# Clinical Assessment of Renal Ischaemic Injury and the role of Cryopreservation; Peritoneal Cooling in Non-Heart-Beating Donation and Topical Cooling for Laparoscopic Surgery

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## Abstract

The project aims focussed on three main areas of study; ischaemic injury assessment, laparoscopic renal cryopreservation and peritoneal cooling for non-heart-beating organ donation. The effects of renal ischaemia represent significant challenges for transplantation and urological surgery in that with sufficient unchecked ischaemic duration, permanent loss of function is inevitable. Prior to consideration of novel approaches to ischaemic protection, aimed at producing improved graft quality for transplantation and an increased safe operating times for partial renal resections, deficiencies in the literature regarding the efficacy of viability testing were targeted. Techniques of ischaemic injury assessment are intended to allow identification of retrieved kidneys which are likely to have lost the potential for adequate function if transplanted. Such organs can then be discarded, thus improving outcomes and decreasing rates of primary non-function.

Results pertaining to ischaemic injury assessment provided support for protocols of viability assessment based on hypothermic machine perfusion. The effect of warm ischaemia on renal viability criteria has been successfully demonstrated in a large animal model, and novel approaches to the use of such assessments have been explored in order to maximise organ resource opportunities and utilisation.

The project has made an important contribution in the technical approach to laparoscopic partial nephrectomy and laparoscopic renal hypothermia. The studies involving the 'Newcastle Laparoscopic Renal Cooling Device' succeeded in achieving 'proof of concept' with demonstration of effective renal cooling and preservation..

Studies relating to preservation interventions in the porcine model of the uncontrolled NHBD have produced striking results. These results strongly suggest that uncontrolled NHBD centres employing cold in-situ perfusion approaches to preservation would be wise to consider supplementary techniques of organ cooling.

# Aims

## Aims

### Ischaemic injury Assessment

To assess the efficacy and potential of ischaemic injury assessment or viability testing within a renal Non Heart Beating Donor (NHBD) transplantation program by:

a) Prospective assessment of the effects of warm ischaemia on retrospectively established measures of organ viability; machine perfusion and perfusate enzyme analysis in a large animal model.

b) Assessment of outcomes of NHBD kidneys with low severity pre-arrest acute renal failure selected using machine perfusion viability testing

c) Assessment of outcomes from dual renal transplantation of 'marginal kidneys' selected using machine perfusion viability testing

### Laparoscopic Renal Cryopreservation

d) To develop a device for laparoscopic renal cooling

e) To develop a large animal model for laparoscopic renal cooling

f) To assess the efficacy of a laparoscopic renal cooling device using transplantation organ viability assessment

### Peritoneal Cooling

g) To develop a large animal model of the uncontrolled NHBD for assessment of the efficacy of additional peritoneal cooling versus current protocols.

h) To establish a human trial of uncontrolled NHBD peritoneal cooling.

# Contents


**Chapter 1**
**Introduction** **1**

1.1 History of renal transplantation 2

1.2 The current situation in renal transplantation 3

1.3 Non-Heart-Beating Donor (NHBD) Transplantation 4
- 1.3.1 History of NHBD transplantation
- 1.3.2 Early NHBD Renal Transplantation
  - 1.3.2.1 The Japanese
  - 1.3.2.2 The Europeans
  - 1.3.2.3 The United States of America
- 1.3.3 The First International Workshop on Non Heat Beating Donation; Maastricht, Netherlands 1995
- 1.3.4 Into the Modern Era of NHBD Renal Transplantation
- 1.3.5 The Advent of Renal NHBD Viability Testing

1.4 Ischaemic Injury in NHBDs 20

1.5 Assessment of Renal Ischaemic Injury 22
- 1.5.1Why Assess Injury?
- 1.5.2 Machine Perfusion Viability Assessment in Kidneys
- 1.5.3 Parameters for NHBD Kidney Viability Testing in Newcastle-upon-Tyne
  - 1.5.3.1 Early Problems
  - 1.5.3.2 The Newcastle-upon-Tyne Pressure Flow Index (PFI)
  - 1.5.3.3 Perfusate Analysis for Renal Viability Assessment
  - 1.5.3.4 Glutathione-S-Transferase (GST)
- 1.5.4 Problems with Viability Testing

1.6 Renal Cryopreservation 28
- 1.6.1 Cryopreservation in Urological Surgery
- 1.6.2 Cryopreservation in renal transplantation
- 1.6.3 Peritoneal Cooling in NHBDs

1.7 Aims 37

**Chapter 2**
**Methods 39**

2.1 Organ Recovery Systems Lifeport ® Kidney Transporter 40

2.2. Machine Perfusion Viability Testing 46
2.2.1 Pressure Flow Index
2.2.2 Measurement of Perfusate Glutathione-S-Transferase (GST)

2.3 Microdialysis 47
2.3.1 CMA 600 Microdialysis Analyzer ®
2.3.2 Assays
2.3.2.1 Lactate
2.3.2.2 Pyruvate
2.3.2.3 Glucose
2.3.2.4 Glycerol

**Chapter 3**
**Renal Ischaemic Injury Assessment 54**

3.1 Introduction 55

3.2 Machine Perfusion Viability Assessment 56

3.3 Viability Testing using Machine Perfusion and Perfusate Enzyme Analysis: Prospective assessment of the effects of warm ischaemia on retrospectively established measures of organ viability 57
3.3.1 Materials and Methods
3.3.2 Results
3.3.3 Discussion

