs. Including this research study, much work was underway to understand the mechanism of action so the use of these drugs could be optimised and new, more specific drugs developed.

The commercial interest in Class I antiarrhythmic drugs all but disappeared from most laboratories after the results of CAST. As discussed above, CAST I was published in 1989 while CAST II was published in 1992. Both trials showed that Class I antiarrhythmic drugs, given after acute myocardial infarction to prevent sudden cardiac death due to asymptomatic ventricular arrhythmias, effectively suppressed asymptomatic ventricle arrhythmias but increased arrhythmic death almost fourfold (CAST Investigators, 1989 and 1992).

26.2.1 The case study approach

The findings presented in this case study are based on a combination of:

1. three face-to-face interviews, including Dr Sheldon (the principal investigator (PI)), Dr Henry Duff (a collaborator) and Dr Stanley Nattel. who did not participate in the case study grant but was suggested to provide an 'honest appraisal' of the research in question
2. documentary analysis of available administrative documents for this grant – only the notification of award and the peer review comments were available as provided by the PI
3. a review of the PI's curriculum vitae
4. documentary analysis of the scientific literature and bibliometric analysis.

Unfortunately, administrative documentation was limited as MRC records dating this far back were not kept. Fuller analysis of the circumstances surrounding the topic identification was therefore not possible.

26.3 Stage 0 – topic/issue identification

The idea for the research project arose from Dr Sheldon's curiosity and sense that the currently held beliefs did not 'make any molecular sense', his previous experience in the area and the research environment he was in. This grant was a renewal of an existing grant; the renewal application was submitted to and funded by the MRC through the open operating grants competition. In this section, we further examine how the research topic was identified based on the three critical factors:

1. the prior training and research paths of the investigators
2. scientific curiosity
3. the climate and environment.

26.3.1 Prior training and research path of the investigator

Dr. Sheldon obtained a PhD. in Molecular Biology from University of Colorado in 1973, completed a postdoctoral fellowship with Dr Sydney Brenner at the Laboratory for Molecular Biology in Cambridge, UK, and then received his M.D from University of Toronto in 1981. He received the majority of his postgraduate research training at The University of Calgary. As a medical student he spent many years undertaking several projects with Dr Phil Seaman, a noted molecular pharmacologist interested in finding a specific receptor for antipsychotic and antidepressant drugs.

This research grant was a renewal, building on Dr. Sheldon's previous research that the MRC had supported since 1988. The research team became aware of Bill Catterall's binding studies in neuroblastoma cells and began applying Catterall's techniques (Catterall, 1977) to cardiology for the first time. Within a year the team had a well-working model.

Dr Sheldon was able to make advances to the research area with his strong molecular background and his clinical training. He felt that his cardiac electrophysiology research was going well and made a career choice to continue in this area.

26.3.2 Curiosity and 'molecular sense'

The ideas outlined in this grant proposal came from the inquisitive nature of Dr Sheldon, who was unwilling to simply accept what the community commonly believed to be the model of action. At that time, it was believed that Class I antiarrythmic drugs were like local anaesthetics. The accepted model suggested that the drugs slotted into the membrane in a non-specific manner that disabled the sodium channel function. Those working in micro-electrophysiological models were working towards the notion of a specific receptor site with specific ligand binding, but few outside that field considered this option. Models existing at the time could not specifically describe how drugs, once bound to the channel, caused blockade of the sodium influx (Sheldon, Cannon and Duff, 1986; 1987). Because existing models could not clearly or accurately describe function, Sheldon was not content to simply accept them.

The field was lacking good quantitative methods to study the interaction of antiarrythmic drugs with their potential receptors or sites of action (Nattel interview, 2008).

In their previously funded 1988 MRC research grant, the research team had identified a predicted receptor in a rat model. This predicted receptor demonstrated saturable, reversible, stereospecific and pharmacologically relevant binding of Class I antiarrhythmic drugs *in vivo* and *in vitro*. This provided rationale for the notion of a specific binding receptor for Class I drugs.

Dr Sheldon's research proposal makes use of the team's recently developed radioligand binding assay. It was submitted for renewal funding in the open competition with the MRC. The proposal built on previous research that showed that a particular class of drugs could bind to a specific receptor.

26.3.3 The climate and environment

There were three main climate and environmental factors that contributed to Dr Sheldon's research focus. These were specific to:

1. the field of cardiology – in the early 1980s when Dr Sheldon was beginning his independent research career, after having completed a PhD in molecular biology and obtaining an MD with specialisations in internal medicine and then cardiology, cardiology was just starting to become 'evidence based'. He described cardiology as the 'first evidence-based specialty'. This shift in the field of cardiology gave a greater recognition for research and its value than had previously existed
2. the institution – the project was based in the Division of Cardiology of the University of Calgary Health Sciences Centre, and was conducted between July 1990 and 30 June 1993. The University of Calgary was one of the first cardiac electrophysiology units in Canada. Dr George Wyse, who became the Chief of Cardiology from 1986 to 1993 and Associate Dean (Clinical Affairs) from 1993 to 1999, began recruiting heavily in 1979. Dr Wyse had an interest in antiarrhythmic drugs. The combined academic and clinical arrhythmia unit led by Dr Wyse made the University of Calgary a good place for Sheldon to undertake his research
3. The team – Dr Sheldon's postgraduate research training at the University of Calgary was primarily under the supervision of Dr Henry Duff. The two had overlapping and synergistic research interests and complementary backgrounds and skills. The opportunity to collaborate on this project along with the two proceeding factors provided a strong and supportive research environment. Dr Duff was a crucial friend, mentor, supervisor, and co-investigator.

26.4 Interface A – project specification and selection

This grant focused on understanding the binding mechanism of Class I antiarrhythmic drugs to receptors on the cardiac sodium channel. The researcher believed that with a better understanding of the binding mechanism, one could design better, more specific drugs.

This grant intended to study three hypotheses:

1. whether all Class I antiarrhythmic drugs share a common allosteric[4] mechanism by which they would block the cardiac sodium channel by stabilising a non-activated state of this channel
2. that the affinity of these drugs at the receptor's sites depends on the conformational states of the receptors (resting, activated or inactivated forms)
3. that the cardiac sodium channel is modulated by lipophilic molecules and the muscarinic receptors.

[4] An allosteric mechanism refers to a mechanism that uses a site other than the protein's active site. The active site of an enzyme contains the catalytic and binding sites. The structure and chemical properties of the active site allow the recognition and binding of the substrate.

The original grant application was not preserved so the specific background and rationale have been recreated through interviews and review of the published literature. Dr Sheldon did not have a complete copy of his grant application but did retain and provide to us a copy of the notification of award letter, complete with peer review comments.

The application received a strong score and therefore strong support as it built on previous funding and was the continuation of an established research programme investigating modes of action of Class I antiarrhythmic drugs. The methods were perceived to be adequate as they were already being successfully used in Sheldon's laboratory and had been published in peer-reviewed journals (e.g., Sheldon, Cannon and Duff, 1986; 1987).

It was felt that the application clearly described anticipated results as well as potential problems. Reviewers also believed that the ongoing collaborations between Dr Sheldon and Dr Duff were advantageous for Sheldon's career development. There were few concerns and/or questions raised by reviewers. There was optimism that collaboration with Duff would lead to evaluation of any promising agents in a dog model of acute myocardial infarction.

Dr Sheldon conducted this research within Dr Duff's laboratory for three main reasons. Firstly, Dr Sheldon was working at another hospital across town, providing all the arrhythmic clinical consults to all the other city hospitals. This collaboration required that he have someone to watch over his basic laboratory while he performed his clinical duties. Secondly, the two researchers got along well and had similar and overlapping interests. In some cases, they were investigating the same things, providing support to each other. Finally, they were able to share and exchange materials and equipment.

The team in the University of Calgary's cardiac electrophysiology unit was a very strong group of basic and clinical scientists, who were all arrhythmia cardiologists. As with any team, there were some interpersonal stresses, but on the whole the team functioned very well together. Sheldon summed this up by stating: 'no matter what methodological or content question was posed there was always someone (local) to ask' (Sheldon interview, 2008).

Sheldon and his team refined and perfected a binding assay developed by Bill Catterall for use in neural tissue (Catterall, 1977) and applied it to the heart. The technique had never been used in the heart before, because it had to be carried out with living cells. Harvesting these living cells was a very fastidious process and required hands-on training. Although the technique is published, one benefits greatly from learning the mechanics firsthand to understand all the nuances.

The research agenda was progressing well and work had clearly established the existence of a specific receptor. The emergence of CAST findings that Class I antiarrhythmic drugs increased the risk of sudden death made the research focus of this grant less 'cutting edge', and less clinically important. CAST had a sobering effect on cardiovascular research and has been referred to as the most important randomised study in cardiology because it taught scientists and clinicians to challenge commonly held beliefs and to use robust methods to investigate clinical effectiveness. Prior to its release there was a general consensus that Class I antiarrhythmic drugs saved lives. This trial based on three Class Ia drugs shed doubt on the entire class of agents. After publication of CAST II, the use of

Class I drugs dropped drastically; they were still used in some conditions but use became much more selective and much more restricted, and interest in the research area faded (Pratt, 1995).

The CAST study had a relatively small impact on the research proposed concerning the identification and description of the sodium channel receptor, as it still had important implications for improving the understanding of the mechanism of action of Class I antiarrhythmic drugs. The research team did show that all the drugs have the same characteristic, specifically that they all bound very strongly to one state. They were then able to determine how tightly the pocket of the receptor binds the drugs and they determined the contribution to the binding energy from the hydrogen atoms. They also explored the effects of temperature and toxins. After that progress, Sheldon reported that 'locally this research vanished' (Sheldon interview, 2008). He described it as elegant research but recognised that given the limited clinical relevance and pharmaceutical interest it was necessary to moved on to what was for him more clinically relevant research. Sheldon credits the funding with allowing him the freedom to re-shape his research interests to an area where there would be more clinical impact.

26.5 Stage 1 – inputs to research

26.5.1 Funding

This three-year MRC grant was a renewal of an earlier grant (1988–1989). The value of the grant was Can$178,824. Funding started in July 1991 and ran to July 1994. An annual breakdown is shown in Table 26-1.

Table 26-1 Funding

Year	Amount (Can$)
July 1990–June 1991	44,706
July 1991–June 1992	59,608
July 1992 – June 1993	59,608
July 1993 – June 1994	14,902
Total funding requested	**178,824**

Dr Sheldon indicated that he has always had adequate funding. It was his view that health research funding was generally available at the time and that the availability of funding was not limited to cardiovascular research. With sufficient grant money, Sheldon had no difficulty hiring a full-time technician. Complemented by a generous start-up grant of Can$150,000 from the Alberta Heritage Foundation for Medical Research[5] (AHFMR) Sheldon was able to purchase the required equipment and supplies.

An illustration of other funds held by the PI can be viewed in Figure 26-1. This chart was created using relevant information found within the grant application and a search of Canadian Institutes of Health Research (CIHR) databases.

[5] Established by the Government of Alberta in 1980, the Alberta Heritage Foundation for Medical Research (AHFMR) (now Alberta Innovates–Health Solutions) supports biomedical and health research at Alberta universities, affiliated institutions and other medical and technology-related institutions.

Figure 26-1 All funds held by the PI from 1988 to 1997

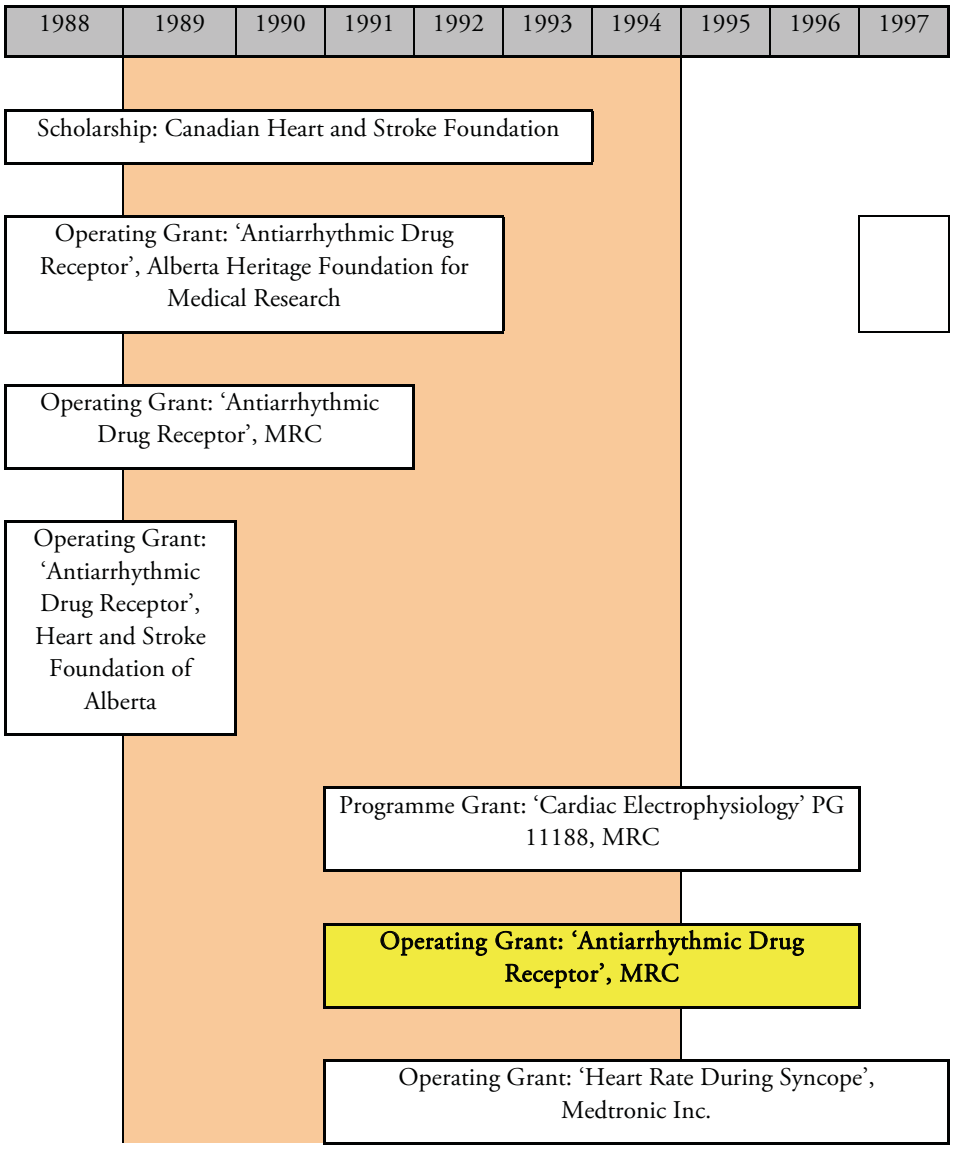


Figure 26-1 helps to illustrate the relative size of the grant in comparison to the overall research programme ongoing within the PI's laboratory over the course of the case study grant, including a two-year period prior to and subsequent to this funding.

26.5.2 Research team

The broader programme grant team in the division of cardiology at the University of Calgary was relatively large, bringing together different levels of experience and expertise and conducting research that spanned basic biomedical and clinical research. This extended team included Dr Sheldon, who had completed a PhD in molecular biology and was a fellow at the time, along with Henry Duff, George Wyse, Anne Gillis, and Brent Mitchell from the Department of Cardiac Sciences, and Bob French and Wayne Giles from the Department of Physiology and Medicine. Dr Sheldon had previously established the research methods he would use for the antiarrythmic drug receptor grant. One peer reviewer wrote, 'The progress clearly indicates that ^{3}H-BTXB [[^{3}H]-batrachotoxinin benzoate] is indeed a very useful probe to study the cardiac sodium channel'. Sheldon's team on this grant included himself and Dr Duff, along with three postdoctoral fellows – Dr Mohammed Taouis, Dr Roger Hill, and Dr Leslie Hill.

26.5.3 Collaborations

Dr Sheldon described the collaborative nature of his department as critical. He worked in an area where there were clinicians and basic scientists, as well as some like Sheldon who were both. He stressed that no matter what content or methodological question he might have had, there was always someone to ask (Sheldon interview, 2008).

26.5.4 Facilities

The research was conducted at the University of Calgary, which was one of the first cardiac electrophysiology units in Canada. At the University of Calgary, there was a strong team of experienced cardiovascular researchers.

The laboratory facilities were excellent and the group was able to maximise their use of very clinical and basic science laboratories. Dr Sheldon, who had moved to Alberta through Toronto and Cambridge, remarked that he could not believe how accessible and available equipment was. This was due primarily to the newly established Alberta Heritage Foundation for Medical Research, which, until 2009 continued to provide significant funds for infrastructure and major equipment in the province. At the time of the grant and until very recently there were little or no resource issues.[6]

26.5.5 Research environment

As described in the previous sections, the research environment at the University of Calgary cardiac electrophysiology unit was vibrant. It included a strong group of researchers who were engaged in collaborative research and had all the necessary equipment. Dr Sheldon described the environment in the following way; 'basic science labs are a strange place of piracy and communism and capitalism. Equipment never lies unused' (Sheldon interview, 2008).

26.5.6 Other facilitators/barriers

Dr Sheldon mentioned that another thing that had helped him was having previously worked with Dr Phil Seaman. He worked with Seaman after his first year of medical

[6] Sheldon commented in a follow-up communication that a new organisation named Alberta Innovates – Health Solutions, has taken the place of the Alberta Heritage Foundation for Medical Research.

school and credits this work experience as one that enabled him to get his grant and start to develop his own research. He believes that working with very credible and reputable scientists early in his career has afforded him lasting benefits.

26.6 Stage 3 – primary outputs from research

The primary output of this research was the refinement and perfection of the Catterall binding technique in the heart. Catterall and his team measured the sodium influx to mouse neuroblastoma cells after scorpion toxin binding. They showed that the toxin enhanced the activation of the action potential in these cells. The results they obtained indicated that the effect on membrane potential due to scorpion toxin binding was due to a membrane potential-dependent conformational change in the binding site (Catterall, 1977). Part of the experimental technique involved microelectrode penetration of the cells. Sheldon explained that Catterall had used the technique in neural tissue but that it was a very delicate and finicky process in part because it had to be performed on living cells. Sheldon and his laboratory perfected the technique and published their methods. They also expended considerable time in training others. Sheldon said they taught anyone who wanted to learn. He stressed the benefit of hands on teaching over simply reading the methods as described in their paper. The nuances and mechanics are difficult to accurately depict in a publication and equally challenging to glean from reading the publication. Sheldon reported that this technique is now used routinely in mouse hearts.

26.6.1 Knowledge production

Dr Sheldon identified 12 publications and 15 abstracts produced from 1990 to 1995 that were 'directly' related to the grant. The following references are for those publications that were identified as directly related to the grant. Of them, we will outline the findings of the following papers in efforts to show the range of findings related to the case study grant as attributed by the PI.

1. Taouis, M., R.S. Sheldon, R.J. Hill and H.J. Duff, 'Cyclic AMP-dependent Regulation of the Number of [3H]batrachotoxinin Benzoate Binding Sites on Rat Cardiac Myocytes', *Journal of Biological Chemistry*, Vol. 266, No. 16, 1991, pp. 10300–10304.
2. Sheldon, R.S., R.J. Hill, M. Taouis and L.M. Wilson, 'Aminoalkyl Structural Requirements for Interaction of Lidocaine with the Class I Antiarrhythmic Drug Receptor on Rat Cardiac Myocytes', *Molecular Pharmacol*ogy, Vol. 39, No. 5, 1991, pp. 609–614.
3. Sheldon, R.S., H.J. Duff and R.J. Hill, 'Class I Anti-arrhythmic Drugs: Structure and Function at the Cardiac Sodium Channel', *Clinical and investigative medicine,* Vol. 14, No. 5, 1991, pp. 458–465.
4. Hill, R.J., E. Thakore, M. Taouis, H.J. Duff and R.S. Sheldon, 'Transcainide: Biochemical Evidence for State-dependent Interaction with the Class I Antiarrhythmic Drug Receptor', *European Journal of Pharmacology*, Vol. 203, No. 1, 1991, pp. 51–58.

5. Taouis, M., R.S. Sheldon and H.J. Duff, 'Upregulation of the Rat Cardiac Sodium Channel by In Vivo Treatment with a Class I Antiarrhythmic Drug', *Journal of Clinical Investigation*, Vol. 88, No. 2, 1991, pp. 375–378.
6. Ranger, S., R.S. Sheldon, B. Fermini and S. Nattel, 'Modulation of Flecainide's Cardiac Sodium Channel Blocking Actions by Extracellular Sodium: a Possible Cellular Mechanism for the Action of Sodium Salts in Flecainide Cardiotoxicity', *Journal of Pharmacology and Experimental Therapeutics*, Vol. 264, No. 3, 1993, pp. 1160–1167.
7. Sheldon, R.S., H.J. Duff, E. Thakore and R.J. Hill, 'Class I Antiarrhythmic Drugs: Allosteric Inhibitors of [3H] batrachotoxinin Binding to Rat Cardiac Sodium Channels', *Journal of Pharmacology and Experimental Therapeutics*, Vol. 268, No. 1, 1994, pp. 187–194.
8. Sheldon, R.S., E. Thakore, L. Wilson and H. Duff, 'Interaction of Drug Metabolites with the Class I Antiarrhythmic Drug Receptor on Rat Cardiac Myocytes', *Journal of Pharmacology and Experimental Therapeutics*, Vol. 269, No. 2, 1994, pp. 477–481
9. Zamponi, G.W., H.J. Duff, F.J. French and R.S. Sheldon, 'Biochemical and Biophysical Studies of the Interaction of Class I Antiarrhythmic Drugs with the Cardiac Sodium Channel', *Drug Development Research*, Vol. 33, 1994, pp. 277–294.
10. Sheldon, R.S. and H.J. Duff, 'The Cardiac Sodium Channel: Insights into Antiarrhythmic Drug Action', In: J. Menon J, ed. Current Topics in Molecular Pharmacology. 1994, pp. 137–145.
11. Sheldon, R.S., and E. Thakore, 'Ring and Link Requirements for Tocainide Binding to the Class I Antiarrhythmic Drug Receptor on Rat Cardiac Myocytes', *Journal of Pharmacology and Experimental Therapeutics,* Vol. 272, No. 3, 1995, pp. 1005–1010.
12. Sheldon, R.S., H. Duff and M.L. Koshman, 'Antiarrhythmic Activity of Quinine in Humans', *Circulation*, Vol. 92, No. 10, 15 1995, pp. 2944–2950.