3.4 Pre-Arrest Acute Renal Failure in NHBD transplantation 61
3.4.1 Methods
3.4.2 Results
3.4.3 Discussion

3.5 Dual renal transplantation for kidneys from 'Marginal' NHBDs 69
3.5.1 Materials and Methods
3.5.2 Results
3.5.3 Discussion

3.6 Conclusions 81

**Chapter 4**
**Laparoscopic Renal Cryopreservation 83**

4.1 Introduction 84

4.2 Design of the Laparoscopic Renal Cooling Device 85

4.3 Evaluation of the ischaemic protection efficacy of the Newcastle Laparoscopic Renal Cooling Device using renal transplantation viability assessment criteria in a porcine model 90
- 4.3.1 Materials and Methods
- 4.3.2 Results
  - 4.3.2.1 Cooling Efficacy
  - 4.3.2.2 Assessment of Ischaemic Injury Severity (Viability Testing).
    - 4.3.2.2.1 Pressure Flow Index (PFI)
    - 4.3.2.2.2 Glutathione-S-Transferase (GST)
- 4.3.3 Discussion

4.4 Conclusions 101

**Chapter 5**
**Peritoneal cooling in NHBD renal transplantation 103**

5.1 Introduction 104

5.2 Materials and Methods 106
- 5.2.1 Peritoneal Cooling Circuit
- 5.2.2 Temperature monitoring
- 5.2.3 Microdialysis
- 5.2.4 Machine Perfusion and Viability Testing
- 5.2.5 Statistical Analysis

5.3 Results 115
- 5.3.1 Renal Cooling
- 5.3.2 Microdialysis – Markers of Ischaemia
  - 5.3.2.1 Lactate
  - 5.3.2.2 Glycerol
  - 5.3.2.3 Pyruvate
  - 5.3.2.4 Glucose
- 5.3.3 Machine Perfusion Viability Testing
- Perfusion Flow Index (PFI)

5.4 Discussion 122

**Chapter 6**
**Discussion 124**

6.1 Ischaemic injury Assessment 125
6.1.1 The Efficacy of NHBD Hypothermic Machine Perfusion Viability Testing
6.1.1.1 Weaknesses and Future Research
6.1.2 Viability Assessment to Maximise Organ Resources
6.1.2.1 Transplantation of Cat. III NHBD kidneys with evidence of pre-arrest Acute Renal Failure
6.1.2.2 Dual Renal Transplantation of 'Marginal Kidneys' selected using Machine Perfusion Viability Testing

6.2 Novel Techniques for Clinical Renal Cryopreservation and Ischaemic Protection 131
6.2.1 Laparoscopic Renal Cryopreservation
6.2.2 Peritoneal Cooling for Uncontrolled NHBDs
6.2.2.1 Limitations of the Porcine Study of supplementary Peritoneal Cooling

6.3 NHBD Organ Preservation - Directions for the future 134
6.3.1 ECMO in Clinical Transplantation
6.3.2 The Current Situation with Uncontrolled NHBD Organ Preservation

6.4 Preservation for Uncontrolled NHBDs - Future Research 140
6.4.1 Optimised Cryopreservation vs ECMO for NHBDs

6.5 Final Conclusions 141

**References 144**

**Appendix 1 158**

**Appendix 2 169**

**Appendix 3 173**

**Publications 180**

## Abbreviations

| | |
|---|---|
| ARF | Acute Renal Failure |
| ATN | Acute Tubular Necrosis |
| ADP | Adenosine-Di-Phosphate |
| AMP | Adenosine-Mono-Phosphate |
| ATP | Adenosine-Tri-Phosphate |
| BSD | Brain-Stem Death |
| CIT | Cold Ischaemic Time |
| CRF | Chronic Renal Failure |
| DBTL | Double-Balloon Triple-Lumen |
| DGF | Delayed Graft Function |
| ESRF | End Stage Renal Failure |
| ECMO | Extra-Corporeal Membrane Oxygenation |
| ECLS | Extra-Corporeal Life Support |
| GFR | Glomerular Filtration Rate |
| eGFR | Estimated Glomerular Filtration Rate |
| GST | Gultathione-S-Transferase |
| HBD | Heart Beating Donor |
| HTK | Histidine-Tryptophan-Ketoglutarate |
| ISP | In-Situ Perfusion |
| LD | Live Donor |
| LPN | Laparoscopic Partial Nephrectomy |
| MP | Machine Perfusion |
| NHBD | Non Heart Beating Donors |
| PC | Peritoneal Cooling |
| PFI | Perfusion Flow Index |
| PNF | Primary Non Function |
| RCT | Randomised Controlled Trial |
| SCr | Serum Creatinine |
| WIT | Warm Ischaemic Time |
| $1^{o}$ | Primary |
| $2^{o}$ | Secondary |

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# Chapter 1

# Introduction

# Clinical Assessment of Renal Ischaemic Injury and the Role of Cryo-preservation; Peritoneal Cooling in Non Heart Beating Donation and Topical Cooling for Laparoscopic Surgery

## Introduction

### 1.1 History of renal transplantation

Solid organ transplantation was first proposed by Alexis Carrel in 1902 in his paper *La technique opérative des anastomoses vasculaires et la transplantation des viscères* in 1902 (Carrel, 1902). It was here that the now well-established technique of end-to end arterial anastomosis was first described. However in most other areas of transplantation medicine and surgery the field has changed significantly.

By 1914 the surgical techniques necessary to perform renal transplantation had been developed in animal models (Carrel, 2001, Carrel, 1983). Vascular anastomosis to the iliac vessels was possible, with an aortic patch described for the arterial anastomosis. The outcome of renal transplantation in these animal trials was invariably acceptable initial function, followed by graft failure and death of the animal at seven to ten days.

The first human kidney transplant was from a living donor. In 1936 in the Ukraine a surgeon named Voronoy performed a renal transplant from an unknown donor to a patient suffering acute renal failure secondary to mercury toxicity (Voronoy, 1936). Both the donor and recipient died within seven days.

After the initially disastrous attempts to treat end-stage renal failure (ESRF) with renal transplantation, in 1944 Kolff gave hope to sufferers with the invention of the dialyser (Kolff *et al*., 1997), built in an enamel factory in occupied Holland. The advent of renal replacement therapy

allowed clinicians the opportunity to manage uraemia. This extremely important, albeit imperfect, intervention continues to save lives to this day.

The deficiencies of dialysis reinforced the notion that complete artificial replacement of normal human renal function was perhaps impossible. Further attempts at human kidney transplantation were made in Boston and Paris in the early 1950s, but the outcomes were depressingly similar to the animal trials of 1914. Explanations for these failures came from Sir Peter Medawar, who postulated that the transplants had undergone 'acute rejection by the immune system' (Holman, 1924, Hume *et al*., 1952, Medawar, 1944, Medawar, 1945). He also showed that this process did not occur in transplants between genetically identical individuals. Renal transplants between identical twins were performed successfully from 1954 (Merrill *et al*., 1956, Murray *et al*., 1958).

The barrier to transplantation was broken when Sir Roy Calne pioneered the use of 6-mercaptopurine(Calne, 1960), azathioprine (Calne and Murray, 1961) and later cyclosporine (Calne *et al*., 1978, Calne *et al*., 1979). The ability to prevent and treat acute rejection allowed renal transplantation to eclipse dialysis as the ultimate renal replacement therapy. Today no single procedure or intervention has been shown to increase patient quality of life (Parsons and Harris, 1997), or be more cost-effective (de Wit *et al*., 1998) than a successful renal transplant for ESRF .

### 1.2 The current situation in renal transplantation

Renal transplantation in the UK is faced with enormous discrepancies between organ supply and demand. The traditional cadaveric, brain-stem dead, heart-beating donor source provides insufficient numbers of grafts for the ever-growing active waiting list. This situation is unlikely to reverse. In fact as existing renal grafts fail over time, and the nephrotoxic effects of long term use of

calcineurin-inhibitors (Gonwa *et al*., 2001, Klein *et al*., 2002) such as cyclosporine (Jankauskiene *et al*., 2001) and tacrolimus (Oka *et al*., 2001) in recipients of other solid organs develop, things may yet get worse. In addition the UK has experienced a significant fall in the numbers of transplants from brain-stem dead cadaveric donors performed annually (UKTSSA, 2003). This is thought to be secondary to improvements in road safety and therefore decreased mortality from road traffic accidents and intracranial haemorrhage (Briggs *et al*., 1997). This appears to be a world-wide phenomenon, with similar patterns in many national registries (UNOS, 2000, Eurotransplant). Transplant clinicians have therefore sought out alternative donor sources, mainly through the development of living-related and unrelated donor programs. However initial data suggests the yield may be relatively low due to exclusions for unsuitability, legal and ethical constraints of unrelated donation, or because the donors change their mind (Saunders *et al*., 2000).

## 1.3 Non-Heart-Beating Donor (NHBD) Transplantation

### 1.3.1 History of NHBD transplantation

Non-heart-beating donors (NHBD) represent the original renal transplantation donor group. Prior to the evolution of the concept of brain-stem death all organ donors were NHBDs. Evaluation of the results obtained in these very early transplants is of limited relevance to current practice due to the rapid evolution of medical and surgical organ transplant techniques. Equally, modern "state of the art" outcome data is presented elsewhere in this thesis. Rather, this section aims to chart the development and progress achieved in NHBD renal transplantation over the last two decades of the $20^{th}$ century.

The NHBD is not a single entity, as recognised by the First International Workshop on NHBDs held in Maastricht, Netherlands in 1995. Under the guidance of the chairman, Professor G.

Kootstra, the original four Maastricht categories of NHBD were developed (Table 1.1). These were later amended to include a fifth category; essentially cardiac arrest and unsuccessful resuscitation in a hospital setting. It is immediately apparent that each category represents part of a wide spectrum of potential organ damage through ischaemic injury. In the early literature, prior to this useful sub-division of the NHB donor group, identification of donor sub-type and therefore comparison of outcome data from different groups is fraught with difficulty.

***Table 1.1***

***The First International Workshop on Non Heart Beating Donation;***

***The Maastricht Categories of Non Heart Beating Donor***

| Maastricht Category | Donor Situation |
|---|---|
| I | Dead on arrival |
| II | Unsuccessful resuscitation |
| III | Awaiting cardiac arrest |
| IV | Cardiac arrest in a brain-dead donor |

At this point, it is useful to introduce a broader dichotomy which exists within the NHBD group; a NHBD situation may be considered as controlled or uncontrolled. Controlled situations are generally expected and occur in hospital settings (categories III and IV), therefore time is available to organise personnel, equipment and undertake legal formalities prior to death. In this way, a well organised retrieval program allows procurement of good quality organs with a relatively short warm

ischaemic time. With the uncontrolled donor (categories I and II) the situation is reversed; the donor comes from the community and the factors necessary for successful organ procurement must be mobilised and effected as quickly as possible. Uncontrolled donors are therefore associated with significantly longer primary warm ischaemic durations and therefore a greater propensity for ischaemic injury. The amended category V donor represents a mixture of the two donor situations and is relatively infrequently used.