We now present a concise overview of select papers identified by the PI as related to this case study grant. All papers deal with some aspect of the sodium channel and its drug receptor.

In the first paper listed above, the research team explored the implication of cyclic adenosine 3',5'-monophosphate (cAMP) regulation of cardiac sodium channels (Taouis et al., 1991). At the time there was evidence to suggest that both the number of sodium channels and their gating mechanisms could be responsive to cAMP. They approached the issue using a radiolabelled sodium channel-specific toxin ([^{3}H]batrachotoxin benzoate ([^{3}H]BTXB)) to measure the number of sodium channels in fresh cardiac myocytes. [^{3}H]BTXB is an alkaloid toxin that preferentially binds to sodium channels that resemble physiologically activated channels (Catterall, 1977). They found that an increase in the intracellular cAMP caused a significant decrease in the number of sodium channel binding

sites. They suggest this indicates the involvement of channel phosphorylation in the decrease in number of sodium channel binding sites.

The second paper describes work to characterise the optimal drug structure for binding within the sodium channel binding site (Sheldon et al., 1991). For this study the team used their radiolabelled ligand assay with a series of closely related lidocaine homologues. Lidocaine is a Class I antiarrhythmic agent. This study suggests that the optimal drug structure for binding to the sodium channel receptor is a single compound with a two-carbon arylamide–amine link and four or more amino-terminal carbons.

The paper by Hill et al. (1991) explores the mechanisms of action of a lidocaine derivative, transcainide. using the same radiolabel assay. At the time of the study transcainide was a new and potent analogue of lidocaine. It had been found to be effective in treating ventricular arrhythmias. There was contradictory data concerning the binding of transcainide being dependent on the state (active versus inactive) of the cardiac sodium channel. The team's results suggest that this lidocaine derivative reversibly binds to and stabilises the non-active state of the cardiac sodium channel.

The sixth paper listed above details research on the function of sodium salts in the modulation of Class I drugs effects (Ranger et al., 1993). This work was done in collaboration with colleagues in Montreal. In the late 1980s a specific Class I agent induced form of proarrhythmic reaction was identified. In the late 1980s sodium salts had been found to effectively treat this Class I drug toxicity. In this current paper the team descries experiments designed to study the mechanisms by which sodium alters the actions of a specific Class I agent – flecainide. Through biochemical and electrophysiological methods the team found that the sodium was able to displace flecainide from its binding site on the cardiac sodium channel, thus modulating its effect. They hypothesised that sodium salts could play a similar antagonist role with other Class I compounds.

The seventh paper listed above describes a study to assess whether Class I antiarrhythmic drugs allosterically inhibit [^{3}H]BTXB binding to sodium channels (Sheldon et al., *Journal of Pharmacology of Experimental Therapeutics*, Vol. 269, 1994, pp. 187–194). The team set out to determine the general nature of the inhibition of [^{3}H]BTXB binding by commonly used Class I drugs and to determine whether the ability to bind to [^{3}H]BTXB-free cardiac sodium channels at pharmacologically relevant concentrations is a common feature of Class I antiarrhythmic drugs. Results suggest two patterns of inhibition by Class I drugs of [^{3}H]BTXB binding; one group that demonstrates non-competitive allosteric inhibition and a second that demonstrates competitive allosteric inhibition. Results indicate that drug affinities for the [^{3}H]BTXB-free channels correlate closely with the half maximal inhibitory concentration (IC_{50}) values of these drugs. This finding suggests the effect is a determinant in their ability to inhibit [^{3}H]BTXB binding.

The eighth paper describes the experiments to determine whether Class I drug metabolites interact with the cardiac sodium channel (Sheldon et al., *Journal of Pharmacology and Experimental Therapeutics*, Vol. 269, 1994, pp. 477–481). Many Class I drugs have electrophysiologically active metabolites. It was unknown whether these metabolites actively contributed to the antiarrhythmic activity. Using radiolabelled [^{3}H]BTXB, Dr Sheldon's team set out to determine whether electrophysiologically active metabolites bind to the receptor at achieved concentrations. Studying 13 Class I agents, they found seven

active metabolites and six inactive metabolites. All active metabolites bound to the receptor at concentrations approaching their clinical concentrations. None of the six inactive metabolites did. The team compared the IC_{50} values and the serum concentrations of the metabolites and found an accurate correlation of whether or not the compound is clinically active as an antiarrhythmic agent. Their results suggest that clinically active drug metabolites may be active as they interact with the Class I drug receptor on cardiac myocytes. It is important to note that the authors end their paper with a note about the CAST trial – they suggest that Class I drugs not be used in patients after myocardial infarction but indicate that their findings are relevant given the continued use of Class I drugs for other patients.

The eleventh paper, similar to the second paper, is a study of drug structure-activity relationships (Sheldon and Thakore, 1995). In this study the group assessed the structural and physiochemical determinants of binding of tocainide and some of its homologs to the Class I cardiac sodium channel receptor. As in previous studies, drug affinity was measured with a radiolabelled binding assay using [^{3}H]BTXB and freshly isolated cardiac myocytes. The study findings are concordant with the notion of a complex receptor with both hydrophobic and hydrophilic domains. These domains are thought to recognise specific areas on the Class I drugs. Again the findings are contextualised in light of the CAST trial. Sheldon stresses that given Class I drugs have been proven effective for suppressing atrial, nodal and reciprocating tachyarrhythmias, and improved ability to define optimal drug structure could improve clinical care and benefit this group of patients.

The final paper examines the antiarrhythmic activity of one Class I drug in humans (Sheldon, Duff and Koshman, 1995). The drug quinine, which blocks the cardiac sodium channel, had been shown to prolong action potential duration in vitro and repolarisation time in vivo in dogs. This suggested that quinine might be an effective antiarrhythmic drug without the risk of excessive prolongation of repolarisation. The team undertook two studies: an open-label dose ranging study to determine the optimal dose and an effectiveness study using the maximum tolerated dose. A total of 24 patients participated in the studies: 17 in study one, ten in study two and three in both studies. The authors report results for both intention-to-treat and treatment-delivered bases. The efficacy of quinine ranged from 65 percent to 92 percent, with the former representing the intention to treat analysis and the latter the treatment delivered analysis. Four of seven patients dropped out of study one due to side-effects. The effective mean daily dose for suppressing spontaneous ventricular ectopy was 927mg It is important to note that both of these studies were conceived and completed before the publication of CAST.

Bibliometric analysis was conducted on ten out of 12 publications identified by the PI as directly related to the case study grant. Two publications have been excluded from the citation analysis as they were not indexed in the Web of Science; they are, however, included in the analysis of case study outputs (see Table 26-2 below).

Table 26-2 Publication output and impact[7]

Number of journal articles:	10
Number of articles included in citation analysis:	10
Total number of citations (all papers):	104
Aggregate relative citation impact:	0.42 (Class II)
Self-citations:	11%

Distribution of publications across relative citation impact classes:

	Class I (uncited)	Class II (>0 and <0.8 citations)	Class III (0.8 to 1.2 citations)	Class IV (1.2 to 2.0 citations)	Class V (>2.0 citations)
Number of publications	1	6	2	1	
Proportion of total output	10%	60%	20%	10%	

Most highly cited publication[8]	Taouis, M., R.S. Sheldon and H.J. Duff, 'Up-Regulation of the Rat Cardiac Sodium-Channel by In Vivo Treatment With a Class-I Antiarrhythmic Drug', *Journal of Clinical Investigation,* Vol. 88, No. 2, 1991, pp. 375–378
Times cited:	31

26.6.2 Dissemination

Dissemination activities were described as relatively straightforward. The research team worked to publish and present their findings at conferences. Sheldon mentioned that he was invited to various meetings to give presentations.

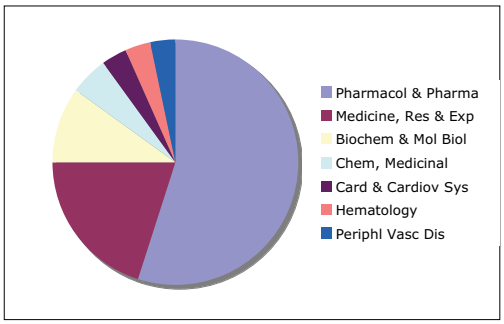
The bibliometric analysis also investigated knowledge diffusion. It was found that Sheldon and his team most commonly publish in the areas of pharmacology and pharmacy, as well as medicine, research and experimentation. Their work is most commonly cited by those working in pharmacology and pharmacy in the United States.

[7] In addition, one publication was indirectly linked to this grant. It was indexed in Web of Science, received six citations in total, giving a relative citation impact of 0.23. It had a relative citation impact class of II and a self-citation rate of 33 percent.

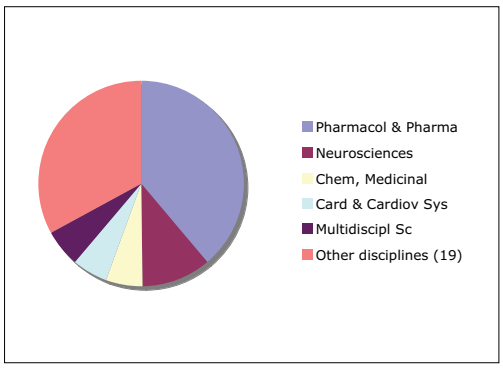
[8] Citation count extracted April 2009

Figure 26-2 Knowledge diffusion: fields of research of output (a), fields of citing papers (b) and countries of citing papers (c)

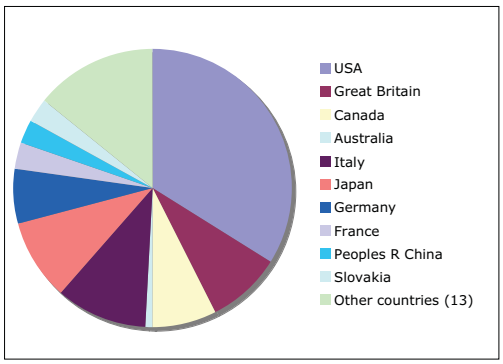
(a)



(b)



(c)



26.6.3 Training and career development

Dr Sheldon credits his research career in part to this grant. He said 'without the grants I probably would have started a practice and I'd be retired by now' (Sheldon interview, 2008).

The grant allowed Dr Sheldon to maintain and further nourish the synergistic relationship with Dr Henry Duff. Sheldon started as a postdoctoral fellow in Duff's laboratory and as Sheldon became an independent researcher the two became colleagues. According to Duff,

they met and discussed how they could help each other. After several years of collaboration they agreed that Duff would work on potassium channel binding and that Sheldon would work on sodium channel binding (Duff interview, 2008). After the CAST trial results were published, Duff indicated that their paths diverged further – his research went more basic and Sheldon's went more clinical. Duff benefitted greatly from participation in this research study and collaborations with Sheldon by strengthening his molecular knowledge base. He continues to draw on his molecular skills and moves molecular concepts to testing and exploration in animal models. Duff remarked during our interview that it is great – a real success – when one sees their students surpass them.

Dr Sheldon also mentored three postdoctoral fellows: Dr Mohammed Taouis, Dr Roger Hill and Dr Leslie Hill. Sheldon believed that Taouis is still active in medical research and is working in Algeria. Dr Roger Hill took the skills he developed and went to work with Pfizer[9]. Sheldon was not sure what the third postdoctoral fellow had gone on to do. He believed that Dr Leslie Hill had taken time off to have a family and thought that she may also have gained employment at Pfizer.

Dr Duff also reiterated that when they started they were the leaders in the area because they had developed a technique to study the binding of these drugs to their receptors called radioligand binding. The team taught anyone who was interested in learning the technique. Neither Dr Sheldon nor Duff were able to estimate the number of people who had come to learn the technique and later taught others.

26.6.4 Benefits to future research and research use

The technique is published but one benefits greatly from learning the mechanics from someone else to understand all the nuances. The technique perfected by the research team was passed on through the laboratory and as staff became more experienced continued to get better. Dr Sheldon stated that the team 'taught anyone who wanted to know' and was successful in doing so; what was once a finicky process can now be routinely carried out on mice. This is important, because although the CAST study dampened interest in Class I antiarrhythmias, the technique was directly applicable to the development of other drugs. Duff in particular has been made advances through applying the techniques to potassium channel drugs. This work has built on the work of grant, resulting in new animal models, better understanding of how channels are regulated and at least one patent in development. Another member of the team has had a successful career at Pfizer, using some of the same skills he developed directly through the grant funding.

26.7 Stage 4 – secondary outputs

The results of the research advanced the understanding of the mechanism of action of sodium channel receptors. Given the CAST results; there was no direct clinical application or development of new, more specific Class I antiarrhythmic drugs.

Dr Sheldon described the research as 'a great idea at the wrong time'. While it was elegant research that advanced the understanding of the mechanism of action of Class I

[9] Pfizer is a research-based biomedical and pharmaceutical company (Pfizer contributors, 2010).

antiarrhythmic drugs on sodium channel receptors, CAST led to a reservation about this group of drugs.

26.8 Stage 5 – adoption by practice and the public

There was no specific adoption of the results of this study by clinical practice or the public. However, the technique perfected by the research team was subsequently used to explore the mechanism of action of potassium channel blockade. Further developed by another researcher, subsequent work has yielded new animal models for heart failure, better understanding of how channels are regulated and patents (ie dofetilide made by Pfizer).

26.9 Stage 6 – broad health and economic outcomes

Dr Sheldon's current research interests have diverged considerably from the work completed in this grant. He had phased out researching antiarrhythmic drugs by 1995 to 1996 for various reasons, including the sobering effect of the CAST results, shifting clinical focus and the tremendous amount of work required to keep up with the literature in an area outside his own area of clinical focus. Sheldon's research interests have followed his clinical interests and have shifted to the area of syncope.[10]

Dr Sheldon stated that this grant provided stability and therefore the ability to investigate and think about other things. This early transition time allowed the clinical research group to stabilize, and it is now a world leader in the field. He stresses that funding bodies should not try to audit projects to determine if researchers followed through solely on what was proposed in their grant applications. He stressed the value of allowing science and researchers to explore important and relevant ideas even if they may fall outside the specifics of the initially proposed research. He commented that being too restrictive would be extremely limiting. He credits this funding with ensuring his continued research career as it afforded him the ability to explore some other relevant and clinically interesting areas in parallel to completing the proposed work.

26.10 Payback

The payback framework describes the process from research to several categories of impact. The categories allow us to compare across different case studies. Table 26-3 shows, in point form and by impact category, some of the impacts, described more fully above, that have emerged from this grant.

[10] Syncope is temporary loss of consciousness, described as 'fainting' or 'passing out'. It is usually related to temporary insufficient blood flow to the brain. It most often occurs when the blood pressure is too low (hypotension) and the heart does not pump a normal supply of oxygen to the brain.

Table 26-3 Payback

Payback category	Impacts from case study
Knowledge production	• 12 related peer-reviewed articles • Meetings both as invited speaker and as participants
Research targeting and capacity building	• Knowledge transfer within laboratory to students and postdoctoral researchers • Three post doctoral fellowships completed; all postdoctoral students are believed to still be working in health research • Refinement of methods or techniques • Techniques taught
Informing policy and product development	• Subsequent work has contributed to a patent being filed
Health and health sector benefits	• None
Broader social and economic benefits	• None

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CHAPTER 27 Biobehavioural influences on hypertension and atherosclerosis

27.1 Overview of case study grant

The grant titled 'Biobehavioural Influences on Hypertension and Atherosclerosis', was funded by the Heart and Stroke Foundation of Canada (HSFC) from 1992 to 1994 and was a renewal of a previous grant that began in 1989. The study hypothesis was that indicators of cardiovascular responses to stress may contribute to the development of atherosclerosis not otherwise accounted for by known risk factors, such as smoking or arterial hypertension, and could provide a focus for preventative interventions. This hypothesis was supported by Dr Spence's previous work, which suggested that increases in systolic blood pressure (SBP) during mental stress were associated with greater increases in left ventricular mass. By distinguishing 'cardiac' reactors from 'vascular' reactors, Spence wanted to interpret the finding and determine if increases in blood pressure during mental stress were associated with more severe artery disease. The team found their hypothesis to be true and the results of this study suggested that cardiovascular responses to stress may contribute to the development of atherosclerosis and may provide a focus for preventative interventions.

27.2 Introduction to case study

Atherosclerosis is a disease affecting arterial blood vessels, commonly referred to as 'hardening' of the arteries. It is a pathogenic process associated with high morbidity due to its role in the development of coronary heart disease and stroke. Atherosclerosis is caused by the formation of multiple plaques within the arteries, causing the arteries to harden. Plaque is a sticky, yellow substance made up of fatty substances such as cholesterol, calcium and waste products from cells (HSF, 2009). Atherosclerosis causes two main problems. Firstly, the arteries enlarge to compensate for the plaques, but eventually plaque rupture leads to narrowing of the arteries (stenosis), reducing blood flow. Traditional risk factors of atherosclerosis, including elevated serum cholesterol concentration, arterial hypertension and cigarette smoking, have been found to account for only some of the variance in the severity of atherosclerosis. This suggested that psychological factors, such as stress, may be involved in the pathogenesis of coronary artery disease. In the mid to late 1980s, research examining the biobehavioural influences on the formation of plaque

focused on coronary artery disease (Manuck et al., 1986). At the same time various other scientists were studying the relationships between personality style and stress with coronary atherosclerosis (for example, Ruberman et al., 1984 and Williams et al., 1988). The results of these studies suggested that psychological factors may be involved in the pathogenesis of coronary artery disease.

Little research had been done regarding the proposed mechanisms by which stress may affect atherosclerosis. One study implicated heightened physiological reactivity to stress as a cause or aggravator of arterial injury, particularly at arterial sites that are subject to sudden and frequent haemodynamic changes (Beere et al., 1984). In 1983, through animal models using cynomolgus monkeys, Kaplan, Manuck and their colleagues had obtained results suggesting that cardiovascular reactivity[1] to social stress correlates with severity of coronary atherosclerosis (Manuck et al., 1983). However, there was limited data available to evaluate this question in humans.

This grant titled 'Biobehavioural Influences on Hypertension and Atherosclerosis', funded by the HSFC, was designed to extend the previous observations made by Kaplan, Manuck et al. to humans. The study hypothesised that indicators of cardiovascular responses to stress may contribute to the development of atherosclerosis that is not explained by known risk factors such as smoking and arterial hypertension and may provide a focus for preventative interventions. This hypothesis was supported by Dr Spence's previous work, which suggested that increases in SBP during mental stress tend to co-occur with more thickening of the heart wall (Spence et al., 1990). By distinguishing 'cardiac' reactors from 'vascular' reactors, Spence wanted to interpret the finding and determine why decreases in heart rate during mental stress were associated with more severe artery disease (ie why stress decreases the heart rate but increases SBP). The particular grant we are studying for this case was funded from 1992 to 1994, although it was a renewal to the research grant first funded by the HSFC in 1989, which was the first study worldwide in which carotid plaque measurements were used as a surrogate outcome and plaque was reproducibly measured.

27.2.1 The case study approach

The key sources of information for this case study were interviews with the principal investigator (PI), Dr John David Spence; a collaborator who has worked with the PI on subsequent projects to further develop the imaging technology, Dr Aaron Fenster; and one of the PI's current colleagues and collaborators, Dr Robert A. Hegele. We have also reviewed Dr Spence's curriculum vitae and a sample of relevant scientific literature and have obtained bibliometric data on publications that he identified as related to this grant.

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[1] The cardiovascular system functions to provide nutrients to systemic tissue beds of the body, as well as to remove waste products of cellular metabolism. In order to do so, the heart and vasculature must work in concert and be flexible enough to respond to a wide range of activities, ranging from quiet rest or sleep to maximal exercise. Thus, the cardiovascular system is continuously 'reactive', depending on the metabolic needs of the organism.

The use of the term 'cardiovascular reactivity' by researchers in the field of cardiovascular behavioural medicine or psychophysiology is usually understood to reflect the physiologic changes from a resting or baseline state to some type of psychological or physical challenge or stressor (Manuck et al., 1989).

Unfortunately, the grant application was not accessible, although a progress report from a related, previously funded grant helped fill in the context, as did some budgetary letters.

27.3 Stage 0 – topic identification

The idea for this project was derived from the PI's previous work and experiences including:

1. the PI's training and prior research experience
2. general misunderstanding within the scientific community
3. personal experiences.

These three factors are elaborated on below.

27.3.1 The PI's training and research experience

Dr Spence says his career did not happen by accident. He said he was interested in research before attending medical school. His interests in atherosclerosis started in medical school in 1970 via lectures by Dr Maria Daria Haust, who was a pathology professor at the University of Western Ontario and the editor of the journal *Atherosclerosis* for 25 years. Spence also trained with Dr Henry Barnett, a doctor with a specialisation in neurology and a reputation for conducting quality stroke prevention research. Barnett is best known for directing many of the most important large multi-centred clinical trials in stroke prevention, including the first randomised trial in 1970 to show that aspirin prevents stroke. Supported by the National Institutes of Health (NIH) of the United States, Barnett showed that a then widely used surgical treatment for stroke patients involving carotid artery bypass was less effective than good medical treatment. Barnett headed up the North American Symptomatic Carotid Endarterectomy Trial (NASCET), the largest NIH-supported trial outside of the United States, and remained active after retirement. Dr Henry J.M. Barnett was awarded the Karolinska Stroke Award for Excellence in Stroke Research in 2008.

After obtaining a medical degree and then completing a residency in neurology and then internal medicine, Dr Spence carried out a fellowship in clinical pharmacology at the Cardiovascular Research Institute in San Francisco from 1974 to 1976, where all but one of the 49 people he trained with spent 11 months in a wet laboratory and one month treating patients. Spence recalls that they called it 'playing doctor' and continues that many of them were great researchers but not truly practicing clinicians (Spence interview, 2008). Spence felt he could not compete with them in a wet laboratory but that he could do much better clinical research.

With a focus on clinical research, and a need for patients, Dr Spence first opened a hypertension clinic at the Victoria Hospital in London, Ontario, in 1980. He added value to his time in the clinic by collecting data to aid his research. Currently Spence has a database containing information from 6,000 patients.

In addition to his training and desire to open the clinic, some unusual events led the PI to believe that stress was a contributing factor in atherosclerosis. In a previous study the research team had tested the effects of certain drugs on atherosclerosis and rabbit aortas.