In the UK today a NHBD is generally assumed to be a category III donor, following withdrawal of treatment in an intensive care setting. Indeed the Newcastle-upon-Tyne group are currently the only UK unit actively retrieving kidneys from uncontrolled NHBDs. However this does not represent the situation found in early modern NHBD renal transplantation. This is because uncontrolled donor opportunities are, and were, far more numerous than those arising in controlled circumstances. As alluded to above, data presented in the eighties and early nineties from NHBD programs around the world describe the outcomes of transplants originating from varying mixtures of NHBD sub-groups. However, it is often possible to determine, or at least infer, the relative donor sub-group composition through close examination of the methods described. The majority of NHBD transplants during this period appear to have come from uncontrolled (mainly cat. II) situations. As shall be seen later, early NHBD procurement protocols and subsequent outcomes are predictably variable.

The brain-stem dead patient currently represents the “ideal” donor situation where retrieval of high-quality organs is possible whilst maintaining a very short primary warm ischaemic time. A sharp transition from warm to cold ischaemia can then be achieved relatively easily using cold perfusion and the introduction of ice. Cryopreservation has formed the mainstay of organ ischaemic protection in modern renal transplantation due to early evidence of its effectiveness (Wickham *et al*.,

1967) and its inherent simplicity. Recent research has demonstrated an increasing interest in more physiological, warm, oxygenated preservation techniques. However it is cryopreservation and the attempts to minimise warm ischaemic duration and sharpen the warm/cold ischaemic interface that have characterised the approach of the transplant community to NHB donation.

As one reviews the literature, it is striking how little the introductions to papers related to NHBD renal transplantation have changed. Invariably, attention is drawn to a rising demand for organs coupled with a static or dwindling supply. The decline in conventional cadaveric (brain-stem dead) organ supply has been attributed to improved road and vehicle safety, and the success of living-related kidney donation programs have as yet proved unable to slake the waiting list's thirst for kidneys. It is this consistent imbalance between supply and demand which has driven interest in marginal donor groups over the last twenty years, including and especially the NHBD.

### 1.3.2 Early NHBD Renal Transplantation

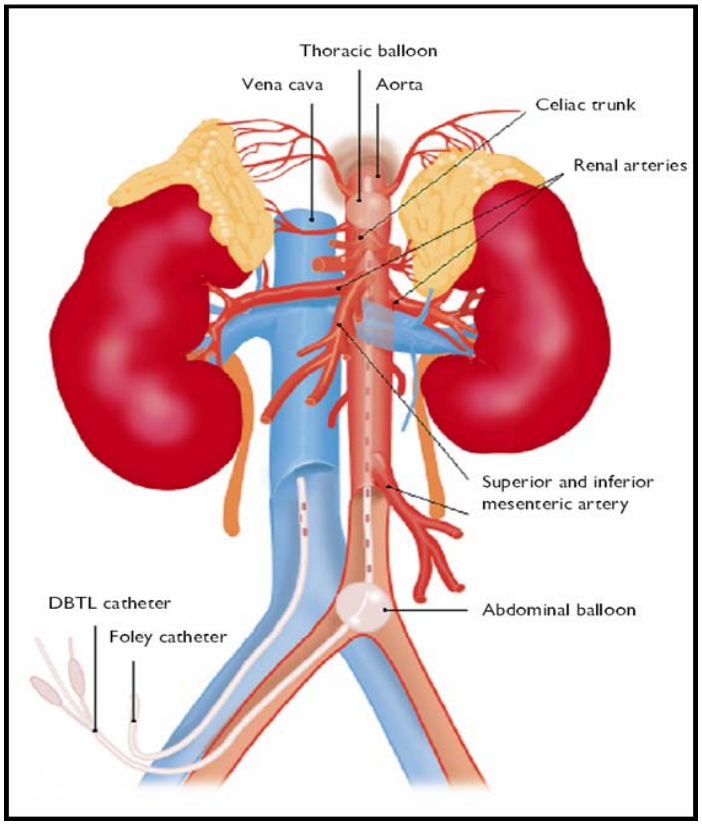
In 1975 Garcia-Rinaldi and Lefrak published details of a method of *in-situ* perfusion of cadaveric kidneys for transplantation (Garcia-Rinaldi *et al*., 1975). This paper introduced the concept of an aortic double-balloon triple-lumen (DBTL) catheter which could be placed via a femoral artery cut-down, in the early phase of the retrieval process. Once in place the two balloons are inflated (one above and one below the renal arteries), so isolating the renal circulation. Cold fluid can then be passed via the third lumen which exits the DBTL catheter through multiple holes between the two balloons (Figure 1.1).

The time taken to get an uncontrolled NHBD to theatre for laparotomy and organ cooling can be relatively long, especially in countries such as the UK where an opt-in system requires prior completion of complex Coroner and family consents. During this period warm ischaemia continues

to impact heavily on organ quality. The technical development of the DBTL catheter allowed transplant surgeons the opportunity to effect cryopreservation much earlier than had been previously possible. Therefore most successful early data from uncontrolled NHBD renal transplants used a form of early cannulation and *in-situ* cold perfusion during procurement.

**Figure 1.1**

**In-Situ Cold Perfusion via Aortic placement of the Double-Balloon Triple-Lumen (DBTL) catheter.**




The DBTL catheter may be introduced via a femoral triangle cut-down procedure; marks on the catheter allow estimation of the length of catheter required to position the superior balloon above the celiac axis, and the inferior below the inferior mesenteric artery. Once inflated the splanchnic circulation is effectively isolated, and with venous venting, cold *in-situ* perfusion may be achieved

#### 1.3.2.1 The Japanese

Brain-stem death was not legally recognised in Japan until relatively recently. As a result prior to this all cadaveric renal transplantation in Japan was performed using kidneys from NHBDs. The majority of donors would fit into the controlled Maastricht categories (III, IV and V), though systems were in place for rapid identification and response in the uncontrolled category II situation. As with most other countries (except more recently in Spain) category I donors were not used due to issues of unknown primary warm ischaemic duration. Modern programs could, and have, learned much from the Japanese approach to the NHBD as for many years they produced results comparable to western heart-beating donor (HBD) programs. An excellent illustration of this is found in the 1993 publication from the Nagoya group (Yokoyama *et al*., 1993). This paper reported a ten-year experience of utilising the DBTL catheter in NHBD kidney procurement and transplantation. In 120 renal transplants they reported a primary non-function (PNF) rate of 6.7% and a delayed graft-function (DGF) rate of 68.3% with one-year graft survival rates of greater than 80% (the contemporary British Transplantation Society guidelines for HBD grafts). However, the excellent mean primary warm ischaemic time of 10.7 minutes suggests the majority of donors were well-controlled and the questionably high DGF rate of 68.3% must be viewed in this context.

#### 1.3.2.2 The Europeans

In Europe, the Maastricht group in the Netherlands led the way with their NHBD program based on DBTL catheterisation and *in-situ* perfusion (van der Vliet *et al*., 1980, van der Vliet *et al*., 1981, Vromen *et al*., 1988). The Spanish also achieved relatively good results (Castelao *et al*., 1988), although overall rates of PNF were higher than is currently acceptable.

In 1993 the Maastricht group called for a standardised protocol for *in-situ* preservation of NHBD kidneys in uncontrolled category II donors (Booster *et al*., 1993a, Booster *et al*., 1993b). The Maastricht approach centred on rapid local availability of DBTL cannulation and perfusion which was achieved by local surgeons who as junior surgeons had usually been trained on the transplant unit to perform the relatively simple technique of femoral cut-down and cannulation. This local expertise was later augmented with the addition of the "NHBD box" containing the necessary equipment and instructions to every Accident and Emergency department in the region. Strict donor criteria were employed to safeguard organ quality. Transplantation was contraindicated from donors with any of the following; age >60yrs, evidence of sepsis, history of intra-venous drug use, hypertension, diabetes mellitus, or cancer. The limit for the primary warm ischaemic time was set at 30 minutes.

In the 98 kidneys retrieved from 49 category II donors between 1980 and 1990, 70 were transplanted within the Netherlands, 16 in Maastricht. Follow-up data was only presented from the Maastricht transplants, compared to 30 well-matched HBD renal transplants. The PNF rate was significantly higher in the NHBD vs. the HBD groups (12.5% vs. 6.7%), as was DGF (75.0% vs. 43.3%). More positively, the graft and patient survival were not significantly different between the groups. Functional outcomes, measured crudely by serum creatinine, also showed no significant differences with regard to NHBD vs. HBD status or immediate function vs. DGF.

It is interesting to note that from 1980-1990 the Maastricht group reported a non-use or discard rate of 28.6%. They, and others, had begun to machine perfuse NHBD kidneys during the cold ischaemic phase in order to achieve better wash-out of blood, and better delivery of preservation solution. This also produced pressure/flow data which might reflect the vascular injury sustained by the organ. Amongst the reported reasons for organ non-use is "poor machine perfusion

parameters" which previews the organ viability testing debate to come. Also notable is that the *in-situ* perfusion solution employed was histidine-tryptophan-ketoglutarate (HTK) which was later shown to be superior to the Euro-Collins solution commonly used at the time (de Boer *et al*., 1999).

In the UK, several groups had commenced NHBD programs. In Newcastle-upon-Tyne a controlled category III program with rapid laparotomy, cannulation and cold perfusion achieved "success rates" (recipient alive and free from dialysis) of 90.5% at one year between 1988-1993 (Balupuri *et al*., 2000c). However, Newcastle's later experience with uncontrolled donors from 1994-98 was not so positive, with an unacceptable PNF rate of 55.5%, resulting in temporary termination of the program. This will be discussed in more detail later. In 1994 both the Leicester and Guy's (London) groups reported high rates of PNF from their early experience. The Leicester group begun their NHBD program in 1992 and reported a PNF rate of 12% in the first year (16 cat II and III transplants) (Varty *et al*., 1994). The London group had started in 1988, aiming to retrieve kidneys from category II donors in the accident and emergency situation as well as from category III / V patients with mainly intra-cranial tumours in hospice settings. In the 27 resulting transplants performed between 1988 and 1991, the PNF rate was 25.9% and the two year graft survival 55%. As a result older age and hospice patients were no longer considered for donation (Phillips *et al*., 1994).

#### 1.3.2.3 The United States of America

NHBD renal transplantation was not prevalent in the USA during the eighties and nineties. However the Pittsburgh and Washington groups were producing interesting results. In early 1995 the Pittsburgh group reported their experience with a protocol based on rapid laparotomy, cannulation and cold perfusion (i.e. no DBTL *in-situ* perfusion prior to laparotomy). 24 NHBD retrievals (14 cat. II, 10 cat. III) were performed during 1989-93. In the category II transplants the PNF rate was 5%

and DGF rate 64%, with a one year graft survival of 86%. In the category III transplants no PNF was observed with a one year graft survival of 82% (Casavilla *et al*., 1995). Importantly the Pittsburgh protocol included a post-retrieval biopsy which was examined prior to transplantation. 18.5% and 15% of category II and III organs were discarded on the basis of the biopsy findings. This process is arguably analogous to the Maastricht viability testing using machine perfusion. In any case results certainly appeared superior to those produced in the UK where no formal assessment of organ quality was undertaken.