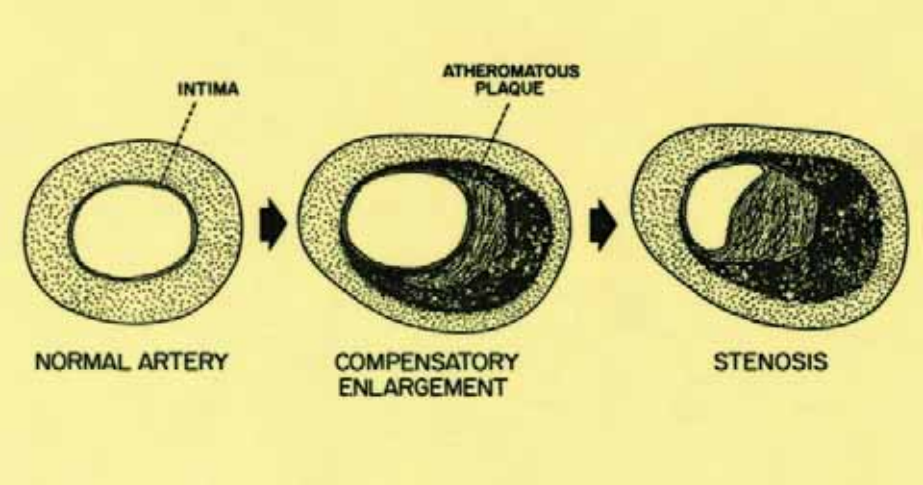
The animal maintenance required two laboratory technicians who each looked after half of the rabbits. One of the technicians was a very kind, nurturing woman who would visit the rabbits in her own time and the other was a man who was visibly afraid of the rabbits. The team observed that the rabbits in the latter group had a higher level of atherosclerosis than those cared for by the woman, even though all other conditions were the same.

Finally, as previously mentioned, the case study grant is a renewal of previous funding of a grant with the same name, received from the HSFC. This funding was received from July 1989 to June 1990. The earlier studies found that patients who had the largest increase in blood pressure during mental stress had the fastest progression of carotid plaque. It was the first study in which carotid plaques were used as a surrogate marker for cardiovascular disease. These findings were published in the *Journal of Hypertension* in 1997 (Barnett et al., 1997).

27.3.2 Scientific misunderstanding

Intima-media thickness (IMT), plaque and stenosis are often grouped together and called 'atherosclerosis' (Spence interview, 2008) although they are biologically different; they are distinct phenotypes. Dr Spence referred to Figure 27-1 to clarify the differences between IMT, plaque and stenosis (Glagov et al., 1987), where IMT is the thickness of the artery lining and adjacent muscle layer, plaque is a growth into the artery wall and stenosis is the narrowing of the artery, due to plaque rupture. Recall that atherosclerosis is the hardening of the arteries.

Figure 27-1 IMT, Plaque and Stenosis



The PI remembers that the above distinction came to him at an American Heart Association Genetics meeting in Hawaii, where there were people from Harvard talking about femoral artery stenosis on angiograms and calling it atherosclerosis. There were other people measuring IMT and calling it atherosclerosis, whereas Dr Spence was measuring carotid plaque and calling it atherosclerosis. These were common misunderstandings and the PI was determined to correct them. Glagov et al. (1987) explain that if you carry out an angiogram on two arteries (such as the first two in Figure 27-1) they may look identical because the space inside the artery, called the lumen, does not change despite the fact that

one may have significant amounts of plaque. This is because the artery will enlarge to accommodate the plaque. During his interview the PI explained that people have tended to think that stenosis was the consequence of plaque growth but that is not the case. His view is that the artery gets bigger as the plaque grows, leaving the lumen unaffected. Spence believes stenosis is almost certainly the consequence of plaque rupture, haemorrhage into the plaque and thrombosis.

Further research has verified these differences. A multiple regression using traditional risk factors (age, sex, blood pressure, cholesterol, smoking, diabetes, etc) explains only 15 percent of IMT, 52 percent of plaque area and 13 percent of stenosis (Spence interview, 2008), indicating that IMT, plaque and stenosis are all different things.

27.3.3 Personal experiences

When Dr Spence started working in the clinic in the mid 1970s he was extremely busy. He was on call for the department of medicine two out of every eight weeks and was on call for neurology another two out of every six weeks; had 45 patients in hospital at any given time; was in clinic two days a week; and was trying to do research. At 35 years of age, with three research grants and numerous patients, Dr Spence suffered a haemorrhage from an ulcer. He realised that stress was likely an important contributor to the ulcer. In the late 1970s, while attending a congress, Spence gave a talk about beta blockers and flow disturbances and heard another talk about stress and atherosclerosis in monkeys. He said 'the whole thing came together' and decided then that he wanted to investigate the relationship between stress and atherosclerosis.

27.4 Interface A – project specification and selection

The original grant application was not preserved, so the specific background and rationale have been recreated through interviews and review of the published literature.

The previous research conducted by the team suggested that the ultrasound measurement of changes in IMT in a single artery does not provide a sensitive index of individual differences in the development of atherosclerosis over a relatively short time intervals (ie less than five years) (Beaudry and Spence, 1989). Thus, this study investigated the effects of cardiovascular reactivity to mental stress on the development of carotid plaque area as measured by ultrasound at baseline and the progression of atherosclerosis over time (two years), after accounting for known atherosclerosis risk factors.

The study proposed to recruit 300 subjects who were patients of an Atherosclerosis Prevention Clinic[2] at the Victoria Hospital in London, Ontario, and volunteers who responded to on-site recruitment and local advertisements with varying levels of atherosclerosis and to monitor the progression of their condition using annual ultrasound measurements. The patients were also tested to find how they reacted to acute stress, (ie did they have a large or small blood pressure increase). The idea was to correlate the reactions to acute reactions to stress with the longer term development of atherosclerosis.

[2] In 1995, Spence moved his clinic from the Victoria Hospital to the Robarts Institute at the University Hospital. The patient population remained the same.

A nurse interviewed the subjects to record age, sex, smoking history, family history of myocardial infarction (MI) and history of vascular events and to take blood for haematocrit and lipoprotein analysis. The assessment procedures included the collection of two key pieces of information: reactivity to stress and the Atherosclerosis Severity Index (ASI), created by Beaudry and Spence (1989). To assess the reactivity, a computerised version of the Stroop Colour Word Interference Task (CWT) was employed as a laboratory stressor. The Stroop effect is a demonstration of interference in the reaction time of a task. When a word such as blue, green, red, etc is printed in a colour differing from the colour expressed by the word's semantic meaning (eg the word 'red' printed in blue ink), naming the colour of the word takes longer and is more prone to errors than when the meaning of the word is congruent with its ink colour (Wikipedia contributors, 2010, 'Stroop effect'). Patients were instructed to relax in the laboratory for 20 minutes prior to performing the CWT. Blood pressure and heart rate were measured using a Dinamapp monitor while stroke volume was recorded. Baseline values and seven values each of the blood pressure, heart rate and stroke volume during the CWT were recorded automatically over 20 minutes at three-minute intervals. Their values were summed and averaged to obtain the baseline and task data points.

The ASI is an aggregate score of clinical manifestations of atherosclerosis and measurement of atherosclerosis by duplex (Doppler and B-mode) ultrasound in the carotid, femoral and popliteal arteries. A history of angina, MI, transient ischaemic attack, cerebral infarction, vascular surgery and angioplasty were recorded and assigned values to reflect their severity. Degree of stenosis was measured by peak frequency and by percent residual lumen. B-mode imaging was used to measure the cross-sectional area of plaque in each artery. Finally, aorta to posterior tibial pulse wave velocity was recorded and included in the aggregate ASI score.

In the first year, the research team refined the atherosclerosis severity score after analysing the data available to date. Principal component factor analysis was used to select the best indicators of atherosclerosis. A single factor solution, which accounted for 31 percent of the variance, was forced. The team observed that the highest loadings were obtained for the plaque areas of the carotids, the common and superficial femorals and the popliteal arteries. The ASI then was a simple additive combination of the standardised values of the areas of atherosclerotic plaques in these arteries.

The availability of a non-invasive device for assessing atherosclerosis, duplex ultrasound, created an opportunity to study arterial lesions in the carotids and the periphery. Cross-sectional analyses were conducted to determine whether cardiovascular reactivity correlates with the severity of whole body atherosclerosis, after accounting for individual differences in certain known risk factors. After removing outliers (ie patients taking beta blockers and those who were older than 71 years of age), multiple regression analysis evaluated the amount of variance in atherosclerosis severity accounted for by indicators of cardiovascular reactivity, after controlling for risk and baseline variables.

With ASI as the dependent variable, age, sex, family history, smoking history and baseline values for SBP, diastolic blood pressure (DBP), heart rate (HR), cardiac output and peripheral resistance were entered into the equation first as a block. Age, sex, smoking history and SBP were found to be significant predictors of ASI. By collaborating with Dr

A. Parbtani, who was the Director of Renal Laboratories at the time, the team obtained measurements of plasma renin activity.

Stepwise regression was then employed to select the reactivity variables that account for most variance in ASI. Change in HR and SBP during CWT were significant predictors of ASI. Changes in DBP, cardiac output and peripheral resistance were not. An inverse relationship between change in HR and ASI was found. Change in SBP co-varied positively with ASI. Together these two variables accounted for an additional 5 percent in the variance of ASI. Secondary analyses using lipoprotein data for 95 subjects revealed highly similar results.

Although it was not possible to analyse comments from grant reviewers, the PI felt that he received reviews from people who did not understand what the research team was doing, suggesting they were either unqualified to review the grant or they were too busy to read the grant carefully. He believes that this was because he was doing novel research at the time, being one of the few researchers measuring plaque, and his techniques and rationale were not well known.

27.5 Stage 1 – inputs to research

The inputs to this research were funds; access to data; staff and their knowledge; skills and experience of colleagues and collaborators; and access to patients, facilities and equipment.

27.5.1 Funding

This two-year grant was a renewal following a three-year grant. The HSF of Ontario (HSFO) approved the PI's application to an open call for a grant in the amount of Can$57,113 for the year 1 July 1992 to 30 June 1993 and recommended Can$59,967 for the second year of the grant commencing 1 July 1993. Table 27-1 shows the details of the budget.

Table 27-1 Funding

Summary	1992–1993	1993–1994
Research assistant	Can$16,470	Can$17,293
Doppler technician	Can$27,143	Can$28,499
Total salary	**Can$43,613**	**Can$45,792**
Equipment	Can$0	Can$0
Total equipment	**Can$0**	**Can$0**
Supplies (VCR tapes, patient reimbursement, electrode tape, blood analyses and processing and data cartridge tape)	Can$13,500	Can$14,175
Total supplies	**Can$13,500**	**Can$14,175**
Other: Travel, statistical analyses	Can$0	Can$0
Total other	**Can$0**	**Can$0**
Budget total	**Can$57,113**	**Can$59,967**

The PI believed he most likely applied for a three-year grant but was only granted funding for two years. The funding letter sent to him acknowledged that the number of requests for funding exceeded the funds available for research, and in accordance with previous years, he was not granted the full amount requested, as per the policies of the foundation. The granted finances included the removal or only partial funding of equipment items, the removal of stipends for summer students and most graduate students, the provision for

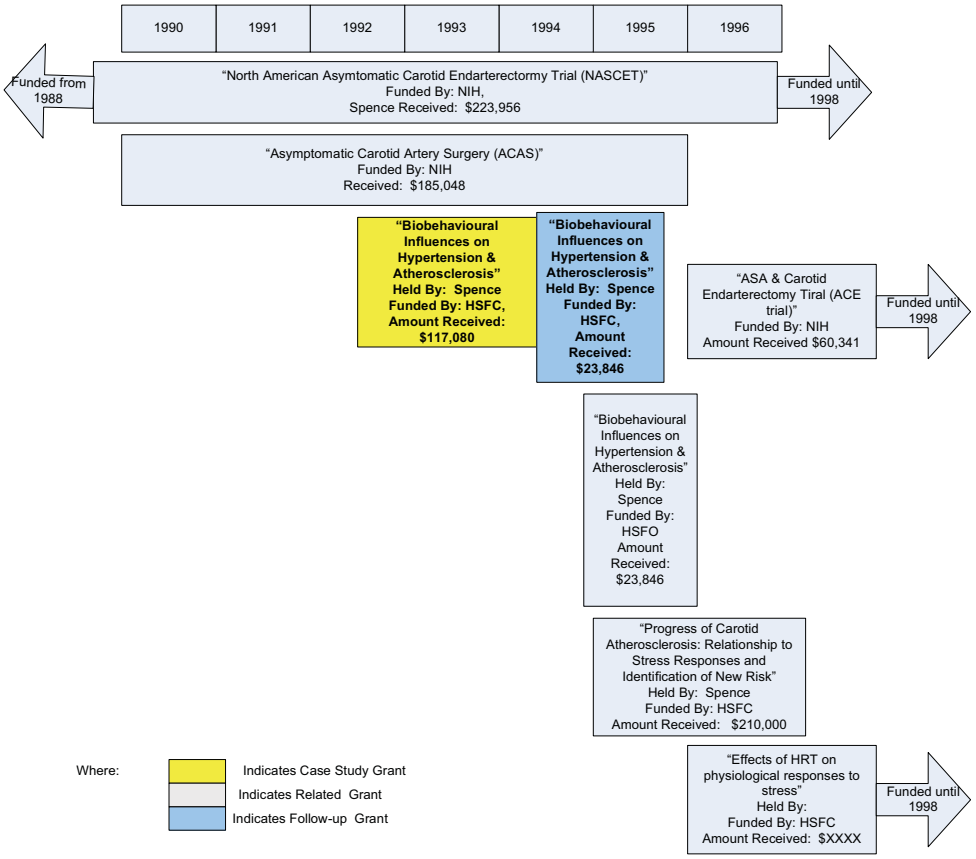
only modest increases in budgets and limited personnel support in relation to previous years. A subsequent application in 1994 received a rating just below that required for ongoing funding from the foundation. In lieu of the amount requested, the PI received Can$23,846 for salaries and supplies to enable continuation of his study through the fiscal year of 1994–1995.

The PI stated that he has never had a grant from the Heart and Stroke Foundation that paid for more than half the cost of the study, except for a grant he currently holds, which pays for about 75 percent of the costs. Both non-PIs interviewed also state that they did not receive enough funding. In order to do research the team has had to cut their costs or find ways to supplement their grants. However, there is a risk to leveraging the remaining funds because the HSFO has regulations that monitor the use of other funds to support the studies they fund and not following the proper procedures (ie knowing upfront of the need for additional funds) can jeopardise a researcher's status with HSFO.[3] Thus in order to do research, the team must often reduce their sample size or do contract work for private industry. Spence stated that he always did industrial contract research and used the profits to subsidise his peer-reviewed grants. He also stressed that the process to attain funding was and is cumbersome. Drafting proposals for funding applications takes a lot of time and effort, and from the 50 percent granted, only half of his research costs are covered. That said, he is grateful to have had continuous funding from the HSFO since 1977.

Figure 27-2 outlines the other projects the PI was involved in and other sources of funds within his clinic for the time period covering the grant plus and minus two years.

[3] As seen in the HSFO regulations governing research grant awards, 'Funds from other granting agencies in support of HSFO-funded grants must be approved through partnered funding process (refer to Section 1. v.). Also, the foundation does not permit funding from other agencies to be supplemented by HSFO-provided funding. All alternate sources of funding must be listed' (HSFO, 2010).

Figure 27-2 Funding obtained by the PI from 1990 to 1995[4]



The PI believed that the worst problem was, and remains, in securing funding for fellows. He said it is extremely difficult to get residents to apply for jobs that may or may not exist. In the Canadian structure, the funding can not be secured ahead of time. Dr Spence proposed a potential solution to this: to let 'good' researchers pick the fellow and hold money for the position so there can be a solid job offer or to set up a guarantor fund, by which the department guarantees the funding and if 20 percent of the fellowships do not come through then the department covers the missing funds. Without a solution, it is Spence's view that he is losing good fellows to other jobs that are more secure.

27.5.2 Access to data

Dr Spence was very strategic in setting up his clinic. He opened up his clinic in 1980 and constructed his practice such that every patient's data was inputted into his database. Thus, Spence maximised his time in the clinic by creating a longitudinal database he could use for his studies. Using this database populated with his patients' information he has participated in various pharmaceutical studies, creating revenue. Each year the team requires about Can$1 million of funding for maintaining research capacity, of which about

[4] During the period from 1990 to 1996, the PI's laboratory also received more than Can$1.6 million from various industry sponsored studies

half comes from industry. Unfortunately, due to increased competition, the profit margin has been decreasing on contract research, thus private funding used to support more than it can now.

27.5.3 Equipment/facilities

The PI explained that his set up was very good. At the time of this research he was at Victoria Hospital, where he had a wing of an old nursing school. He had lots of room and local support has been adequate.

In 1995 the PI moved to the Robarts Research Institute, which housed one of the core genetics laboratories for the region, as well as providing access to physicists who were developing new ultrasound machines and imaging technology. Collaboration within the institute was said to be strong, its physical proximity to the PI's clinic (and data) provided a natural fit to develop imaging techniques. He also explained that being located in London, Ontario, versus a larger city such as Toronto may have helped in his ability to recruit research subjects. Being located in a smaller city means less traffic, potentially shorter distances to travel and ample parking for patients, which are common obstacles in recruitment for clinical studies and challenges faced in larger cities. In addition, Dr Spence explained that there is a feeling of civic pride in the medical centre. The team proposed to recruit 300 patients for this study but were able to include data from 351 subjects with a wide range of types of atherosclerotic disease.

27.5.4 Collaborations

The team collaborated with Dr Parbtani, whose laboratory provided the plasma renin activity (PRA) measurements. Parbtani's laboratory had provided PRA measurements for routine clinical diagnosis purposes, as well as for various research projects for more than 15 years.

While not on the research team, Maria Di Cicco, Registered Vascular Technologist, was invaluable to the success of this study. As Dr Spence's technician in the early 1990s, she made Spence aware of the software, originally meant for monitoring tumours, that was embedded in the ultrasound machine and allowed cross-sectional images of anything you could see. Thus using the ultrasound machines one could draw two-dimensional images of plaque consistently over time. Spence stated it was she who invented two-dimensional measuring of plaque.

27.5.5 Research team

The research team's core members were Dr Spence MD, Stephen Manuck, PhD, J.R. Jennings, PhD, from the University of Pittsburgh, Anne Viswanatha, MSc, and Peter Barnett, PhD.

Jennings and his co-investigators in Pittsburgh developed the model for inducing mental stress and measuring blood pressure. Spence described the Pittsburgh team and Manuck as 'very experienced researchers'.

Dr Peter Barnett, PhD, a psychologist with statistical expertise, joined the study in 1992. He had not been involved in research projects prior to this study. With his assistance the team was able to study additional domains including Type A personality, hostility and anger using standard instruments.

27.5.6 Research environment

The PI reflected that the research environment was very hard at the time, in that he did not have enough time or money to conduct research. In order for him to do research he had to forego about 40 percent of his potential income (ie his income would have been higher had he just worked on his clinical practice). Still today, because of the demands from the clinic, the PI does most of his research between 4am and 8am.

The PI said that this is changing and that in the past several years the University of Western Ontario has started to pay clinical researchers what they would make if they had a full-time practice. This is a positive step, because otherwise promising researchers, when put on call, can be attracted to the income they receive for those hours. They soon realise that if they do not go to clinic they receive less income. Again, this is why Dr Spence strategically organised his practice around his research.

27.6 Stage 3 – primary outputs from research

The results of the multiple risk analysis, during the stressful task, suggested that changes in the HR and SBP accounted for significant proportions of the variance of ASI above and beyond that accounted for by the risk factors and baseline variables. The total amount of variance in ASI accounted for by age, sex, smoking history and SBP was 47 percent. These results suggested that older men with some hypertension who smoke are likely to have more severe atherosclerosis. Via stepwise regression, changes in HR and SBP during stress were found to be significant predictors of ASI, whereas changes in DBP, cardiac output and peripheral resistance were not. The beta for change in SBP was positive, whereas change in HR had an unpredicted inverse relationship to ASI. Together, these variables accounted for an additional five percent in the variance of ASI.

Secondary analyses using lipoprotein data for 95 subjects revealed highly similar results. Control and baseline variables, including total cholesterol and triglycerides, accounted for 55 percent of the variance in ASI, although none of the lipoprotein variables was found to be significantly related to ASI. Changes in HR and SBP during the CWT were significant predictors, as in the main analysis. The direction of these relations was the same as in the main analysis.

These results were consistent with the hypothesis that cardiovascular responses to stress may contribute to the development of atherosclerosis and may provide a focus for preventative interventions. The results suggested that increases in SBP during mental stress tend to co-occur with more severe atherosclerosis. Less interpretable was the finding that decreases in the HR during mental stress were associated with more severe artery disease. The research team thought that further analyses, in which 'cardiac' reactors distinguished from 'vascular' reactors, would help explain this result.

The study concluded its findings based on 178 subjects for whom complete data (not including lipoprotein data) were available. There were 96 men and 82 women in the sample, with a mean age of 49.7 years. The findings were considered to be preliminary on two accounts. First the team was still collecting data and hoped to include a total of 300 subjects. Second, this study was designed to examine the influence of cardiovascular

reactivity on the development of atherosclerosis. As such, it required a longitudinal study, with subjects being re-examined annually for three years.