With varying rates of success and failure in evidence across the world, the First International Workshop on NHBDs was convened in Maastricht, Netherlands.

### 1.3.3 The First International Workshop on Non Heat Beating Donation; Maastricht, Netherlands 1995

Chaired by Professor G Kootstra, the workshop covered the spectrum of issues concerning NHBD at the time. In many cases these issues remain relatively unchanged today. Topics included; the ethics of uncontrolled NHBD retrieval and invasive measures of organ preservation prior to consent, the role of reinstitution of CPR techniques after death, DBTL *in-situ* perfusion versus rapid laparotomy, and the potential role of viability testing using machine perfusion and perfusate enzyme data.

In terms of results, several groups reported their experience to date. The Leicester group had reached the 3 year point in their category II program, and presented data from 1992-95 (Dunlop *et al*., 1995). Using donor exclusion criteria similar to the Maastricht group (Age <60 yrs etc.), DBTL *in-situ* cold perfusion with Coroner's permission (prior to family consent for donation), often with the reinstitution of ventilation and cardiac massage with a 'thumper' device, Leicester retrieved 44

kidneys. 30 were transplanted locally with a PNF rate of 10%, DGF 100%, and a one year graft survival of 93.3%. These creditable results, despite 100% DGF, were achieved without viability testing. However, they came at another cost, namely effort and resource. The Leicester group responded to 73 potential donors during the study period from which only 24 actually proceeded to retrieval.

In Maastricht, Kootstra's uncontrolled NHBD work had continued apace. In the two years since their last group data publication, they increased the number of locally transplanted kidneys from 16 to 57 (Wijnen *et al*., 1995). From these transplants (1980-1992), with a mean follow-up of 85 months, data was presented at 1, 3, and 5 years post implantation compared with a matched group of 60 local HBD transplants. PNF rates were <3% in both study groups, though the DGF rate was predictably significantly higher in the uncontrolled NHBDs (60% vs. 35%). No significant differences were seen regarding graft survival or functional outcome.

The Madrid group were also producing strong results, but in category III donors. The protocol at the time involved perfusion with Euro-Collins via a central venous line at cardiac arrest, followed by rapid transfer to theatre, laparotomy, cannulation and cold perfusion. 52 NHBD transplants were compared to 98 HBD. No significant differences in PNF or nine-year graft survival were observed. DGF (67% vs. 46%) and serum creatinine at 1 year were higher in NHBDs, but this difference became non-significant by 5 years (Gonzalez Segura *et al*., 1995).

This was clearly extremely encouraging data, but difficult to reconcile with contrastingly poor results elsewhere. In reply to the Lancet paper by Wijnen described above, Rene Chang from London highlighted what he felt to be an "over-optimistic gloss" regarding the potential results that could be achieved using NHBDs (Chang, 1996, Andrews *et al*., 2001). He based this assertion on the results of his local uncontrolled program, reiterating the group's early results as described above.

The Maastricht group may well have pointed out that although their results had improved, their organ non-use rate had increased from 28.6% to 34%. The reason for this was the Maastricht viability testing protocols, soon to be published, which had the potential to select out retrieved organs which were likely to result in PNF. Although we can assume thorough subjective assessments are likely to have occurred in other centres, arguably it was the objective, formalised, quantitative assessment which produced the impressively low rates of PNF seen in Maastricht, albeit at the cost of an expensive non-use rate.

### 1.3.4 Into the Modern Era of NHBD Renal Transplantation

In the UK by 1998, the London group had ceased retrievals from uncontrolled NHBDs and Leicester were possibly having doubts. In their HBD comparison paper of 1997, the Leicester group examined 30 category II and III transplants against 114 HBD. Against the HBD group, the DBTL in-situ cold citrate perfusion NHBD protocol, with a 40 minute primary warm ischaemic cut-off, produced a PNF rate of 13% (mainly in uncontrolled donors) and an inferior serum creatinine at 2 years (Butterworth *et al*., 1997, Nicholson *et al*., 1997). More telling is the Elwell paper of the same year concerning the outcome of Leicester NHBD referrals (Elwell *et al*., 1997). From 144 NHBD referrals made between 1992 and 1997, there were only 48 resulting retrievals. The group bemoaned the waste of time, effort, and resource, which must have been extremely frustrating. Leicester later published some excellent uncontrolled results (PNF 7%, 8 year-graft survival better than HBD) (Metcalfe *et al*., 2001), demonstrating a learning curve and possibly an experienced leadership with an eye for which kidneys and/or donor situations were likely to end in PNF. However, the Leicester uncontrolled NHBD program was later terminated.

Elsewhere many groups continued to produce good results using both controlled and uncontrolled NHBDs (Pokorny *et al*., 1997, Tanabe *et al*., 1998). Possibly the most impressive uncontrolled NHBD data to date was produced in the USA by Dr Light's Washington group. In 1994 they established a rapid organ recovery program (RORP) for NHBDs. The protocol involved DBTL *in-situ* cold perfusion and hypothermic machine perfusion following retrieval (similar to the Maastricht protocol). However the Washington group were the first to use additional peritoneal cooling, via two abdominal trocars, one for inflow and one outflow of iced saline (Light *et al*., 1997). Initial data (1994 and 1997) reported retrieval of 29 kidneys of which 23 were transplanted. 5 kidneys functioned immediately, 16 after a period of delayed graft function and 2 experienced PNF.