27.6.1 Knowledge production

The PI identified 64 papers, three editorials, five chapters, seven letters and 33 abstracts as directly related to the case study grant and the subsequent work stemming from the case study grant. Below is a selection of those publications that Dr Spence identified as the most important:

1. Barnett, P.A., J.D. Spence, S.B. Manuck and J.R. Jennings, 'Psychological Stress and the Progression of Carotid Artery Disease', *Journal of Hypertension,* Vol. 15, No. 1, 1997, pp. 49–55.
2. Spence, J.D., P.A. Barnett, W. Linden, V. Ramsden and P. Taenzer, 'Lifestyle Modifications to Prevent and Control Hypertension. 7. Recommendations on Stress Management. Canadian Hypertension Society, Canadian Coalition for High Blood Pressure Prevention and Control, Laboratory Centre for Disease Control at Health Canada, Heart and Stroke Foundation of Canada', *Canadian Medical Association Journal,* Vol. 160, Suppl. 9, 1999, pp. S46–S50.
3. Spence, J.D., P.A. Barnett, R.A. Hegele, A.J. Marian, D. Freeman and M.R. Malinow, 'Plasma Homocyst(e)ine, but not MTHFR Genotype, is Associated with Variation in Carotid Plaque Area', *Stroke,* Vol. 30, 1999, pp. 969–973.
4. Spence, J.D., P.A. Barnett, D.E. Bulman and R. Hegele, 'An Approach to Ascertain Probands with a Non Traditional Risk Factor for Carotid Atherosclerosis', *Atherosclerosis,* Vol. 144, 1999, pp. 429–434.
5. Spence, J.D., M. Eliasziw, M. DiCicco, D.G. Hackam, R. Galil and T. Lohmann, 'Carotid Plaque Area: A Tool for Targeting and Evaluating Vascular Preventive Therapy', *Stroke*, Vol. 33, 2002, pp. 2916–2922.
6. Spence, J.D., M.R. Ban and R.A. Hegele, 'Lipoprotein lipase (*LPL*) Gene Variation and Progression of Carotid Artery Plaque', *Stroke,* Vol. 34, 2003, pp. 1178–1182.
7. Landry, A., J.D. Spence and A. Fenster, 'Measurement of Carotid Plaque Volume by Three-dimensional Ultrasound', *Stroke*, Vol. 35, No. 4, April 2004, pp. 864–9.

Papers 3–7 represent the subsequent research conducted by Spence and his research team.

Dr Spence said that the first key publication with retest reliability in which the team examined the relation between cardiovascular reactivity (the response of the cardiovascular system to psychological stress) and the severity and progression of carotid atherosclerosis resulting from this grant was published in 1997 (Barnett et al., 1997). The team used duplex ultrasonography and measured the change in the area of all detectable plaques in the extracranial carotid arteries during two years. Cardiovascular reactivity was assessed by measuring changes in haemodynamics during a frustrating cognitive task – the CWT. Established risk factors for atherosclerosis were measured by interviewing patients, undertaking a physical examination, and performing blood assays for 351 subjects with a wide range of types of atherosclerotic disease. The team found that atherosclerotic plaques

were present in the carotid arteries of 273 (78 percent) subjects. In a forward stepwise multiple regression analysis, it was found that greater age, a history of hypertension, use of lipid level-lowering agents, a longer history of smoking, a larger cholesterol:high-density lipoprotein ratio, a smaller change in HR during the task and a higher resting SBP were associated significantly with a greater plaque area. In 136 untreated subjects who were followed up for two years, a greater change in SBP during the task, a higher total cholesterol:high-density lipoprotein ratio and a lower body mass index were significant predictors of the change in atherosclerosis, after controlling for age and initial plaque area in a stepwise multiple regression analysis. These results supported the hypothesis that haemodynamic responses under conditions of mental stress may influence the progression of atherosclerosis.

The second publication listed above is a guideline, available nationally from the *Canadian Medical Association Journal*, in which the authors intended to provide updated evidence-based recommendations for healthcare professionals concerning the effects of stress management on the prevention and control of hypertension in otherwise healthy adults (except pregnant women) (Spence et al., *Canadian Medical Association Journal*, 1999). Through a systematic search of the literature for the period 1966–1997 (which yielded, among other sources, three meta-analyses where articles were reviewed, classified according to study design and graded according to level of evidence) and other relevant evidence obtained from the reference lists of the articles identified, the personal files of the authors and contacts with experts, the team made the following recommendations: 1) In patients with hypertension, the contribution of stress should be considered; and 2) For hypertensive patients in whom stress seems to be an important issue, stress management should be considered as an intervention. Individualised cognitive behavioural interventions are more likely to be effective than single-component interventions. These recommendations were reviewed by all of the sponsoring organisations and by participants in a satellite symposium at the fourth International Conference on Preventive Cardiology, although they were not clinically tested. The evidence for stress management is quite strong and has been shown to reduce blood pressure, but, to date, no one has done large enough longitudinal studies to show that it reduces strokes, heart attacks or death.

In the third publication, the team tested to see if elevated plasma homocyst(e)ine [H(e)] concentration was associated with premature atherosclerosis (Spence et al., *Stroke*, 199)). At the time numerous studies had found a link between elevated H(e) concentration and vascular disease. A common cause of elevated plasma H(e) concentration was known to be a mutation in the gene encoding methylenetrahydrofolate reductase (MTHFR). In this study the team sought to determine if plasma H(e) or MTHFR would be more strongly associated with carotid plaque area (CPA). The team studied 307 patients by measuring their CPA with two-dimensional ultrasound and determining traditional atherosclerosis risk factors and plasma H(e) concentration and MTHFR genotypes. They concluded that plasma H(e), but not MTHFR genotype, is significantly associated with carotid atherosclerosis, suggesting that a biochemical test may be sufficient to identify patients who may be at increased risk of atherosclerosis.

The fourth publication then examined the roles of H(e) in a linear regression model of the determinants of CPA when accounting for traditional risk factors (Spence et al., 1999). Patients with 'unexplained atherosclerosis' were identified as those who deviated from the

regression line relative to the overall study sample. H(e) was then examined in those with excessive amounts of CPA and a significantly higher mean plasma concentration of H(e) was found. These results again suggest that monitoring plasma concentrations of H(e) might be useful in identifying subjects with non-traditional determinants and a predisposition to atherosclerosis.

The team then moved on to look at CPA as measured by ultrasound images of cross-sectional longitudinal views of plaques (Spence et al., 2002). The team thought that doing so could identify patients at increased risk of stroke, myocardial infarction and vascular death. The team followed patients for up to five years, measured CPA in 1,686 patients and concluded that carotid plaque area and progression of plaque measurements were very strong predictors of cardiovascular risk. This indicated that plaque measurement may be useful in targeting preventative therapy and evaluating new treatments.

The sixth paper investigated the role of lipoprotein lipase (*LPL*) gene, which had been associated with lipoprotein phenotypes and vascular disease risk, in CPA development, again using the cross-sectional measurements of plaque (Spence, Ban and Hegele, 2003)). Through this study of 452 patients the team concluded that *LPL* gene variation may be a determinant of atherosclerosis, as estimated by static baseline CPA and by progression of CPA.

The final paper on the above list marks the team's move from two-dimensional ultrasound imaging to three-dimensional ultrasound imaging of carotid plaque (Landry, Spence and Fenster, 2004). Two-dimensional ultrasound imaging produces variable results because technicians must localise an image plane in the artery, which is difficult to reproduce. Three-dimensional imaging improves the visualisation, reduces variation in measurements and has the potential to allow quantitative monitoring of plaque changes, which can provide accurate and reliable information about plaque response to therapy.

The initial list of directly related publications provided by the PI included 45 papers. Bibliometric analysis was conducted on 38 of them,[5] including all peer-reviewed publications but excluding book chapters and e-publications that are ahead of print. Of the 38 publications included in the analysis, 29 publications were indexed with the Web of Science and so were included in citation analysis. The results of this analysis are presented in Table 27-2.

[5] Upon his review the PI wished to include 64 publications, as stated earlier, however due to timing and resources the bibliometric analysis could not be updated.

Table 27-2 Publication output and impact

Number of journal articles:	38
Number of articles included in citation analysis:	29
Total number of citations (all papers):	454
Aggregate relative citation impact:	1.24 (Class IV)
Self-citations:	36%

Distribution of publications across relative citation impact classes:

	Class I (uncited)	Class II (>0 and < 0.8 citations)	Class III (0.8 to 1.2 citations)	Class IV (1.2 to 2.0 citations)	Class V (> 2.0 citations)
Number of publications	4	11	4	6	4
Proportion of total output	14%	38%	14%	21%	14%

Most highly cited publication[6]:	Hackam, D.G., J.C. Peterson, J.D. Spence, 'What level of plasma homocyst(e)ine should be treated? Effects of vitamin therapy on progression of carotid atherosclerosis in patients with homocyst(e)ine levels above and below 14 mu mol/L', *American Journal of Hypertension*, Vol. 13, No. 1, Part 1, 2000, pp. 105–110.
Times cited:	76

27.6.2 Dissemination

Dissemination activities were relatively straightforward and involved papers in both mainline journals for the field and high-impact journals, talks, poster and platform presentations. The PI habitually attends the International Atherosclerosis Society meeting, which is held bi-annually, and the International Hypertension Society meeting. During the period of this grant, Dr Spence presented abstracts and papers at various meetings, including the annual meeting of the American Heart Association, the International Stroke Congress, the annual meeting of the International Hypertension Society and the World Neurology Congress. For example, in 1996, Dr Spence was a keynote speaker in Glasgow at the annual meeting of the International Hypertension Society and conducted a platform presentation at the American Heart Association's Inteernational Stroke Congress, where he presented his findings on atherosclerosis and psychological stress; in 1994, at the International Symposium on Atherosclerosis held in Montréal, Spence did a poster presentation titled 'Phenotyping of Premature Atherosclerosis by Ultrasound Measurement of Carotid Plaque: a Tool for Studying Genetics of Atherosclerosis'.

Dr Spence also presented his work locally via lectures and seminars in postgraduate training. In addition, the team has written editorial commentaries and contextual reviews. The team's work was distributed to multiple different stakeholder groups, including patients (the subjects in the studies received standardised feedback) and practitioners and policymakers who attend the conferences and meetings. Commercial stakeholders were also made aware of this research via Fenster's imaging unit at the Robarts Research Institute. After this grant, but in subsequent, related work, Dr Spence has been involved

[6] Citation count extracted April 2009

with pharmaceutical companies who are interested in measuring plaques to test their compounds. This will be discussed further in a later section.

The PI suggested that informal networks have played a big role, meanwhile Hegele remarked that formal networks, such as the Canadian Stroke Network, have also been a huge help for this project in putting the team in touch with other collaborators or clinics who have patients with high risk for heart disease or stroke.

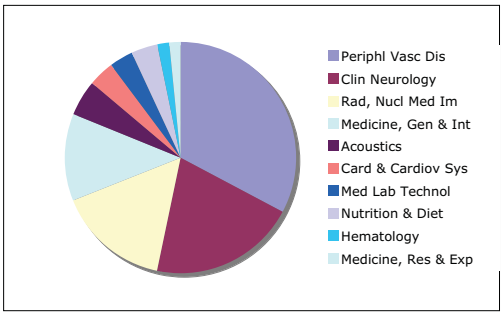
Dr Spence has also written a book titled *How to Prevent Your Stroke*, published in 2006, about atherosclerosis, which arose from brochures and leaflets he had written as information for patients (Spence, 2006). It is available internationally, online and in bookstores. Dr Spence explained that in part he chose an American publisher because there is a portion of the book that discusses the increased incidence of hypertension in black people; the rate of stroke is double in this population. Thus Spence wanted to reach the African American population.

Plaque measurement was covered by the media as a result of Spence's presentation in Vienna at the International Stroke Conference in 2008, where Dr Spence shared his findings on the reduction of events that come from treating plaque versus risk factors. Spence's work is also communicated to the public via two to three lectures a year, which are organised by the HSFC. Spence also attended a stroke conference for the public in Kitchener, Ontario, in 2008, and in Cambridge in 2009.

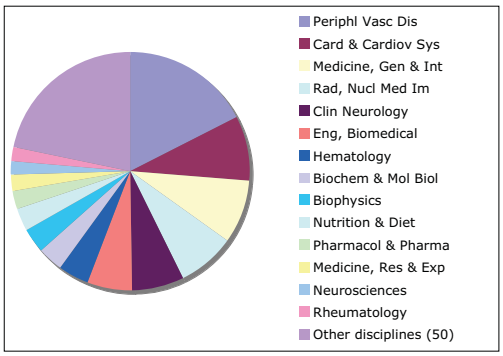
Further bibliometric analysis examined the knowledge diffusion of 29 articles and the results are depicted in Figure 27-3. It found that Dr Spence most commonly publishes within the fields of vascular disease and clinical neurology. His work is most commonly cited by those within the field of vascular disease and 'other disciplines' from the United States, United Kingdom or Canada.

Figure 27-3 Knowledge diffusion: fields of research of output (a), fields of citing papers (b) and countries of citing papers (c)

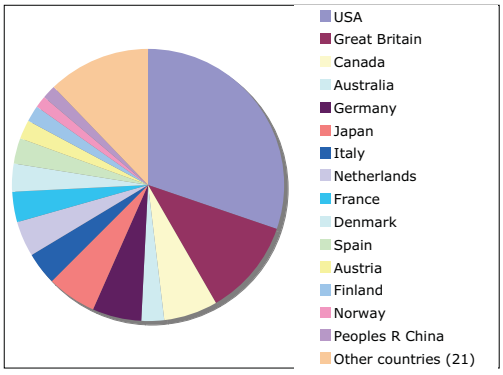
(a)



(b)



(c)



27.6.3 Training and capacity building

The PI said that this research and his subsequent research on plaque measurements have fed into the training of students and practitioners via textbooks and teachings at the undergraduate and graduate level.

There were no students involved in this grant; the team members involved already had their credentials. Dr Spence explained that his problem in assisting in this type of capacity building is in his inability to promise a salary to potential research fellows. Rather, they must apply for a research fellowship and often during the time between the application and

the granting of the fellowship, bright neurology students are attracted to the guaranteed salaries offered by clinical practice. However, since this grant, and arising directly from it, Spence has published papers with six medical students involved in the Summer Research Training Programme, nine imaging students in the Robarts Imaging Research programme and three stroke fellows.

Dr Spence did feel that this work has improved his ability to recruit researchers to his laboratory. For instance, Dan G. Hackam, PhD, one of the co-authors on the 2002 *Stroke* paper was recruited back to the team after finishing his training in internal medicine at McMaster University. In addition to research, Hackam currently teaches in the Department of Epidemiology and Statistics at the University of Western Ontario and works within the Division of Pharmacology at the university's hospital.

Dr Spence also believes that his ability to measure plaque has expanded his Premature Atherosclerosis Clinic. Most of the patient referrals are driven by the plaque measurements. He also believes that doctors within southern Ontario are used to seeing the plaque measurements and are more likely to refer patients than doctors elsewhere.

27.6.4 Benefits to future research and research use

The PI did not pursue his work on the relationship between stress and atherosclerosis beyond this grant, although all of the team's subsequent research has revolved around plaque measurements. In the early 1990s the team was measuring plaque area with two-dimensional measurements. Dr Aaron Fenster, Head of the Imaging Department at the Robarts Research Institute, was working on developing three-dimensional imaging, mainly for prostate cancer. Fenster heard about Dr Spence's interests in measuring carotid plaque. The two met, and their discussions led to Spence leaving the Victoria Hospital and coming to Robarts in 1995 in order to work more closely with Fenster on three-dimensional ultrasound. Robarts initially funded the 4,000 square foot clinical research unit that was built to accommodate Spence's clinic; the funds were paid back to Robarts from the overheads of industrial research conducted within the clinic.

This research conducted by the case study grant led the team and their collaborators to further understand the differences between and the biology of IMT, stenosis and plaque. The PI has found that atherosclerosis is focal and not continuous along the length of an artery, as many believed. Yet a common method used to monitor IMT is to measure it at pre-specified locations and then calculate the average of all measurements taken. For example, it is possible to take eight measurements along the artery, of which only one may intersect plaque. By averaging the measurements, the result is to dilute the impact of the plaque.

The team discovered in 1997 that plaque grows along the artery 2.4 times faster in length than in width. Thus, using the above example, if the same patient undergoes IMT measurement a year later, there could then be two intersects where plaque is found. The IMT would change much less than the plaque area, in part because the two sites with plaque have been averaged with the other measurements where there is no plaque. The PI stated that this makes IMT an ineffective way to assess atherosclerosis burden and change. Another major flaw with IMT measurements is that annual changes in a given individual

are often impossible to detect. The IMT is very thin, and even though resolution on the ultrasound is 0.3mm, it cannot identify annual changes that are often only 0.015mm.

The PI and his research team have also shown in a follow-up study that there can be regression of plaque. Atherosclerosis is not necessarily progressive, although this is not yet widely accepted (three years ago a student working in Dr Spence's laboratory was told by his Pathology professor that atherosclerosis never regresses). That said, despite treatment, half of Dr Spence's patients still had progression of plaque. His clinic has since shown that those patients with plaque progression, after adjusting for all traditional risk factors, had twice the risk of cardiovascular events than the others who experienced stable plaque or regression.

Despite this evidence and other theories regarding IMT measurement, there was and continues to be a great deal of work investigating the physiological differences between IMT and plaque. For instance, a recent review by Dr Robert Hegele and his graduate student, Rebecca Pollex, showed that IMT and plaque are genetically distinct (Pollex and Hegele, 2007).

Another path of study stemming from the case study research uses software and traditional risk factors to predict an individual's amount of plaque. Graphically the team showed three lines: those who have the predicted amount of plaque, those who have more plaque than predicted and those who have less than predicted. The two 'unpredicted groups' are very interesting scientifically as clinicians do not understand them. The team is looking for the genes that protect against the plaque and those that cause plaque, in the hope of one day identifying new plaque therapies.

In 2004, the team, through collaborations with Dr Fenster, published for the first time their ability to monitor plaque in patients in practice by measuring three-dimensional plaque volumes. The three-dimensional ultrasound uses methods derived from topographical mapping software to obtain a measure of carotid plaque roughness by applying the contours. With funding from HSFC the team is doing a prospective study that takes patients with the greatest (the top two percent) plaque area (70 percent of all events occur in this high-risk population) and asks how categorical variables such as IMT, plaque area, three-dimensional plaque volume and plaque roughness compare in how they predict cardiovascular outcomes. Dr Spence believes the volumes can be used to assess plaque therapy (ie treating the arteries as measured by plaque progression or regression).

In 2007, Dr Spence became involved in the Canadian Atherosclerosis Imaging Network (CAIN) study. The CAIN is a pan-Canadian imaging network involving ten institutions across the country. It is funded through grants from the Canadian Foundation for Innovation (CFI) and the Canadian Institutes of Health Research (CIHR), as well as other sources. The CAIN research programme involves the creation of a national network focused on in-vivo imaging of vessel wall disease, combined with imaging of occult end-organ disease, as well as the acquisition of clinical and pathological endpoints. The network enables unprecedented cross-sectional and longitudinal clinical studies of patients with atherosclerotic disease in coronary or carotid vascular beds and has established an international resource for studying the natural history, progression and regression of atherosclerosis and novel therapeutic interventions aimed at atherosclerosis. Vascular imaging expertise and infrastructure in all major Canadian cities are linked in this novel

multidisciplinary team to form a core clinical research network; patients are recruited from qualified sites across the country to enable unique research into the vascular biology of atherosclerosis, imaging technology assessment and clinical vascular imaging.

Dr Jean-Claude Tardif, the CAIN study leader, is a cardiologist who does intravascular ultrasound (IVUS) of the coronary artery to look at coronary plaque. He and Dr Spence discussed the possibility of applying for a strategic grant in atherosclerosis imaging. Both agreed that if they could show that carotid plaque volume was closely correlated to coronary plaque volume, which has similar relationships to risk factors, and response to therapy, then Canadian clinics could be the target for pharmaceutical companies wishing to test their new atherosclerosis drugs. One of the main projects within the CAIN study will involve 2,000 subjects across Canada who are having coronary IVUS to monitor the plaques within their carotids. This is intended to help the CAIN group determine the necessity of intracoronary IVUS, which involves putting a catheter into the coronary artery. If both measurements are found to yield the same results then Spence would argue that IVUS should no longer be done as it is quite invasive and could be replaced by carotid ultrasound. Spence says his involvement in the CAIN study is directly related to the case study grant, which was when the team started measuring carotid plaque. Spence and Fenster are both members of the CAIN Steering Committee.

Other researchers around the world are also starting to use the three-dimensional measurements in their studies. UK Biobank is an enormous multi-million pound epidemiological study being run from Oxford University by Rory Collins; it is expected to involve 500,000 people (recruitment is continuing until 2010). The overarching objective of the study is to investigate the separate and combined effects of genetic, environmental and lifestyle factors on major morbidity, mortality and health. Collins invited Dr Spence to speak to the Wellcome Trust last September about measuring three-dimensional carotid plaque in 100,000 of the study subjects.

Dr Spence noted that more and more people are using plaque measurements as a research tool, although it will not be widely accepted for clinical use until proven by a randomised controlled trial. He continued by saying that Americans and Canadians are resistant to three-dimensional plaque volume measurement; they still use IMT measurement despite the finding reported in the 2002 paper in *Stroke*. These conclusions were validated in 2007 in *Stroke* by a population-based study conducted in Norway in which plaque area was measured. This study confirmed that plaque area predicted coronary events. IMT, which is more commonly measured, was found not to be a predictor of coronary events (Johnsen et al., 2007).

Outside of the field of research, brothers Rob Brook in Ann Arbor and Jeff Brook in Toronto are conducting a study for Environment Canada using the PI's data and findings. Brooks is studying air pollution by postal codes and is creating a map of Ontario so that Spence can then look into his extensive database to see whether air pollution contributes to carotid plaque burden. They will do a retrospective study first to justify a prospective study.

Overall, the case study grant has led to increasing awareness about measuring plaque volumes in patients via three-dimensional imaging, which can be used to assess the effectiveness of treating the arteries as measured by plaque volumes. As stated by one of the

non-PIs interviewed, the research of the original grant has expanded; it is not winding down. The team's network is bigger than ever and involves various international collaborators.

27.7 Stage 4 – secondary outputs

Plaque measurements are now being used in pharmaceutical industry-led studies. Wyeth is currently conducting a study using three-dimensional plaque measurements to investigate atherosclerosis, while Roche is funding a natural history study on plaque progression in patients with rheumatoid arthritis with a view to investigate their anti-arthritis drug and its effect on this population and the development of atherosclerosis. Roche is also funding a major clinical trial through the CAIN, which will involve measurement of coronary plaque by IVUS and IMT; CAIN will add to this study the measurement of three-dimensional carotid plaque volume. The PI stated that Merck, AstraZeneca and Pfizer are all interested in the imaging of the arteries and are all talking about using three-dimensional plaque volume measurement to study new drugs for atherosclerosis.

The PI believes three-dimensional plaque measurement is about to become a widely used technology in the development of new therapies. The imaging team has gone on to develop various companies and patents based on software required to take the three-dimensional plaque measurements, which is available commercially through TomTec. A current focus of the imaging team and industry is to streamline the imaging process, making it easier to do as well as making the imaging technology accessible. The imaging team is currently pursing creating a company that would receive the images electronically and do the analysis centrally. Spence is involved with this project and has been training a group in Holland to do the analysis of three-dimensional ultrasound imaging, using the research team's software. He has also collaborated with a group in Israel to study regression of carotid three-dimensional vessel wall volume by diet.[3]

Regulatory approval of the ultrasound equipment has been attained. To date, the regulatory agents who look at pharmaceuticals have not approved plaque measurement as a surrogate outcome for drug effects. The team is hoping the CAIN study will help facilitate these advancements.

The insight gained from the grant, that IMT, plaque and stenosis are all different things, led to an approach to using carotid plaque measurements as a powerful tool for genetic research. Dr. Spence and colleagues first published that concept in 1999.[4] More recently, in collaboration with Dr. Nik Schork of the Scripps Institute in San Diego, they published evidence that plaque measurements, when adjusted in multiple regression for traditional coronary risk factors, could identify two quantitative traits (unexplained atherosclerosis and

[3] Shai I.,J.D. Spence, D. Schwarzfuchs, Y. Henkin, G. Parraga, A. Rudich, A. Fenster, C. Mallett, N. Liel-Cohen, A. Tirosh, A. Bolotin, J. Thiery, G.M. Fiedler, M. Blüher, M. Stumvoll, M.J. Stampfer for the DIRECT Group, 'Dietary Intervention to Reverse Carotid Atherosclerosis', *Circulation*, Vol. 121, No. 10, 2010, pp. 1200–1208.

[4] Spence J.D., P.A. Barnett, D.E. Bulman, R. Hegele, 'An approach to ascertain probands with a non traditional risk factor for carotid atherosclerosis' *Atherosclerosis*, Vol. 144, 1999, pp. 429–434.

unexplained protection from atherosclerosis) that can markedly reduce the sample size required for genome-wide association studies.[5]

27.8 Stage 5 – adoption by practice and the public

The PI has changed the paradigm in his clinic from treating risk factors to treating arteries. He now believes that treating arteries without treating plaque would be like treating hypertension without measuring blood pressure. Without measuring plaque volume, Dr Spence believes that it is impossible to know what is happening in the arteries.

In his own clinic, when Dr Spence observes plaque progression or a lot of plaque, he knows he needs to treat the patients more intensely. If plaque is observed to be regressing over two years, the patient is sent to a general practitioner for monitoring, as Spence concludes treatment is working. If the plaque is progressing, the PI will keep them as his patients and turn to more aggressive therapy. He continues to study these patients and is trying to understand what these people have that causes the plaque to progress. His current research investigates genetic factors. The PI concludes that IMT measurement is an ineffective way to monitor the arteries because plaque volume is more related to coronary artery disease than IMT.

That said, plaque volume is still not the standard method for monitoring arteries, although this technology is starting to gain greater acceptance and spread. Although it is not a very sensitive to acute changes, IMT measurements are easy to do and have an established, long history of use. Plaque volume provides a more accurate measurement of what is happening within the arteries but it is more difficult to do. Although many Americans and people in Western Europe are starting to measure plaque volumes, there has been resistance to the PI's findings, despite increasing evidence.[6]

27.9 Stage 6 – broad health and economic outcomes

To date there has been no documented widespread health gain or cost savings associated with plaque measurements, although Dr Spence believes that patients whose care is guided by plaque measurement do receive better medical treatment and advice. Within his own clinic, Dr Spence has observed that by changing the paradigm in his clinic to treating the arteries, as measured by plaque volume, there is no plaque progression among his patient population but there has been overall regression since 2005. Among patients with

[5] Lanktree M.B., R.A. Hegele, N.J. Schork, J.D. Spence, 'Extremes of unexplained variation as a phenotype: an efficient approach for genome-wide association studies of cardiovascular disease', *Circulation Cardiovascular Genetics*, Vol. 3, 2010, pp. 215–221.

[6] In a follow-up communication Dr Spence noted that this is beginning to change: A large HMO in Argentina is now using plaque measurements in treatment of patients for vascular prevention, and a group in India visited the Robarts Imaging Group in July, 2010 to discuss implementing 3D ultrasound measurements of atherosclerosis in vascular prevention. At a meeting in Los Angeles in August 2010, the Society for Heart Attack Prevention and Eradication endorsed the measurement of carotid plaque area, in addition to IMT and coronary calcium, for vascular prevention.

asymptomatic carotid stenosis, microemboli were reduced from 12.6 percent to 3.7 percent of patients and events were reduced from 17.6 percent to 5.3 percent of patients. The latter finding was in press at the time of writing this case study.[7] Another study of the change in rate of plaque progression in his clinic population between 1997 and 2007 was submitted for publication at the time of writing this case study.[8] The PI continues to follow more than 4,000 active patients, with 900 new patients per year with stroke or transient ischaemic attacks (TIAs). A grant application for a clinical trial to test treatment of arteries based on plaque measurements (versus usual care, based on treatment of risk factors) is being prepared for submission.[9]

27.10 Payback

The payback framework describes the process from research to several categories of impact. The categories allow us to compare across different case studies. Table 27-3 shows, in point form and by impact category, some of the impacts, described more fully above, that have emerged from this grant.

[7] This has now been published in: Spence J.D., V. Coates, H. Li, A. Tamayo,C. Muñoz, D.G. Hackam, M. Dicicco, J. Desroches, C. Bogiatzi, J. Klein, J. Madrenas, R.A. Hegele, 'Effects of Intensive Medical Therapy on Microemboli and Cardiovascular Risk in Asymptomatic Carotid Stenosis', *Archives of Neurology*, Vol. 67, No. 2, 2010, pp. 180–186.

[8] This has now been published in: Spence J.D., D.G. Hackam, 'Treating arteries instead of risk factors: a paradigm change in management of atherosclerosis', *Stroke*, Vol. 41, No. 6, 2010, pp. 1193–1199.

[9] In a follow-up communication Dr Spence remarked that in July 2010, CIHR approved a grant for Dr. Spence and colleagues to validate that 3D ultrasound measurements of plaque volume can be carried out at multiple sites.

Table 27-3 Payback

Payback category	Impacts from case study
Knowledge production	• 11 related peer-reviewed articles • Cardiovascular responses to stress may contribute to the development of atherosclerosis and may provide a focus for preventative interventions • For the first time, 2-D imaging of carotid plaque was used in a clinical study, which over time led to the discovery and use of 3-D imaging • 2D plaque measurement established for patient management and genetic research • 3D ultrasound measurement of plaque volume and vessel wall volume established for evaluation of new therapies
Research targeting and capacity building	• Imaging laboratory has expanded from 3 to 5 people in the early 1990s to 250 people. Both the clinic and the imaging laboratory have involved a great number of trainees over the years who have continued on as technicians, in academia and in medicine • Creation of new clinical research facility at the Robarts Research Institute • This successful area of research has allowed the team to have continuous funding from Foundations as well as federal funders and the private sector
Informing policy and product development	• The team has had a great deal of involvement with industry testing the efficacy of atherosclerotic pharmaceuticals • Guidelines, available nationally, in which the authors provide updated evidence-based recommendations for health care professionals concerning the effects of stress management on the prevention and control of hypertension
Health and health sector benefits	• Potential for widespread health gain/cost savings from the improved monitoring and management of atherosclerosis using three-dimensional carotid plaques measurements, as witnessed in the PI's patient population
Broader social and economic benefits	• Near future potential economic gains from widespread use of the imaging technology

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CHAPTER 28 Prolonged heart and lung allograft preservation

28.1 Overview of case study grant

The grant of interest to this case study, titled 'Prolonged Heart and Lung Allograft Preservation' was funded by the Heart and Stroke Foundation of Canada (HSFC) from 1989 to 1991 and was a renewal of funding which was awarded to Drs Patterson and Weisel from 1987. With a larger team, Weisel and his co-applicants, Patterson, Dr Wu and Dr Feindel, proposed to assess whether improved conditions of cold storage would facilitate functional restoration after 12 hours of preservation and if modifying the conditions of reperfusion would restore heart and lung function early after transplantation. Through the team's evaluation of various solutions that can be used to preserve the heart during transplantation, carried out at Toronto General Hospital, the team concluded that using a cold crystalloid cardioplegic solution and topical hypothermia for heart–lung allograft preservation provided the same level of protection as more complicated techniques.

In part, as a result of the studies conducted in Weisel's laboratory, through the funding investigated in this case study grant and subsequent research that arose from it, cardiovascular surgeons now protect the heart for transplantation using blood cardioplegia solutions. The heart is now better protected prior to, and throughout, transplantation and other surgeries. Scientific advances in heart surgery have reduced mortality, and increased long-term survival rates of patients undergoing heart transplants. The results for lung transplantation, which is rarer, have also improved.

28.2 Introduction to case study

By the late 1980s both heart and lung transplantation had become an accepted procedure for patients whose hearts and/or lung(s) had become irreparably damaged. However, the transplantation programmes were limited by the supply of available donor organs and safe preservation techniques, which at the time limited organ preservation to four hours. By

improving methods of heart and lung preservation to prolong graft survival, the number of donor organs could be increased.

In the grant application titled 'Prolonged Heart and Lung Allograft Preservation' Dr Weisel, the principal investigator (PI), and his co-applicants proposed an extensive evaluation of alternative methods to preserve the heart and lungs for a 12-hour period in order to improve donor organ supply and increase the number of patients who could benefit from transplantation. The team hypothesised that improved techniques of cold storage would increase survival of donor hearts and that modifying conditions during reperfusion following transplantation would facilitate immediate metabolic and functional recovery after prolonged preservation. To conduct these studies, the team was awarded a two-year grant from July 1989 to June 1992. The work was conducted at Toronto General Hospital (TGH), which is affiliated with the University of Toronto.

As this grant was a renewal, the PI had already established important collaborations at TGH, primarily with Drs Donald A. Mickle and Ren-Ke Li, who assisted in developing the human myocyte cell cultures, and the other co-applicants, Dr Patterson and Dr Feindel. Together the team had previously used cell cultures to assess storage solutions for prolonged (12 hours) hypothermic (4°C) preservation. The team had originally planned to conduct canine studies as well but due to lack of finances focused only on the cell cultures.

There were two main developments that made this research plausible. First was the conversion from crystalloid solution to blood cardioplegic solutions. In preparation for cardiac transplant, cardiac activity of the donor heart needs to be temporarily and reversibly stopped – this is referred to as cardioplegia. Cardioplegic solutions for cold storage of organs were first proposed in the 1960s, although scientists were unsuccessful finding the appropriate additives to make cardioplegic solutions effective. Heart surgery was in its infancy and some fundamental knowledge was still unknown, such as the appropriate doses of potassium required. Potassium is required to help ensure that the heart does not use up the energy stores during the period of heart isolation. In the 1970s crystalloid-based solutions were introduced and found to be somewhat effective for organ storage. Blood cardioplegia (introducing blood into the solution to act as a buffer and provide nutrients to the heart during the procedure) was introduced in the early 1980s by Dr Buckberg. A large randomised controlled trial investigating its use was conducted by Steve Fremes in 1984 (Fremes et al., 1984) and demonstrated the benefit of blood cardioplegia use for patients who are undergoing coronary bypass surgery. Second was the team's demonstrated ability to grow human ventricle myocytes and human arterial and venous endothelial cells. By using these cells the team proposed to evaluate different solutions and optimal temperatures that could be employed to store donor organs for transplantation.

In separate studies, Dr Paterson and his research fellow, Dr Shaf Keshavjee, defined the best solutions to protect the lungs from ischaemic injury during the preservation period prior to lung transplantation. Both the heart and lung teams also evaluated continuous blood perfusion of the heart and/or the lungs to improve the recovery of function after transplantation. However, at the time, technical difficulties limited the clinical application of this technology.

The team expected that the work of this grant would identify the best conditions for prolonged (12 hours) cold storage and reperfusion. After establishing the optimal conditions in the laboratory, results were to be applied to the ongoing clinical transplant programmes within TGH in order to improve the results of heart and lung transplantation. The overarching rationale for this work was that improved availability of donor organs could decrease the number of patients dying on the transplant waiting lists and permit improved recovery of those patients who do undergo transplantation.

In 1989, Weisel, the PI of the case study grant, was an associate professor of surgery at the University of Toronto. He had completed a bachelor of arts in 1965 from Yale University, followed by a medical degree from the Marquette Medical School (MMS) in Milwaukee, Wisconsin. He then completed eight years of additional training first at MMS and then at the Boston University Medical Center before starting a senior residency in thoracic and cardiovascular surgery at the University of Toronto. In 1978 he was awarded a research fellowship from the Canadian Heart Foundation (now the HSFC) at the University of Toronto.

28.2.1 The case study approach

The findings presented in this case study are based on a combination of: three face-to-face interviews, including Dr Weisel (the PI)), Dr Ren-Ke Li (a student who contributed to project), and Dr Vivek Rao, who did not participate in the case study grant but subsequently carried the research forward; a review of the original grant application and supporting documentation; a review of the PI's curriculum vitae; and documentary analysis of the scientific literature and bibliometric analysis.

28.3 Stage 0 – topic/issue identification

When Dr Weisel applied for this grant he had previously held a number of grants related to transplantation, was extremely active in the field of research and had his own laboratory.

The idea for this grant was derived from a series of factors including:

1. scientific interest
2. the PI's prior research experience
3. gaps in the current body of knowledge relating to optimal conditions for organ transplantation

These three factors are elaborated on below.

28.3.1 Scientific interest

Dr Weisel told us that in the late 1960s and early 1970s the feeling among cardiac surgeons was that if *any* transplant patients survived (since most died), the programme was successful (Weisel interview, 2008). Weisel began practising cardiac surgery in 1978 after completing his residency and with his colleagues started to evaluate their clinical results. They found that a major cause of mortality following transplant was that the heart was not adequately protected prior to and during surgery due to poor preservation techniques and lack of knowledge. Thus, Weisel began to look at alternatives for protecting the heart.

Dr Weisel stated in his interview that he thought this was an important area and that to simultaneously investigate lung preservation was logical and interesting. Much of his previous work had involved lung transplant surgeons so this grant was a natural progression. Weisel explained that there are a lot of cross-fertilisations that could be maximised by teaming up with the lung transplant surgeons, as well as a lot of differences that were worth exploring. The preservation of both the heart and the lung involves preserving the endothelial functions. While they are different organs with different responses, many of the agents or interventions used in the heart are successful in the lung and vice versa. The overall concept of preserving endothelia function is a common thread and allowed the team to investigate the two organs together.

28.3.2 Prior research experience/need

In the late 1980s, the techniques used for cold storage worldwide, developed for both heart and lung preservation, came from Norman Shumway who has since retired as the head of transplantation at Stanford University. The technique involved the cooling down of the organs with a crystalloid solution and putting them in ice for transport. However many of the organs did not function properly after transplantation, resulting in death. There was a clear need for better ways to preserve the organs.

Need was also highlighted by a lack of safe storage for transport of donor organs. In the late 1980s organs were being shipped between Canada and the United States (this no longer happens for adult organs). Prior to this study, transplant teams were using a fish tank filled with a blood solution for heart and lung organ preservation, they kept the organs aerated by ventilating the lungs. Weisel recalled two occasions where the pump malfunctioned and created massive complications. On one flight, the fish tank developed a hole. The surgeon on board gave his own blood to ensure an adequate volume of the blood solution. In another case the pump's motor stopped working and at 2am the plane had to make an emergency landing in Cleveland, where, with the assistance of the local police, those on board purchased a new motor from a pet store. Weisel and his team thought there must be a better way and so pursued research aimed at protecting the heart longer for transplant.

28.3.3 The Climate and Environment

Through previously conducted studies Dr Weisel had already carried out preliminary work in this field and developed important collaborations between the heart and lung transplant surgeons, as well as his basic science colleagues at TGH. Through collaborations with Drs Donald Mickle and Ren-Ke Li, Weisel and his team had demonstrated their ability to grow human ventricular myocytes and human arterial and venous endothelial cells in cultured media. At the time of the application, they were also growing type II alveolar pneumocytes.

The case study grant was a renewal of HSFC funding from 1987. The previous project had allowed the team, again with input from Dr Mickle, to propose a series of studies to assess alternative techniques for four-hour preservation of heart-lung allografts (Fremes et al., 1984). The previous grant funding also allowed the team to perform preliminary studies using human myocyte cell cultures to evaluate 12-hour preservation, which established the feasibilities outlined in the case study grant.

In other studies, the team had established a protocol of ischaemia and hypoxia that could simulate the conditions of organ storage (Fremes et al., 1989). They had also developed a cell-culture method to evaluate the effects of storage on human myocytes (Fremes et al., 1991) and a canine model that could be used to elucidate the effects of hypothermic cold storage and evaluate heart–lung preservation on metabolic and functional recovery. This work was done via an extensive collaboration with the Department of Clinical Biochemistry. In addition, Weisel developed collaborations with Dr Alex Patterson and his research fellow, Dr Shaf Keshavjee, who were evaluating alternative solutions to protect the lung for lung transplantation. The two groups were collaborating to employ the information learned to the benefit of both teams.

Around the same time, in the early 1990s, a new paradigm was emerging throughout all of science, which is now known as 'preconditioning'. In cardiology, evidence was forming to indicate that the heart contains an endogenous mechanism that allows it to protect itself from injury naturally. The hypothesis was that if one can put the heart through three cycles of brief ischaemia for a period of about five or ten minutes, followed by three periods of brief reperfusion it will become resistant to prolonged ischaemia. Weisel and others believed that if you could complete this process you could prevent ischaemic injury. Preconditioning has been attributed to James M. Downey, who was publishing papers on reperfusing rabbit hearts after periods of ischaemia (Ytrehus et al., 1994).

28.4 Interface A – project specification and selection

Dr Weisel wrote this application with his co-applicants, G. Alex Patterson (an assistant professor of surgery), Tai-Wing Wu (a professor of clinical biochemistry) and Christopher Feindel (an assistant professor of surgery), all at the University of Toronto.

Together the team proposed to test the following hypotheses:

1. improved conditions of cold storage will facilitate functional restoration after 12 hours of preservation
2. modifying the conditions of reperfusion will restore heart and lung function early after transplantation.

To test their hypotheses, the team proposed to conduct two types of experiments:

1. cell culture studies and
2. canine studies

For the cell-culture studies, the team prepared cultures of myocardial cells, pulmonary alveolar Type II pneumocytes and vascular endothelial cells. The team proposed to use these cultured cells to determine the optimal storage temperature for prolonged heart-lung grafts. The team also planned to investigate the effects of various storage solutions on heart–lung preservation. Canine studies were to be carried out to determine the effects of reperfusion on the heart–lung grafts. After donor extraction, the heart–lung preparations were to undergo 12 hours of cold storage before transplantation. The team proposed to assess the benefits of slow oxygenation during reperfusion using a modified blood cardioplegia technique and the effects of antioxidants on the incidence of reperfusion

injury using standard assessments of metabolic and functional recovery to determine the effect of those interventions. The conditions of reperfusion were to be altered to determine the optimal method of reperfusion.

Right and left ventricular functions were to be assessed by nuclear ventriculography, a technique widely used by Dr Weisel and his group at the time, and by measurement of haemodynamic variables. Myocardial metabolism was to be assessed by measuring adenine nucleotides and their degradation products. The effect on cell membranes was also to be assessed.

Rapid cooling induced by the infusion of various cold cardioplegic solutions during the period of cold storage were thought to induce a variety of metabolic abnormalities, such as increased cellular permeability. In addition, perfusion after implantation of the graft following prolonged cold storage was thought to result in sudden cell injury due to the accumulation of oxygen-free radicals metabolised during the period of anoxic metabolism prior to the implant. The sudden increase in oxygen tension with reperfusion was believed to paralyse temporarily cellular mechanism that ordinarily deals with oxygen-free radicals. The team thought that restoration of oxygen tensions or the employment of antioxidants may decrease the risk of reperfusion injuries.

One reviewer commented that, as written, the team's protocol was very complex because of its distinction into two units and it would be unlikely that the team could complete all they had proposed in the two years of funding requested in the grant application (CHF, 1988). Overall, the reviewers' comments were very good to excellent, including praise to Dr Weisel for developing state of the art techniques to evaluate effectively myocardial metabolism. Weisel and his team were perceived as highly productive researchers.

The improvement of myocardial reperfusion and lung reperfusion following transplantation was thought to also be applicable and successful in reducing morbidity and mortality in short-term organ preservation situations. The team expected that the cell-culture and canine experiments would identify the best conditions for prolonged (12 hours) cold storage and reperfusion. After establishing optimal conditions in the laboratory the team intended to test them within the clinical transplant programmes at TGH in order to improve results of heart and lung transplantation.

The project was modified to exclude the canine studies after the proposal had been sent to HSFC. Using a large animal is very expensive and the team did not acquire sufficient funding to study this large animal model. In a group meeting, one of the team members, Ren-Ke Li, who had extensive experience in the field of cell cultures, suggested using a simple, cheaper in vitro model to identify the optimal conditions of time, temperature and other compositions. The team would still be able to find the best conditions to use in the animal studies but his model saved time and money. His suggestion was well received and the team carried forward using only a cell-culture model. However, it should be noted that the cell cultures were not a true representation of the intact heart.