From donor 22 onwards the Washington method was refined by use of a peritoneal cooling circuit involving a heat-exchange coil in a sub-zero ice/alcohol fluid bath. Improved intra-peritoneal temperatures were subsequently achieved (10°C after 8 minutes cooling). Excellent clinical results were observed. No further PNF was seen and the DGF rate fell from 71% to 50% (Light *et al*., 2000a). From a critical stand-point however, the Washington donors were mainly young trauma (gun-shot) victims and therefore the results obtained have limited validity for older, often co-morbid European donor groups.

### 1.3.5 The Advent of Renal NHBD Viability Testing

By the early 2000's a dichotomy in NHBD practice had formed in Europe. Groups could be divided into those retrieving from controlled NHBDs without viability testing protocols, and those retrieving from both controlled and uncontrolled NHBDs with machine perfusion viability testing. The Maastricht group had published widely regarding their methods of identification of damaged organs (Daemen *et al*., 1997b, Daemen *et al*., 1997c, Kievit *et al*., 1997). These viability criteria were based

on impaired flow and high resistance during machine perfusion, and measurement of enzymatic markers of ischaemic injury. The main marker chosen was alpha glutathione-s-transferase (GST), released by damaged proximal tubular cells and washed-out into the perfusate.

Newcastle-upon-Tyne had experienced disastrous uncontrolled PNF rates between 1994 and 1997, which resulted in the termination of the program. During this period no machine perfusion or viability testing protocols had been employed. From 1998 the uncontrolled program was reinstituted (phase III) using a modified Maastricht protocol of DBTL *in-situ* perfusion and a locally produced machine perfusion device. Viability testing was performed using pressure / flow characteristics and perfusate GST. Early data from phase III was vastly improved. From 11 uncontrolled retrievals, 7 kidneys were discarded on the basis of viability testing and 15 renal transplants were performed. The PNF rate was 1/15 (6.7%), with a 92% "success rate" (patient alive and free from dialysis) at one year (Balupuri *et al*., 2000c, Balupuri *et al*., 2000b, Balupuri *et al*., 2000a). By 2002, 88 NHBD transplants had been performed (cat. II 56%, cat. III 39%, cat. IV 5%) and were compared to a well-matched group consisting of the next consecutive local HBD transplant. The kidney non-use rate was high at 47.7%, but in return no significant differences were observed with respect to PNF, 3 year graft / patient survival, or 3 year eGFR (estimated glomerular filtration rate) functional outcomes (Gok *et al*., 2002b).

With the Maastricht and Newcastle evidence mounting, many felt the case for machine perfusion and viability testing in NHBD kidneys was well-made. Indeed, writing in the Lancet, Yves Vanrenterghem of the Leuven group stated that;

"The development of a non-heart-beating program is no longer acceptable if machine perfusion and viability testing are not available"(Vanrenterghem, 2000).

This was an understandably troublesome statement for groups successfully transplanting controlled NHBD kidneys with simple static cold storage. Letters sent to the editor of the Lancet included responses from the Leicester and Guy's groups. The Leicester group (Metcalfe and Nicholson, 2000) argued that the reduction in PNF seen in the Newcastle data could be analogous to the reduction seen during the first and second halves of the Leicester program. They attributed this to the "learning curve" phenomenon, which undoubtedly plays an important role in the evolution of any NHBD program. With experience (and likely previous bad experiences with PNF) program leaders become more discerning regarding which organs they are prepared to transplant. This itself may amount to a form of subjective "viability testing" in the following way. An experienced NHBD surgeon will collate information regarding ischaemic duration, donor demographics, quality of perfusion, macroscopic appearance etc. and come to a conclusion regarding the probability of a successful outcome. The development of such individuals within a program will result in improved outcomes. However problems may arise when such people leave or are unavailable. Also, the Leicester group pointed out that the cautious subjective approach may result in a high non-use rate (1.4 transplanted kidneys per retrieval in Newcastle vs. 0.5 in Leicester) and welcomed a reliable objective test.

The Guy's group also contested Yves Vanrenterghem's statement (Gerstenkorn *et al*., 2000). They argued that only half the discarded organs had been excluded on the basis of viability tests alone, and that the Maastricht / Newcastle tests were unvalidated as it could not be proven that the discarded organs would not have functioned. In conclusion they sensibly suggested that current NHBD programs should continue if, as per British Transplantation Society guidelines, the one year graft survival was ≥80%.

If Yves Vanrenterghem's statement had read; "The development of an ***uncontrolled*** non-heart-beating program is no longer acceptable if machine perfusion and viability testing are not available" it is likely that few would have argued.

Success in NHBD renal transplantation is defined by low rates of PNF, and rates of graft survival and function comparable to those of HBDs. DGF is a very common, if not routinely expected, complication of NHBD kidney transplantation. However, medium-term evidence suggests that following recovery, DGF grafts will go on to perform comparably with non-DGF grafts (Light *et al*., 2000b).

Explanations for poor outcomes can be attributed to two main areas. These are excessive ischaemic injury (which can be due to long primary WIT cut-offs or ineffective preservation interventions), and failure to identify damaged organs which should not be transplanted (lack of effective viability testing). In the controlled NHBD situation poor outcomes can be avoided with proper attention to effective retrieval techniques. As primary warm ischaemic times (WITs) are short, it is possible to run successful programs without formal viability testing. In the uncontrolled situation, where primary WITs are much longer, effective cryopreservation interventions and viability testing are essential. In this way, through the identification and non-use of excessively damaged organs, the PNF rate is kept to acceptable levels.