28.5 Stage 1 – inputs to research

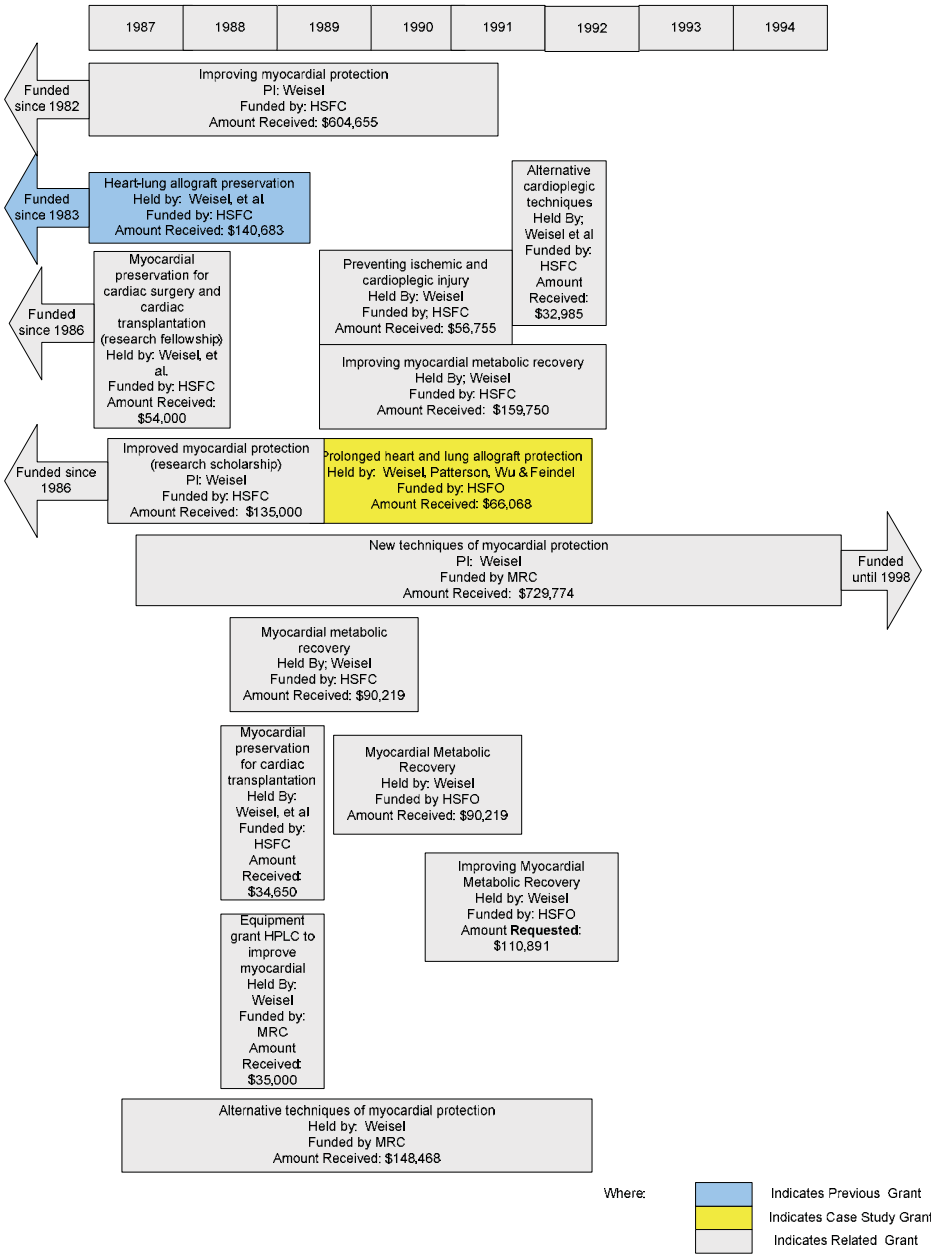
28.5.1 Funding

For this renewal grant, the team requested a total of Can$291,272: Can$141,390 over the fiscal year of 1989 to 1990 and Can$149,881 for 1990 to 1991. The team did not receive the requested funds; they were awarded Can$33,029 for both years, a total of Can$66,058 in their first attempt to secure funding.

Dr Weisel claimed in his interview that if this project had not been funded by HSFC it probably would not have happened. The team was able to get funding from a variety of different sources but Weisel called this a 'fringe project in terms of heart and lung transplantation'. He carried on saying that had the team not received the funding from HSFC, they would have focused on the other things they were doing. With that said, Weisel claimed that this work has been 'very successful in terms of generating more funding and more people doing this research and people being interested in it. It's still very viable' (Weisel interview, 2008).

An illustration of other funds held by the PI can be viewed in Figure 28-1. This chart was created using relevant information found within the grant application and a search of CIHR databases. This chart helps to illustrate the relative size of the grant in comparison to the overall research programme ongoing within the PI's laboratory over the course of the case study grant, including a two-year period prior to and subsequent to this funding.

Figure 28-1 All funds held by the PI from 1987 to 1994



28.5.2 Research team

The review committee report stated that 'a highly skilled and productive group of investigators [had] been assembled to manage the various elements of the proposed study' (Scientific Review Committee Report, 1988). The heart and lung transplant surgeons at the University of Toronto enlisted the support of basic scientists to evaluate extensively alternative techniques for prolonged heart and lung preservation. Weisel said that it was 'essential to have input from both sides when looking at mechanisms and applications...The interaction is very helpful. The clinical groups I deal with understand

the need to bring in basic scientists. You need people who understand all the components you don't in order to do it. Clinicians or basic scientists cannot do everything on their own. We need to understand the problems as well as the alternate solutions'.

As previously mentioned, there were three co-applicants, Drs Wu, Patterson and Feindel, who worked with Weisel and the other team members. Wu was a biochemist who had helped the team develop many compounds. The cell-culture laboratory was run under Wu's direction. Patterson was Head of Thoracic Surgery and is known as the first surgeon to conduct a successful double lung transplant worldwide. He did so in 1986, with Dr Cooper. Feindel was a young staff member in cardiac surgery at the time of the case study grant.

The team members mentioned in Grant Application Section 1, in addition to Dr Weisel and his co-applicants, were Ms Mindy Madonik, Mr Fred Arbour and a summer student. Madonik had been a research assistant for the Division of Cardiovascular Surgery since 1981 and was said to have 'extensive experience in organizing [the team's] animal research studies...[and] extensive experience in evaluating respiratory function'. Her previous experience involved managing the cardiopulmonary bypass circuitry, which was required for the heart and lung transplants, as well as participating in nuclear ventriculography and intraventicular pressure measurements. Arbour was said to be an 'experienced biochemical technologist…[with] extensive experience with biochemical measurements [and] cell culture technique.' Thus, he was ideally suited to grow the myocytes, pneumocytes and endothelial cells required for the transplantation studies (Weisel, 1988).

Dr Weisel also mentioned two full-time students, Fred Keeley and Ren-Ke Li, as team members on this project. Keeley was a PhD biochemistry student and Li was an MD and PhD student in biochemistry (the equivalent to what would be called laboratory medicine and pathobiology) from China. As previously mentioned, Li had extensive experience in cell cultures and is responsible for shifting the team's direction from animal studies to focus solely on in-vitro models. Doing so saved the team various expenses associated with doing large animal studies. Weisel explained that the University of Toronto has various options enabling PIs to hire summer students through programmes available in Toronto such as the Schultz Science Student Scholarship programme for undergraduate students via the HSFC, MRC/CIHR funding for medical students or the Wills programme for high school students.

Drs Patterson and Weisel were young staff surgeons at TGH working in heart and lung transplantation and preservation. They had two trainees who were also involved in this research. Steve Fremes, who worked with Weisel, was interested in the heart. The surgical programme at TGH offers residents time out of their clinical training to do basic science training. Steve was part of this programme and had taken two years out of his clinical training to do a master of science degree before finishing his cardiac surgical training. He is now Head of Cardiac Surgery at Sunnybrook. The other trainee, Shaf Keshavjee who was interested in lungs and worked with Patterson, continued to do a MSc and is now Head of Thoracic Surgery at the University of Toronto, as well as head of the lung transplant programme.

The applicants wrote in their grant proposal that 'the heart and lung transplant surgeons at the University of Toronto have secured the support of a group of excellent research

scientists' (Weisel, 1988). Dr Weisel said the research team assembled a critical mass of transplant surgeons and basic scientists permitting an intensive evaluation of heart and lung preservation.

28.5.3 Collaborations

An important collaborator and mentor for Dr Weisel was Dr Donald A.G. Mickle, who was Head of Biochemistry at TGH at the time. Weisel had worked with Mickle since 1978, until Mickle retired in 2003–2004. Weisel said another great advantage of the collaboration, in addition to Mickle's experience and guidance, was that they pooled everything in their respective laboratories. Mickle was also a colleague of Wu's and Li had worked with Mickle as a PhD student in 1988. Mickle and Li developed the human myocyte cell cultures used to assess alternative storage solutions for prolonged hypothermic preservation and trained an additional technologist for the combined team.

The team also collaborated with Mario D'Costa, PhD and clinical biochemist, whose laboratory assisted Weisel's team with measurements of surfactant phospholipids and surface active properties of the secretions

Dr Weisel also indicated the collaborations between the heart and lung groups to be extremely valuable. At the time Weisel was working very closely with Joel Cooper, former head of the transplant programme, who did the first lung transplant at TGH, and Alex Patterson, who subsequently led the lung preservation and transplantation programme. Both the heart and lung groups continue to collaborate.

28.5.4 Facilities

This research was conducted at Toronto General Hospital, which housed excellent palliation for patients with cardiac and pulmonary diseases as well as a growing heart and lung transplant programme (Weisel interview, 2008).

The concept of clinicians and basic scientists working together was facilitated by a decision made around 1980 by Alan Hudson who was the Surgeon-in-Chief and later became the Chief Executive Officer of the hospital. Hudson led a change that directed that allocation of clinical resources be based on academic productivity. Dr Weisel claimed that was a tremendous facilitator to the development of academic excellence in the institution. It was a paradigm shift from focusing on clinical care to also doing research. Weisel said that the university has been supportive of the concept. The Toronto General Research Institute (TGRI) as part of the University Health Network, Canada's largest hospital and a major teaching hospital of the University of Toronto, was just starting in the early 1990s as the arm for graduate development. The institute has developed dramatically.[1]

Dr Weisel recalled that the laboratory space was good, although the quality of the equipment was variable and depended on what was needed and available within his laboratory or the laboratories of those willing to share. In the early 1990s there was no institution regulated or formal review process involved in obtaining resources. Weisel

[1] In 2007, the TGRI housed 188 researchers, 304 trainees and 366 technical/support staff over 244,000 square feet of research space. TGRI received Can$67 million in external funding.

recalled that his desk was in close proximity to the head of medicine's desk, which was 'very beneficial to get space'.

Dr Weisel claimed that the community, networking and sharing at TGH was very good and better than it was in Boston, where he had trained. One challenge at TGH then and now is that it is so big, and there are so many people working there, that it can be difficult to find who and what one needs. Li also mentioned that the collaborative environment at TGH was excellent and a strength of their group.

28.5.5 Research environment:

The research environment at the University of Toronto, where Dr Weisel had to do all of his ethics approvals, was much more relaxed than it is now. In the early 1990s the 'ethics process' involved a few colleagues who would get together and discuss their projects. Decisions were made on what seemed logical, there were no hard and fast rules. Weisel claimed the process is now more bureaucratic, with more paperwork, but reasonable as it affords necessary precautions.

28.5.6 Other facilitators/barriers

Dr Weisel mentioned that facilitators to this and subsequent research projects have been the networks within the Canadian Cardiovascular Society (CCS) and the American Heart Association (AHA). The AHA more so than the CCS was an opportunity to bring together basic and clinical scientists, allowing people to look at the same problems from different angles. It also included people from different domains such as stroke engineers, electrophysiologists and biomechanics.

A barrier to this research project and others is the difficulties clinicians face in finding time away from their clinical duties to do research. Dr Weisel said this was and still is a major challenge.

28.6 Stage 3 – primary outputs from research

The overall finding of the study confirmed the value of the techniques used in Toronto. Cold crystalloid cardioplegia and topical hypothermia for heart–lung allograft preservation provided the same level of protection as more complicated techniques. Through their evaluation of various solutions that can be used to preserve the heart during transplantation, their work suggested that a blood solution was better than a saline-based solution. The University of Wisconsin solution (UWS) was found to be the best solution to use as it can preserve the heart cells much longer in cold conditions.

28.6.1 Knowledge production

The PI indicated that 28 papers listed on his curriculum vitae were directly related to this grant funding, although it should be mentioned that this funding contributed to a much larger research programme and body of work, thus it was difficult to attribute publications to a specific grant. Of them, we will outline the findings of the following papers in efforts to show the range of findings related to the case study grant as attributed by the PI, where the latter two show the progression from the in-vivo model to an animal heart model and then to a clinical trial involving thousands of patients.

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5. Ikonomidis, J.S., T. Shirai, R.D. Weisel, B. Derylo , V. Rao, C.I. Whiteside, D.A.G. Mickle and R-K. Li, 'Preconditioning Cultured Human Pediatric Myocytes Requires Adenosine and Protein Kinase C', *American Journal of Physiology*, Vol. 272, No. 3, Pt. 2, 1997, pp. H1220–H1230.

The first paper (Fremes et al., *Journal of Surgical Research*, 1991) references previous studies conducted by the team using human cell cultures that suggested the UWS may be preferred for prolonged cardiac storage because it contains adenosine, which was believed to maintain adenine nucleotides better than other storage fluids. Using human cardiomyocytes isolated from left ventricular biopsies, the team tested the preservation abilities of four solutions: Stanford cardioplegia, phosphate-buffered saline, modified Euro-Collins and UWS. The team found that Euro-Collins was not the best solution to flush and cool the lungs. The high potassium, intracellular solution may have induced pulmonary endothelial injury. The team concluded that more work was required to develop an extracellular solution to flush the lungs and permit more rapid cooling of the lung allografts prior to transplantation. This paper concluded that UWS does preserve adenine nucleotides better than other storage fluids and may improve clinical results of cardiac transplantation.

In the second paper referenced above (Fremes et al., *Journal of Thoracic Cardiovascular Surgery,* 1991), they acknowledge that ideal storage conditions (solutions and temperature) could extend the current limits of hypothermic storage of cardiac allografts when times are greater than four hours. Using human endothelial cells and ventricular myocytes, the team screened the same four solutions and various temperatures (0, 4, 8, 24 and 36 °C) to evaluate hypothermic storage. These studies concluded that myocytes were more sensitive to prolonged preservation than endothelial cells. The techniques used were thought to be helpful as a model of prolonged hypothermic storage.

The third paper (Tumiati et al., 1994) describes a method to study ischaemic and reperfusion damage in cultured ventricular cardiomyocytes. The cardiomyocytes were made 'ischaemic' by oxygen deprivation and volume restriction. Reperfusion was simulated by bathing cells in a large volume of normally oxygenated phosphate-buffered saline with

glucose (PBSG)[2] following 'ischaemia'. The team observed that 90 minutes of 'ischaemia' caused cell structural changes and alteration of metabolism, while a prolonged 'ischaemic' interval of 120 minutes exacerbated these abnormalities. The team concluded that their in-vitro model closely mimicked in-vivo conditions.

The 1995 paper written by Fremes et al. describes the results of a study that evaluated whether adenosine pretreatment is cardioprotective for prolonged cardiac storage and whether the presence of adenosine in the storage media affects the results. Rat hearts were subjected to normal perfusion or to a solution supplemented with adenosine (UWS) for ten minutes followed by adenosine-free perfusion (St. Thomas' Hospital II solution) for ten minutes. Hearts were then stored for eight hours at 0°C in either an adenosine or an adenosine free solution. This study suggested that the beneficial effects of adenosine pretreatment were independent of which storage solution was used. These studies suggest that adenosine pretreatment improves recovery after prolonged hypothermic storage and that the presence of adenosine in the preservation solution does not alter the results. The experiments provide further evidence that extended myocardial protection is better enhanced with UWS than with St. Thomas' Hospital II solution.

By 1997, the research team had moved to study clinical myocardial protective techniques by reviewing cases of 7,334 patients undergoing coronary artery bypass grafting (CABG) between 1982 and 1986. Since many institutions had switched from unoxygenated crystalloid cardioplegic solutions to oxygenated blood cardioplegia solutions in the mid 1980s[3], this reflective study allowed the team to see the longer term effects of using the blood solution. The paper by Ikonomidis et al. 1997, demonstrates that continuous cardioplegic strategies may help resuscitate the ischaemic myocardium and reduce operative complications. The paper does recommend further refinements in cardioplegic solution temperature, direction of delivery and additives to 'precondition' the myocardium against ischaemic damage would further improvement myocardial protection.

Bibliometric analysis was conducted on 24 out of 28 publications identified by the PI as directly related to the case study grant. Publications have been excluded from the analysis if the publication type is not a primary research publication (ie article, review, notes). 21 out of 24 articles were included in the citation analysis as three articles that were included in the analysis of case study outputs were not indexed in the Web of Science and thus could not be included in the citation analysis. Table 28-1 shows the results of the publication output and impact.

[2] PBSG is a solution made of calcium chloride, magnesium chloride and glucose.

[3] TGH converted from crystalloid to intermittent cold blood cardioplegia in 1985.

Table 28-1 Publication output and impact

Number of journal articles:	24
Number of articles included in citation analysis:	21
Total number of citations (all papers):	327
Aggregate relative citation impact:	0.85 (Class III)
Self-citations:	18%

Distribution of publications across relative citation impact classes:

	Class I (uncited)	Class II (>0 and < 0.8 citations)	Class III (0.8 to 1.2 citations)	Class IV (1.2 to 2.0 citations)	Class V (> 2.0 citations)
Number of publications	1	11	2	4	3
Proportion of total output	5%	52%	10%	19%	14%

Most highly cited publication[4]:	Ikonomidis, J.S., T. Shirai, R.D. Weisel, B. Derylo , V. Rao, C.I. Whiteside, D.A.G. Mickle and R-K. Li, 'Preconditioning Cultured Human Pediatric Myocytes Requires Adenosine and Protein Kinase C', *American Journal of Physiology*, Vol. 272, No. 3, Pt. 2, 1997, pp. H1220–H1230
Times cited:	51

28.6.2 Dissemination

Dr Weisel said that the knowledge created by his team and his colleagues at TGH 'was ahead of the international community.' His team disseminated their work through peer-reviewed papers; the cardiac surgery journals were the usual targets as cardiologists did not pursue publications in journals outside of their area.[5] They would also disseminate their findings via meetings, most commonly the Canadian Cardiovascular Society (CCS), the Society for Thoracic Surgeons (STS), the American Heart Association (AHA) and the American Association for Thoracic Surgery (AATS), as well as bi-annual cardiac surgery meetings. These meetings typically included users of the research such as clinicians, scientists, commercial entities and policymakers. In addition Weisel presented 'Cardiac Preservation for Transplantation' at the National Disease Research Institute (NDRI) meeting in Washington, DC, in September 1990, 'Myocardial Preservation for Coronary Reoperation' at the 25th postgraduate programme for the STS, Orlando, FL, February 1992 and 'New Developments in Cardioplegia' as a visiting professor to a research seminar at the University of Ottawa Heart Institute in 1993. Weisel and/or members of his team conducted poster presentations and were invited speakers nationally and internationally. Weisel also claimed to have presented the findings related to the case study grant to non-

[4] Citation count extracted April 2009.

[5] There has been a major, recent shift in that cardiologists are no longer just publishing in their area of select specialty journals. This has dramatically improved the ability of cardiologists to communicate beyond their speciality. Now they are using all of the available technology and influences (ie stem-cell research) and as a group are very involved in what scientists are doing.

academic audiences during a presentation to the Montreal Heart Institute where family doctors and community based health care providers were in attendance.

Knowledge was also disseminated to international trainees who worked at TGH. At the time Dr Weisel remembered two trainees from Japan who would have known about his team's studies, progress and techniques. Dr Li mentioned that he promoted the in-vitro model, telling his colleagues about the good model and its abilities to yield results in the cardiovascular field[6]. The team shared their data nationally and internationally.

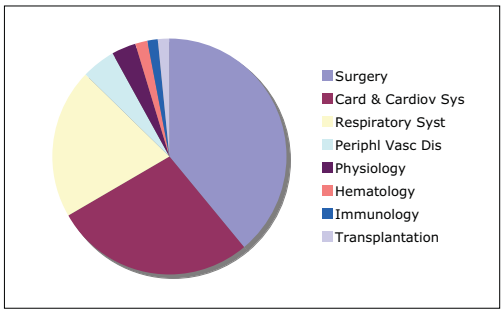
Dr Weisel and his team do not make an effort to disseminate their findings to patient groups. Weisel believes dissemination to patients would occur through physicians providing tertiary care.

The bibliometric analysis also investigated knowledge diffusion. It was found that Weisel and his team most commonly publish in surgical journals. Their work is most commonly cited by those working in cardiology and cardiovascular systems in the United States.

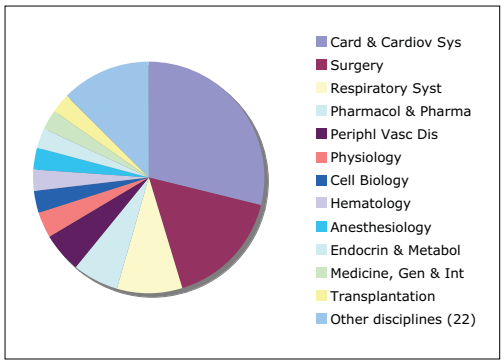
[6] Dr Li published two articles about his methods (Tumiati et al., 1995, and Li et al., 1996).

Figure 28-2 Knowledge diffusion: fields of research of output (a), fields of citing papers (b) and countries of citing papers (c)

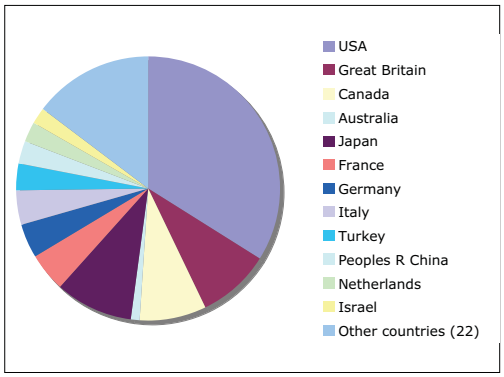
(a)



(b)



(c)



28.6.3 Training and career development

Dr Weisel said that his participation in this grant definitely did influence his career as he became much more involved in transplant preservation than he had been in the past, due to the focus on ischaemic reperfusion, now known as 'preconditioning'. Weisel has since left this area of research and now focuses on stem cell research; however the area of preconditioning remains a large part of the cardiac research programme at TGH. A key player in this area currently is Vivek Rao, one of the case study interviewees, who is the present head of the transplant programme at TGH. Rao took over the research programme started by Weisel and his colleagues and remains well funded.

Of the co-applicants, Dr Feindel subsequently became a professor of cardiac surgery and continues to work on preconditioning. Dr Patterson has since left TGH and now resides in the United States where he is President of the AATS and also works for the International Society for Heart and Lung Transplantation (ISHLT).

Ren-Ke Li, MD, PhD, received his faculty position in July 1993. From 1992 to 1993 he was a postdoctoral fellow working for Dr Weisel. Li is currently a senior scientist within the Division of Experimental Therapeutics at TGRI. Due to the limited number of transplants annually and the limited opportunity for influencing practice, Li decided to change the focus of his research, now focusing on finding new therapeutic means to improve cardiac function after heart attack. In 1993 he started a new programme called 'cell therapy' that used a patient's own cells to improve their own heart. Now this is widely known as 'cardiac regeneration'. Li explained that cardiac regeneration could be simpler that transplantation and mean no rejections and no preservations of organs. He mentioned in his interview that at the time of this grant he was looking to collaborate with many people as he was starting his laboratory. He also commented that participating in this successful project was helpful in that it generated many published papers.

Of the other members of the research team, Fred Arbour, who was a student at the time, went on to have a successful career at Sick Kids Hospital in Toronto. He is now retired.

Ren-Ke Li completed his fellowship and is now a full professor at the University of Toronto and a senior scientist and Head of Space Allocation of the Research Institute at TGH, where he directs the allocation, development and utilisation review of research space; and leads the space planning and development initiatives for the Research Institute. Dr Keeley is a senior scientist in the cardiovascular research programme at the Hospital for Sick Children, and he is a professor in the departments of biochemistry and laboratory medicine and pathobiology, and a member of the Research Advisory Committee of the Heart and Stroke/Richard Lewar Centre of Excellence at the University of Toronto. He is a former member of the board of directors and the Research Policy Committee and a current member of the finance committee of the Heart and Stroke Foundation of Ontario.

Of the two students, Steve Fremes, who worked with Dr Weisel, is now Head of Cardiac Surgery at Sunnybrook. Shaf Keshavjee who was interested in lungs and worked with Dr Patterson, continued on to complete a MSc and is now Head of Thoracic Surgery at the University of Toronto, as well as head of the lung transplant programme.

This research programme has also led to citations in textbooks intended for the teaching of healthcare practitioners and scientists. For instance, as seen in *Cardiac Surgery in the Adult*, in the chapter on myocardial protection, Dr Weisel and his colleagues are referenced three times, outlining some of the team's major findings from reperfusion and optimal temperatures for cardioplegia solutions required for best possible organ function (Verma et al., 2002; Teoh et al., 1986 and Hayashida et al., 1994).

28.6.4 Benefits to future research and research use

Through this study the team identified which solution of four was better for cardiac preservation. From there the team advanced to animal models, which gave the team further insight into optimal conditions of preservation. The project has continued to progress to this day and in part has led a successful ever-improving transplantation programme that

still exists at TGH, despite there being a relatively small target population (ie it remains a challenge to match donors to recipients and thus the number of annual transplantations is small). Within Canada there are 40,000 heart operations a year and 162 transplants yet it remains an important area research. A key player in this area of research is Vivek Rao, who has found that better protection of the heart can help circumvent the need for late graft vasculopathy, which is the major cause of morbidity and mortality after heart transplantation. Dr Weisel explained that in the 1990s, surgeons used to put the organ in, see if the patient would recover, and then in patients who did recover, observe complications within 20 years due to rejection (vasculopathy). It has since been found that better protection of the heart may reduce the later onset of vasculopathy. The team is still searching for optimal methods of protection, preservation and transplantation of donor hearts (Sheshgiri et al., 2008, and Delgado et al., 2009).

Within the bounds of the case study grant, Dr Weisel and his team were using the cultures to test different agents. The fact that the tissue cultures did grow in the heart brought a new paradigm, stimulating the team to investigate what the mechanisms of the heart cells were and what they do. Weisel said that the 'tissue cultures [used in this study] were very successful and have expanded dramatically' (Weisel interview, 2008). While the team did not create the technique for the tissue cultures they did adapt it for this use. Weisel believes he and his team were the first to use human tissue cultures for this type of intervention. Dr Li has built on the techniques and ideas of the case study grant and proposed to grow heart cells and transplant them back into the heart and see if they will continue to grow. The team first published this proposal and their initial results with in-vivo transplanted rat cardiomyocyte (Li et al., 1996). Li is now growing human heart cells and working to transplant them back into the heart and see if they will continue to grow (Al-Radi et al., 2003). This was the initial concept of cell transplantation, dating back to the 1990s, which has spawned millions of dollars of funding in every heart centre globally. The studies funded by the index grant stimulated the researchers (led by Dr Li) to perform an extensive series of investigations that are credited with demonstrating that cell transplantation improves heart function after a myocardial infarction. Initially, the group believed that the implanted cells became functioning cardiomyocytes, but subsequent studies by the group demonstrated that heart function improved because of the paracrine release of factors that prevent cardiac thinning and dilatation. In addition to using the technology to develop the concept of cardiac cell transplantation, the team's revised techniques are now used in protocols to treat diabetes, joint disease and cell therapy for Parkinson's and Alzheimer's diseases.

Dr Rao, who now leads the programme on heart preservation, said that in the mid 1990s this research programme also explored various myocardial protection studies, where the team investigated peri-operative myocardial protection in low-risk patients who were having bypass surgery.

In 1997 Dr Weisel participated in a large blood crystalloid trial at the University of Toronto. Through this trial the team proved that the new technique, which involved perfusing the heart with a blood cardioplegia solution, was substantially better at protecting the heart than the old method, which involved a crystalloid solution, although technically it is much more difficult. The team also learned that the heart did not have to be as cold, as Dr Shumway, who would put the heart on ice, had initially thought. It was

found that if the organs were kept warmer, metabolism can continue and is more receptive to perfusion during the time when it is not working (Christakis, et al., 1997).

In the late 1980s and early 1990s, the many cardiologists, including Dr Weisel and his team hypothesised that by putting the heart through three cycles of imposed ischaemia for a brief period (about five or ten minutes) followed by reperfusion, it becomes resistant to prolonged ischaemia. Simultaneous research has revealed that the heart (and likely the lungs as well although this has still not been verified empirically) has an endogenous mechanism that allows it to protect itself from injury naturally. This process is known as preconditioning and it is now known to happen naturally within the heart. Thus surgeons believe that if you can complete this process you can prevent ischaemic injury from happening. Pharmaceutical companies are interested in preconditioning and are working to create a drug that would artificially induce the process, thus making the heart and perhaps the lung resistant to ischaemic injury. The team has identified some potential drugs. They have even conducted some very large clinical trials at various phases in the production cycle. At this point in time results are promising, although they do not meet expectations of preclinical work.

Industry has also been involved in the blood cardioplegia studies, exploring potential additives to optimise results. No patents have arisen from this grant, although some discoveries made in subsequent projects looking at mechanisms have been patented.

28.7 Stage 4 – secondary outputs

Through the Cardiac Care Network of Ontario (CCN) and the Canadian Society of Transplantation (CST), Weisel and his team have made recommendations to the government about funding various programmes and best practices for other institutions, based on consensus. The AHA, the American College of Cardiology (ACC), American College of Surgeons (ACS) and the Transplantation Society produce guidelines pertaining to procedures that have proven to be beneficial.

Dr Weisel and subsequently Dr Rao's work has been referenced in three guidelines produced by the AHA and the ACC guidelines on protecting the heart for cardiac surgery:

1. The Management of Heart Failure
2. Listing and Management of Transplant Patients
3. Artificial Heart Technologies.

The recent peri-operative guideline also includes many references to Weisel's work.

Dr Weisel is cited in the 'ACC/AHA 2006 Practice Guidelines for the Management of Patients with Valvular Heart Disease'.

The three clinicians involved in this research project, Drs Weisel, Feindel and Patterson, have been involved in guideline preparations at both the national and international level. Weisel explained that most of the guidelines for use within Canada originate from American sources, such as the AHA or ACC, which are then adapted for the Canadian environment through Canadian guidelines. Weisel believed that Feindel was the member

of the executive committee of the CNN and the CST, although the author could not confirm this statement.

This research has also spawned another group at Sick Kids Hospital who are looking at paediatric transplants. The information produced by Dr Weisel and his colleagues was helpful to the paediatric surgeons who needed information on how to better protect transplant organs. In the 1990s 'the results of infant transplantation were terrible and now they are superb – we are a world leader here' (Weisel interview, 2008).

28.8 Stage 5 – adoption by practice and the public

The key finding from the case study grant, and subsequent studies, was that optimal storage temperature of the heart was not as cold as Dr Shumway had originally thought. Dr Rao explained that depending on the location of the donor, he often does not take heart down to four degrees Celsius, on ice. If the donor is coming from 1-2 hours away he will put the heart in a cold preservation saline solution, without ice. The team has found convincing evidence in these studies that freezing the heart is detrimental, however, if the heart is coming from far away (ie 4-6 hours away), surgeons at TGH do still cool the heart to nearly four degrees Celsius.

One of the solutions that were evaluated during the case study grant for lung preservation is known as low molecular weight dextran solution. It was subsequently validated clinically by Dr Patterson's group, as well as six other groups who were looking at similar approaches. It is now used not only at TGH but worldwide as preservation solution of choice (Yamazaki, et al., 1990 and Maccherini et al., 1991).

Blood cardioplegia is used by most transplant centres in North America, although researchers are still looking to optimise results by exploring different additives and their appropriate doses. Solutions and approaches need to be adapted for the type of operation to ensure feasibility and safety. This surgical intervention has been tested at various levels from small-scale trials to large-scale trials and is now widely used. The concepts used by the team with regard to the vascular endothelial cells have been further adapted to different situations, such as preserving the brain during heart or even brain surgery, since the brain also involves endothelial functions.

28.9 Stage 6 – broad health and economic outcomes

All of this material from the PI's laboratory as well as other laboratories worldwide has resulted in tremendous improvements in the outcomes of cardiac and lung transplantation and surgery.

In part, as a result of the studies conducted in Dr Weisel's laboratory, through the funding investigated in this case study grant and subsequent research that arose from it, cardiovascular surgeons now protect the heart for cardiac surgery using blood cardioplegia solutions. The heart is now better protected for surgery and transplantation. Scientific advances in heart surgery have reduced the mortality rate from about ten percent to a current rate of about one to two percent. Results for transplantation have also improved

progressively. Problems of organ rejection and immunosuppression still remain for heart transplant recipients, affecting long-term mortality. Better preservation of endothelial function at the time leading up to and during transplantation has been shown to improve long-term results. Furthermore, in the late 1980s and early 1990s when heart transplantation was new, there was about a ten to twenty percent incidence of primary graft dysfunction, where the heart did not function well and required a lot of support. This grant was initially submitted in this time period when clinicians were trying to better preserve the organs. Weisel told us that the current incidence of graft dysfunction at TGH is down to about five to ten percent. This is due in part to better management of recipient and donor and better selection criteria of both recipient and donor.

The ISHLT posts on their website various statistics collected worldwide from various centres around the world that perform heart and/or lung transplants[7]. As reported to the ISHLT, the success rate of heart transplants has increased since the 1980, as shown in Figure 28-3. The success rate of lung transplants has also increased although in more modest numbers, as shown in Figure 28-4. Survival was calculated using the Kaplan-Meier method, which incorporates information from all transplants for whom any follow-up has been provided. Since many patients are still alive and some patients have been lost to follow-up, the survival rates are estimates rather than exact rates because the time of death is not known for all patients. The half-life is the estimated time point at which 50 percent of all of the recipients have died. Survival rates were compared using the log-rank test statistic.

It is expected that further health outcomes will be observed in the future, as data is collected for 10–20 years after transplant. These findings will help researchers understand how to better affect long-term outcomes at the time of transplant.

[7] Since 2003, approximately 200 centres worldwide reported carrying out heart transplants to the ISHLT, while just over 100 centres worldwide reported conducting lung transplants. In 2006, about 30 centres worldwide reported doing a heart–lung transplant.

Figure 28-3 Adult heart transplantation: survival by era

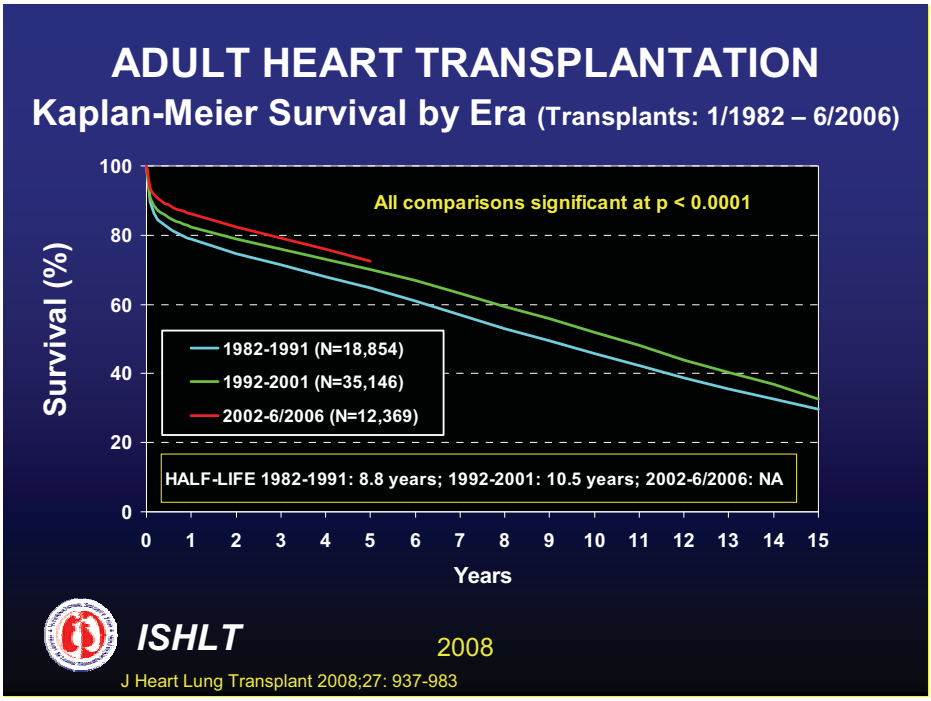
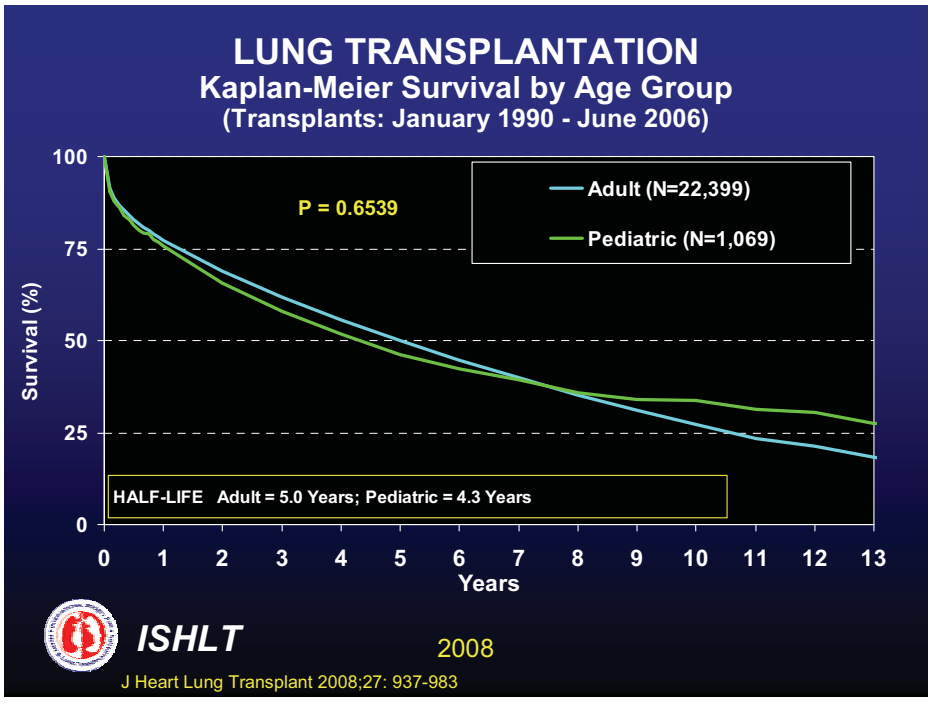


Figure 28-4 Adult lung transplantation: survival by age



28.10 Payback

The payback framework describes the process from research to several categories of impact. The categories allow us to compare across different case studies. Table 28-2 shows, in point form and by impact category, some of the impacts, described more fully above, that have emerged from this grant.

Table 28-2 Payback

Payback category	Impacts from case study
Knowledge production	• 24 directly related peer-reviewed articles • Found blood cardioplegia solutions to be more effective than crystalloid solutions
Research targeting and capacity building	• Presented findings at various meetings across North America including AHA and CCS, and meetings directed at various audiences • Knowledge transfer within laboratory to students (domestic and foreign), post docs • 2 PhDs obtained • Techniques taught by Mickle and Li to fellows • Refinement of techniques adapting to future needs within science
Informing policy and product development	• Working with industry to make the blood cardioplegia solution and its additives are widely available • Work referenced by national and international guidelines • Working with pharmaceutical companies to develop drug that will artificially induce preconditioning process • Widespread use of dextran solution in lung preservation
Health and health sector benefits	• Overall reduction in mortality following cardiac surgery and cardiac transplantation
Broader social and economic benefits	• None

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CHAPTER 29 Stroke prevention in the elderly in primary care

29.1 Overview of case study grant

29.1.1 Overview

Dr (now Professor) Peter Whincup and his co-applicant Professor Shah Ebrahim thought that the number of strokes suffered by older people could be reduced by addressing stroke risk factors, especially high blood pressure, in primary care settings. Whincup and Ebrahim, were clinical epidemiologists at the Department of Public Health and Primary Care, Royal Free Hospital School of Medicine, London. The department hosted a long-running cohort study, the British Regional Heart Study (BRHS), which had started in 1975 with a sample consisting of almost 10,000 middle-aged men selected from 24 towns around Britain. The main drivers for Whincup and Ebrahim were their clinical interest in reducing the prevalence of strokes through the better application of existing evidence and the timely opportunities provided to use the institution's database. They developed a multi-faceted proposal and applied to the Stroke Association (SA) for £139,630 to conduct a three-year study. The aim was to examine the management of risk factors for stroke in primary care in patients aged 60 years and older. The SA agreed to fund the project but only for the first two years at a cost of £91,019.

Some elements of the project proceeded exactly as planned but others did not, partly as a result of emerging findings and partly because of the reduced time and funding. The first of two journal publications that resulted from this project described a simple scoring system that was based on their analysis and could be used in general practice to identify men who would benefit from further intervention. The second article described the results of a survey of current practice among general practitioners (GPs) about the identification and management of risk factors for stroke. This showed that there was scope to increase the benefits of stroke prevention in primary care by focusing on the management of patients at high absolute risk. The team built on the original project in a follow-up randomised controlled trial (RCT) that evaluated the implementation of an approach they devised aimed at addressing the need they had identified for improved management of risk factors for stroke in primary care. Although the intervention did result in better identification of the risk factors, it did not seem to bring about the improvements in management that they had identified as being desirable. Nevertheless, the original project made an impact in various other ways, including on other further research. For example,

while the risk-scoring system had not been developed specifically to be a research tool, the team used it in at least one further important piece of research, as did some other research teams. The project had some impact on the career development of the PI and the research fellow, Dr William Coppola. Members of the team gave a range of conference presentations and engaged in other dissemination activities aimed at particular healthcare providers. Ebrahim used the findings both in a major systematic review, where they provided important evidence, and in a textbook on stroke. The findings from the original project probably had some impact, albeit relatively minor, on policies in several ways. For example, the research informed both a guideline from the international Systolic and Pulse Pressure Working Group (Black et al., 1999) and the relevant protocols of some local health authorities in the UK. As a result of these and other activities, the project possibly had a slight impact on some practitioners. Therefore, the project possibly made a small contribution to the reduction in mortality and morbidity from stroke as a very minor part of the evidence about how to recognise risk factors for stroke and the need to improve management, but this is impossible to quantify.

29.2 Introduction to the case study

29.2.1 Background

Stroke was known to be a major cause of mortality and morbidity, and calculations at the end of the 1980s suggested that stroke patients occupied a total of 5.78 million hospital bed days annually and accounted for 3.9% of the National Health Service (NHS) budget, not counting community support services (Office of Health Economics, 1988). While mortality rates from stroke were falling, in the early 1990s it was thought likely to remain a major burden on health service resources for the foreseeable future (Whincup and Ebrahim, 1991). Several key risk factors for stroke had been identified; among them were high blood pressure, which was thought to account for 60% of all strokes (Dennis and Warlow, 1991), smoking and pre-existing heart disease.

This project examined the possibility of addressing risk factors for stroke in primary care settings. Dr (now Professor) Peter Whincup, the PI, and his co-applicant, Professor Shah Ebrahim, were medically trained clinical epidemiologists based at the Department of Public Health and Primary Care, Royal Free Hospital School of Medicine, London. In the early 1990s, the Royal Free Hospital was the most prolific centre for publications on cardiovascular disease (CVD) in the UK according to some background analysis conducted for the current project. The Department of Public Health and Primary Care hosted the BRHS, which started in 1975 following a proposal successfully submitted to the Medical Research Council (MRC) from Professor Gerry Shaper and colleagues (Walker, Whincup and Shaper, 2004). The MRC supported the BRHS from 1975 to 1985; since then it has been funded by the British Heart Foundation (BHF) and the Department of Health in England, with other funders supporting specific studies linked to the BRHS. The sample, which consisted of almost 10,000 middle-aged men, was selected from 24 towns around Britain. In this prospective study, potential risk factors for CVD were measured at initial entry to the study, and consultation patterns of individual subjects with their GP have been recorded.

The initial focus of the BRHS was on the prevalence and incidence of CVD and their relationship to established behavioural and biological risk factors (Walker et al., 2004). A strategy for use in general practice to identify men at high risk of heart attacks had been developed as early as 1986 (Shaper et al., 1986). With increasing duration of follow-up, a wider range of outcomes was introduced and other conditions were added to the BRHS. A later study, the Nine Towns Study of Blood Pressure, provided additional data on risk factors for CVD in women in nine of the 24 towns (Bruce et al., 1990).

Dr Whincup had been at the Royal Free Hospital since 1985, and by the time of this grant was starting to become an independent researcher. From 1985 to 1990 he was a Wellcome Training Fellow in Clinical Epidemiology and conducted work on the early development of heart disease. This led to various well-cited publications, including one in the *British Medical Journal* on blood pressure in children aged 5–7 years, which has been cited more than 100 times (Whincup, Cook and Shaper, 1989). He also worked with colleagues in the Department of Public Health and Primary Care on projects linked to the BRHS, including work published in the *Lancet* on the association between serum albumin and CVD, which has been cited almost 200 times (Phillips et al., 1989). From 1990 to 1991, he was a BHF Intermediate Research Fellow and became Senior Lecturer in the department just before submitting the application in 1991.

29.2.2 The case study approach

For this case study the PI and the research fellow were interviewed. Documentary and bibliometric analysis was conducted of: the papers from the research team describing the research, various papers that cited the original research, a small number of documents identified by searches of citations received by the papers and of Google/Google Scholar, and copies of various presentations made by the research fellow. The original application was available by the SA, and the PI provided an abridged version of his curriculum vitae.

29.3 Stage 0 – topic identification

The main drivers behind the research were the PI's clinical interest in reducing the prevalence of strokes through better application of existing evidence and the timely opportunities provided to use the institution's database.

29.3.1 Clinical interest

Whincup and Ebrahim believed there were inadequacies in the management of stroke risk in the population and potential opportunities to reduce the prevalence of strokes through better application of the existing evidence. In particular, it looked very much as if older people were not being offered treatment for the degrees of high blood pressure that would have led to treatment in people of middle age and yet older people had a much higher risk of developing CVD. The team therefore felt there was a potential inequality in the management of this health problem (Whincup interview, 2008).

In 1990 Professor Ebrahim had argued that effective management of high blood pressure in subjects older than 60 years could reduce the incidence of stroke by up to 20% (Ebrahim, 1990). In their research proposal, Whincup and Ebrahim highlighted recent trials that had also shown the health gains that could result from treating hypertension in

elderly people (Whincup and Ebrahim, 1991). Examples included the Swedish Trial in Old Patients with Hypertension (STOP-Hypertension) (Dahlöf et al., 1991) and the Systolic Hypertension in the Elderly Program (SHEP) trial (SHEP Collaborative Research Group, 1991). They also referred to observational studies that suggested that the risk of stroke was lower in ex-smokers than in current smokers, which implied that the risk of cigarette smoking is likely to be at least partly reversible.

In the proposal, Whincup and Ebrahim argued that primary care represented the most obvious setting for a strategy of stroke prevention (Whincup and Ebrahim, 1991). They claimed that little was known 'about the criteria for treatment of high blood pressure in primary care, especially in elderly patients, for whom antihypertensive treatment has not usually been recommended in the past'. Furthermore, the feasibility had not yet been established of altering smoking, drinking and exercise patterns in those at risk of stroke by health promotion in primary care.

29.3.2 Timely opportunities provided to use the institution's database

It was seen as particularly timely to conduct research to explore issues around the management of stroke in primary care in more detail – a point confirmed by the inclusion of stroke as a key target in the government's then recently published health policy green paper *The Health of the Nation* (Department of Health, 1992). Professors Whincup and Ebrahim recognised that the BRHS, which was based at their department, would provide an excellent setting for various parts of the project. Indeed, the proposal was developed with extensive discussions with Professor Gerry Shaper, who was still Director of the BRHS and who recognised the possibilities of using the BRHS framework for this study.

29.4 Interface A – project specification and selection

Dr Whincup, with support from Professor Ebrahim, developed a multi-faceted proposal and applied to the SA for £139,630 to conduct a three-year study. The aim was to examine the management of risk factors for stroke in primary care in subjects aged 60 years and older. The specific objectives were (Whincup and Ebrahim, 1991):

1. to develop a system for identifying subjects who are at high risk of stroke, which could be applied in both men and women
2. to examine current practice in the measurement and control of risk factors for stroke, particularly high blood pressure, in an existing network of general practice
3. to examine the feasibility of using non-pharmacological measures (including particularly smoking cessation, weight control, moderation of alcohol intake and the introduction of regular physical activity) to reduce the risk of stroke in high-risk subjects
4. to develop a protocol for the prevention of stroke in subjects aged 60 years and older in a primary-care setting, which can be monitored and evaluated in an RCT.

The final objective envisaged that the RCT would be undertaken in a further project, but even the first three elements involved a variety of approaches to data collection and analysis.

The SA agreed to fund the project but only for the first two years at a cost of £91,019. It was suggested that the team would be eligible to apply for the third year's funding but that no guarantee of success could be given. No guidance was given as to any parts of the study that could be reduced or cut out.

29.5 Stage 1 – inputs to research

29.5.1 Facilitators

The SA provided £91,019 for the first two years; no application seems to have been made for the third year's funding, but, as discussed below, an application was successfully made to the SA for funding to undertake a proposed follow-on RCT. The funding was mainly to pay for a clinical research fellow, who it was hoped would have some experience of primary care. Whincup and Ebrahim used some of their medical school-funded research time to work on the project. There was also some support from the BRHS in the form of its administrator, Mary Walker, and the statistician, Olia Papacosta.

At the time of the application Professor Ebrahim was a more senior researcher, but in 1992 Dr Whincup was to become a co-applicant on the next round of funding for the BRHS, which received about £100,000 a year in total, mostly from the BHF but with a separate stream of funding from the Department of Health.

29.5.2 Knowledge and expertise

The applicants were both clinical epidemiologists, with Professor Ebrahim having rather more experience, especially in relation to stroke research, than Dr Whincup, who received his PhD in 1991. The expertise available through colleagues in the BRHS was considerable. Furthermore, the research fellow recruited for the project, Dr William Coppola, was a GP and so brought that valuable experience to the team; there were also other GPs in their academic department who could be consulted.

29.5.3 Techniques

The range of techniques included: analysis of relevant data from the BRHS; a survey of GPs; an investigation of current practices through case note review in surgeries; a questionnaire survey of the attitude of patients randomly selected from the registers of the practices included in the survey of GPs; and a review of the findings to develop a proposal for an RCT. There was not thought to be anything particularly groundbreaking in the specific techniques that were used. In combination, however, the planned techniques provided a comprehensive approach.

29.5.4 Samples/study recruits

The data for the first part of the study came from the BRHS, and the original plan was that the GPs to be included in the survey would also come from those involved in the BRHS. The BRHS practice network was also used to identify half the practices to include in the case note review, with other practices from the same town making up the other half. The patients in the final survey were taken from the same practices.

29.6 Stage 2 – research process

Some elements of the project proceeded exactly as planned, but this was not the case for all elements of the project. When interviewed Professor Whincup explained that he felt that they had had to prioritise, probably because of the reduced funding and the licence it implicitly gave them, and that partly as a result of the emerging findings they decided to concentrate on the early parts of the proposal.

The data collected through the BRHS – during the initial data entry at the time the cohort was recruited and at five and 11.5 years of follow-up – was analysed to produce a scoring system to identify men at high risk of stroke. A regression analysis was undertaken on the data.

The second element of the study involved a review of current practice in the measurement and management of high blood pressure in the primary care sector. The team decided they wanted to give greater attention to this element of the work, partly because the development of the risk-scoring system suggested that the people who were at high risk could be identified very clearly, which meant that it might be most productive to put the emphasis on what could be done by GPs rather than focusing on developing health promotion programmes that would follow on the survey of patients described below (Whincup interview, 2008). The survey of GPs that formed the first part of this element was therefore more extensive than originally planned: 1,000 GPs were surveyed rather than the 200 GPs and 50 associated practice nurses originally described. To recruit this number of GPs, they had to use the services of a commercial company rather than the practice network of the BRHS.

The processes used for the remaining parts of the project are not described in any articles, but, according to a brief note in the archival material provided by the SA, case-note review, which constituted the second half of the second element, covered rather fewer practices than originally intended. Furthermore, only 1,600 patients were surveyed in the third element than the 4,800 planned.

As described later, the final part of the proposed work was incorporated into the follow-on RCT, which it had always been hoped would be funded.

29.7 Stage 3 – primary outputs from research

29.7.1 Knowledge

The two publications linked to this project are listed below:

- Coppola, W.G.T., P.H. Whincup, M. Walker, O. Papacosta and S. Ebrahim, 'Scoring System to Identify Men at High-Risk of Stroke – a Strategy for General Practice', *British Journal of General Practice*, Vol. 45, 1995, pp. 185–189.
- Coppola, W.G.T., P.H. Whincup, M. Walker and S. Ebrahim, 'Identification and Management of Stroke Risk in Older People: A National Survey of Current Practice in Primary Care', *Journal of Human Hypertension*, Vol. 11, 1997, pp. 185–191.

Coppola et al. (1995) described the simple scoring system that was derived from the analysis. Using age, systolic blood pressure, current cigarette consumption and evidence of anginal chest pain the analysis showed the highest quintile of the score identified 82% of all strokes occurring within five years. The authors claimed that: 'based on readily measured variables, this scoring system could be used in general practice to identify men at high risk of stroke who would benefit from further intervention. Effective identification of high risk individuals requires assessment of the combined effects of multiple risk factors…the work involved in intervening in the top 20% of the population has the potential for dealing with the bulk of stoke prevention required in a practice' (Coppola et al.,1995).

Coppola et al. (1997) described the results of the survey of current practice among GPs in the identification of stroke risk, management of hypertension and use of other interventions to reduce the risk of stroke. Although most GPs reported that they specifically identified patients at risk of stroke, less than one third used either age or pre-existing CVD as an indicator. Thresholds for drug treatment of hypertension increased markedly with patient age, with many GPs having a much higher threshold for treating hypertension than those recommended in the British Hypertension Society's guidelines (Sever et al., 1993). The authors concluded that: 'the results suggest that there is scope for increasing the benefits of stroke prevention in primary care, by focusing on the management of patients at high absolute risk, in whom the greatest treatment benefits are likely to be obtained' (Coppola et al.,1997).

Members of the research team regarded the second paper (Coppola et al., 1997) as probably being the more important, because it gave indications as to how management of stroke risk could be improved. This was especially important as their research clearly showed that patients older than 60 years were least likely to receive attention for high blood pressure, yet existing research showed that those with the highest blood pressures were most likely to benefit from interventions.

Table 29-1 shows the bibliometric analysis that has been conducted for this case study using the standard approach for all the case studies in this project. This involves comparing the number of citations with those received by papers in similar journals and of a similar age. During interview, Professor Whincup explained that although the team regarded these as important papers, other work he had done was published in journals with a higher impact. These papers were aimed at journals that would be most likely to be read by their target audience, ie GPs and those with an interest in hypertension, irrespective of their impact factor. Although most of the citations come from the United States and the UK, other citations come from a wide geographical spread.

Table 29-1 Publication output and impact of directly related publications

Number of journal articles:	2
Number of articles included in citation analysis:	2
Total number of citations (all papers):	40
Aggregate relative citation impact:	0.71 (Class II)
Self-citations:	15%

Distribution of publications across relative citation impact classes:

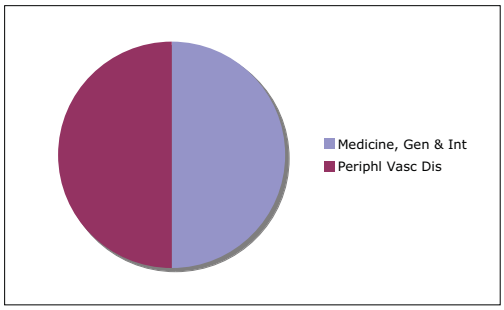
	Class I (uncited)	Class II (>0 and <0.8 citations)	Class III (0.8 to 1.2 citations)	Class IV (1.2 to 2.0 citations)	Class V (>2.0 citations)
Number of publications		1	1		
Proportion of total output		50%	50%		

Most highly cited publication[1]:	Coppola, W.G.T., P.H. Whincup, M. Walker and S. Ebrahim, 'Identification and Management of Stroke Risk in Older People: A National Survey of Current Practice in Primary Care', *Journal of Human Hypertension*, Vol. 11, 1997, pp. 185–191
Times cited:	22

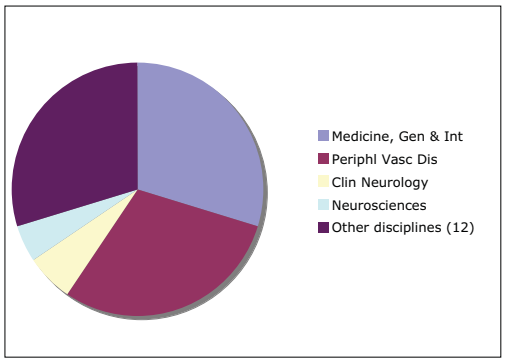
[1] Citation count extracted April 2009.

Figure 29-1 Knowledge diffusion: fields of research of output (a), fields of citing papers (b) and countries of citing papers (c)

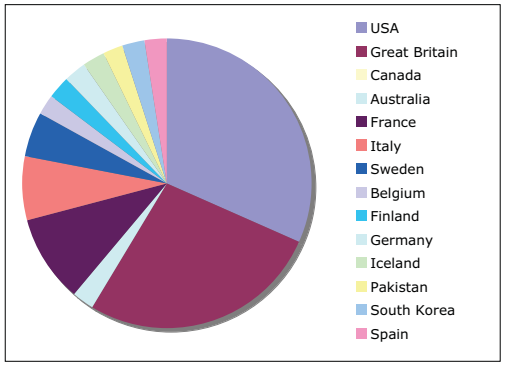
(a)



(b)



(c)



29.7.2 Benefits to future research and research use

Capacity building and career development

The study was [one of] the first Whincup had undertaken as PI, and in that regard it was an important contribution to his career development, but it did not precipitate any dramatic change in his career. Ebrahim was already a professor and it seems unlikely the project made any significant contribution to his career development. It was the first clinical research project on which Dr Coppola had worked, and it therefore brought him into academic medicine. Although for various reasons he did not develop the work into a higher degree, it did develop his research skills and statistical understanding. He remains in

the department, contributing in particular to the medical education field – for example, teaching critical appraisal where the background of having been involved in research is useful (Coppola interview, 2009).

Perhaps as a result of changes in the work undertaken in response to the reduction in funding to two years instead of three, and as a consequence of an inflation increment remaining largely unused, there was some money remaining at the end of the project. The SA agreed to a request from Professor Ebrahim for the money to be used to fund a statistician/programmer to help complete the analysis of this project and to help with setting up the follow-on project described below. The money helped retain the services of this researcher, who had been helping as part of the wider team, until she received a North Thames Regional Health Authority Research Fellowship to undertake a doctor of philosophy (PhD) degree.

Targeting further research

The research team reviewed their findings, which had led to the development of a way of identifying individuals at high risk of stroke and demonstrated the scope for better management through the application of existing evidence. They developed a proposal to conduct an RCT to evaluate a focused intervention for the prevention of stroke in patients aged 60 years and older in a primary-care setting. Professor Ebrahim led on this proposal with Dr Whincup, and the SA agreed to fund a three-year project, with additional funding from Camden and Islington Multidisciplinary Audit Advisory Group (MAAG). Dr Coppola again worked on the project. The project was completed and the findings were reported to the SA. This RCT found that there was better recording of data on risk factors in the intervention arm, but neither case-note review nor patient recall provided evidence of a change in practice in the management of the risk factors for stroke. It was suggested that perhaps the RCT would have made greater impact had a more intensive approach been used to change the way GPs managed risk factors for stroke in older people (Coppola et al., 1999). The findings were described in the SA's magazine, but the production of articles describing the project became a lower priority. This reflected both the nature of the results and the break up of the research team as members took up other roles. Furthermore, although issues about how to improve the management of stroke remained important to Whincup and the others, the main focus of his subsequent research continued to be surveys of children in relation to the development of CVD and studies linked to the BRHS, of which he has been Principal Clinical Investigator since 1998. Specifically in relation to stroke, he focussed particularly on secondary prevention and identifying the need for greater action to tackle the under-management of prevention in people who had already developed CVD.

The stroke risk-scoring system developed in the original project was not specifically designed to be a research tool. Nevertheless, it has been used in several subsequent research studies, including a study based on the BRHS of carotid plaque, intima-media thickness and CVD risk factors, which was led by Professor Ebrahim. It was funded by the SA and the researchers included Dr Whincup and other members of the BRHS team. It resulted in a publication in *Stroke* that has been cited more than 200 times (Ebrahim et al., 1999). Other research teams also used the risk-scoring system in their own studies – for example, the data collection in a study about what Italians at high risk knew about ischaemic stroke

used a risk profile determined by using Coppola et al.'s scoring system (De Dominicis et al., 2006).

29.8 Interface B – dissemination

All three members of the research team disseminated the findings as widely as possible at various conferences and on other occasions. Dr Whincup made about six presentations: four academic and two to GPs. He also worked with local health authorities. Dr Coppola also gave presentations at various conferences and meetings, including presenting the findings from the GP survey at a meeting of the European Society of Cardiology and Epidemiology Working Party of the European Society of Cardiology in Venice in 1994 and the findings and risk score at the Cardiovascular Disease Prevention conference of the British Hypertension Society in 1998. The audiences for these meetings were academics and clinicians. The article based on the findings in the SA magazine was more aimed at end-users.

Professor Ebrahim also used the findings in presentations and included them in various publications, including a major systematic review he undertook for the NHS Health Technology Assessment programme. In this review, titled 'Detection, Adherence and Control of Hypertension for the Prevention of Stroke: A Systematic Review', evidence from the GP survey provided the largest source of data in a table showing the median blood pressure thresholds and proportion of GPs treating hypertension in different patient age groups (Ebrahim, 1998). Ebrahim also included the findings in a textbook on stroke (Ebrahim and Harwood, 1999).

29.9 Stage 4 – secondary outputs

The original project, and the publications from it, possibly had a slight impact at a national and international level as a small part of a much wider body of evidence behind an increasing number of policies about how to recognise stroke risk in older people and the importance of improving the management of stroke. It is impossible to quantify this impact, but Professor Ebrahim was a member of various important groups and committees, including, for example, the Third Joint Task Force of European and other Societies on Cardiovascular Disease Prevention in Clinical Practice. This task force produced European guidelines (De Backer et al., 2003) that have been cited more than 650 times, and they seem to have cited Ebrahim's systematic review (Ebrahim, 1998).

In addition to this possible impact on major policy statements, there are a number of specific, but more restricted, policies on which the findings did explicitly have an influence. Firstly, a range of medical academics from the United States, France, Australia and Italy came together to form a, perhaps temporary, professional grouping called the Systolic and Pulse Pressure (SYPP) Working Group (Black et al., 1999). It drew on Coppola et al. (1997) as the main evidence to support the statement in its first report, or guidelines, that 'the dangers of elevated SBP levels are under-appreciated' (Black et al., 1999). Secondly, Dr Whincup had an honorary public health contract with the then local health authority, Enfield and Haringey Health Authority, in the part of London in which

the Royal Free Hospital is situated. He, and Dr Coppola, did some presentations to local GPs, and the project informed the protocol drawn up by the health authority for the management of hypertension in primary care (Whincup interview, 2008). Thirdly, Coppola was also told by a colleague that the project informed the policy guidance in the West Midlands region on risk factor modification for the prevention of stroke (Coppola interview, 2009).

Dr Whincup, and even more so Professor Ebrahim, played a role within the SA. Whincup acted as an advisor to them in the mid to late 1990s and was consulted about surveys they conducted to inform the development of policies within the SA.

All team members have drawn on this work to some extent in their teaching of medical students, and the findings helped to inform the teaching curriculum, in that Whincup ensured there was a session on the prevention of heart disease and stroke.

Finally, the growing attention given to stroke prevention at that time means that it might be worth considering the barriers to greater uptake in policy guidelines of the specific stroke risk-scoring system developed in this project. There does seem to have been a reluctance to adopt such risk-scoring systems[1], as witnessed by the fact that the one developed for heart attacks from the BRHS as far back as 1986 (Shaper, 1986) had still not been widely adopted at the time. In particular, there seemed a reluctance to adopt a separate scoring system for stroke, even though the evidence indicated that the risk factors were not identical to those for heart attacks.

29.10 Stage 5 – adoption by practice and public

There has been a gradual improvement in blood pressure control in older people as indicated by regular reports by the health surveys for England and by other agencies (Whincup interview, 2008). It can again be suggested that the original case study project possibly played some part, however modest, in that. Although it is impossible to quantify any impacts on practitioners, there are several reasons for suggesting there could have been some at least some impact:

- In the health authorities in which the researchers worked with local GPs, it is likely that there was some impact directly through contacts with GPs and indirectly through the local policies informed by the research.
- Where the project made an impact on policies, as in the other examples given above, it is possible that those policies, in turn, made some impact on practitioners in the healthcare system.
- The activities within the SA and through teaching that were informed by this study might have made some impact on practitioners and/or the public.
- Several other authors wrote pieces promoting action based partly on the findings. Two publications in the *British Medical Journal* might have been particularly

[1] When interviewed, Professor Whincup described the importance of the Framingham risk-scoring system, but also explained that there was resistance to specific risk-scoring systems, especially ones for stroke.

important, because it is the main journal that clinicians in the UK claim to read in order to, among other things, inform their clinical practice (Jones et al., 2008). Firstly, the study was cited in an article titled, 'Information Needed to Decide About Cardiovascular Treatment in Primary Care' in the 'Information in Practice' series in the *British Medical Journal*, where it was referenced as one of three scoring systems available for stroke (Robson, 1997). Secondly, an editorial in the *British Medical Journal* in 2000 calling for a radical rethink on isolated systolic hypertension used Coppola et al. (1997) as the sole evidence to support the statement: 'patients with isolated systolic hypertension remain under-recognised and undertreated' (Wilkinson, Webb and Cockcroft, 2000). This editorial closed with a strong call for action: 'It is about time that we recognised isolated systolic hypertension as an important clinical condition and changed our practice accordingly'.

29.11 Stage 6 – final outcomes

As noted, one of the main drivers behind this project was the desire to ensure greater use of the clear evidence that appropriate interventions to manage risk factors for stroke could make a big impact in terms of health gain. The account above indicates that the project might have had some impact, however small, on policy and on healthcare practice, by supplying a minor part of the evidence on how to recognise stroke risk and improve management of the risk, especially in relation to treatment for hypertension in older people. If this is correct, then the project has possibly made a very small contribution to the considerable reduction that has occurred in the mortality and morbidity caused by stroke, but this is impossible to quantify.

29.12 Payback

The payback framework describes the process from research to several categories of impact. The categories allow us to compare across different case studies. Table 29-2 shows, in point form and by impact category, some of the impacts, described more fully above, that have emerged from this grant.

Table 29-2 Payback

Payback category	Impacts from case study
Knowledge production	• Two direct peer-reviewed articles
Research targeting and capacity building	• Research team built on the original project in a follow-up RCT that attempted to implement some of the findings • Research team and other researchers have used the risk-scoring system in further research • Project had some impact on the career development of the PI and research fellow
Informing policy and product development	• Informed both guidelines in a working group statement and local protocols that could be considered to be minor policies • Possibly had a slight impact as a small part of a much wider body of evidence used in the increasing number of major policies about how to recognise stroke risk and improve management, but this is impossible to quantify
Health and health sector benefits	• Possibly had a slight impact on the reduction in mortality and morbidity from stroke as a very small part of the evidence about how to recognise risk and improve management, but this is impossible to quantify
Broader social and economic benefits	• It is not possible to identify any broader social or economic impacts

29.13 References

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