## 1.4 Ischaemic Injury in NHBDs

For the reasons outlined earlier, transplantation clinicians fear the high PNF and DGF rates previously associated with NHBD grafts (Balupuri *et al*., 2000c). The pathophysiological processes producing these unwelcome clinical syndromes are, in the main, acute tubular necrosis (ATN) and vascular injury secondary to warm ischaemia. The severity of the damage sustained determines whether a transplant will result in immediate function, DGF, or PNF.

If post-transplantation DGF occurs, the recipient will require dialysis until the graft is functioning, and for this he must remain in hospital. Both dialysis and increased hospital stay is expensive, and patients with DGF can suffer significant psychological morbidity. The consequences of PNF are clearly unacceptable. Therefore the most important interventions in any NHBD situation act to prevent or limit the effects of warm ischaemia.

In many NHBDs cardiac arrest may be preceded by a period of hypoxaemia and inadequate organ perfusion secondary to the pathological processes causing death. In this way ischaemic injury may begin prior to the point that renal oxygen delivery ceases completely and the warm ischaemic period officially begins. At arrest the kidney is at normal temperature and hence renal cells remain metabolically active and continue to use ATP. Without oxygen and aerobic respiratory substrate delivery, intracellular ATP concentrations begin to fall. This stimulates the enzyme phosphofructokinase, causing a shift to anaerobic metabolism of glucose which produces ATP, if only in much reduced quantities. In the context of anaerobic respiration, toxic metabolites accumulate and result in intracellular acidosis and functional enzyme inhibition (Trump, 1982).

If the cells remain warm, ATP levels will continue to fall. Without sufficient availability of ATP the $Na^+/K^+$-ATPase transporters in the cell membrane cease to function, leading to a cellular

influx of sodium and efflux of potassium along concentration gradients. $Ca^{2+}/Mg^{2+}$-ATPase dysfunction results in vastly raised intracellular calcium and decreased magnesium levels. $Mg^{2+}$ is important for enzyme function, and deficiency leads to mitochondrial failure and impairment of membrane repair mechanisms. Calcium ions entering the cell with greatly increased intracellular $Na^{+}$ concentrations result in osmotic cell swelling, loss of plasma membrane integrity and ultimately cell death (Trump, 1982).

Once ATP is exhausted, energy may be released from the conversion of ADP to AMP, and subsequently from the conversion of AMP to adenosine. Adenosine is not conserved within the cell and hence if this state is reached, the possibility of ATP regeneration on reperfusion is much reduced. When cells die toxic free radicals and lysosomal enzymes are released damaging nearby cells adjacent cells and contributing to tissue necrosis.

Human metabolic rate will halve with every 10°C reduction in temperature (Wickham *et al*., 1967). Thus by reducing organ temperature from 37°C to 7°C the metabolic rate, and thus ATP consumption, can be reduced by 87.5%. This is a major aim of the cold in-situ perfusion techniques discussed below. In addition, perfusion preservation solutions contain similar electrolyte concentrations to intracellular fluid, in order to limit the effects described above if ATPase transporters fail. Some perfusion fluids go further still, with the inclusion of adenosine to limit cellular loss, glucose as a metabolic substrate, free radical scavengers, and colloids to reduce tissue oedema.

## 1.5 Assessment of Renal Ischaemic Injury

### 1.5.1Why Assess Injury?

NHBD kidneys have invariably sustained a degree of warm and cold ischaemic injury, with the most extreme cases seen in uncontrolled donor situations. Renal injury can be considered as a spectrum, ranging from mild reversible damage, to irreversible injury such that an organ will never regain function. The presence of certain factors (e.g. short ischaemic times, young healthy donor, and effective cryopreservation) will reliably suggest minimal injury and the potential for a good transplantation outcome. Equally, experienced personnel might become very good at predicting likely organ quality, hence avoiding PNF. It could be argued that such factors are essentially subjective viability assessments. However, in uncontrolled donors subjective assessments are too weak to allow confident practice. Objective methods of assessing ischaemic injury and viability must be sought.

### 1.5.2 Machine Perfusion Viability Assessment in Kidneys

The aim of machine perfusion is to improve upon static cold storage of donor organs through improved delivery of cold preservation solution and better wash-out of donor blood. In this way, more cells are more rapidly exposed to the beneficial cooling and metabolic effects of the preservation solution, and immunogenic factors within the donor blood are removed. In NHBDs the parameters involved in hypothermic machine perfusion can also be used as indicators of organ injury and hence viability. Such techniques follow from Kootstra's research into methods of preservation in the 1980's and 1990's (Booster *et al*., 1993b, Daemen *et al*., 1995, Kootstra, 1988). The machine perfusion system he used was principally the Gambro® machine which was modified by using steel containers with glass lids, into which the kidneys were placed. The perfusion system re-circulated a

University of Wisconsin solution® with supplementary oxygen delivery but without using an oxygen carrier compound. Some centres, including Newcastle-upon-Tyne used locally built systems. In the United States, later development produced the Waters® machine and later the Organ Recovery systems Inc. LifePort® kidney transporter. The Lifeport® is now commonly used throughout Europe and the US, and has been used in Newcastle-upon-Tyne since 2003.

### 1.5.3 Parameters for NHBD Kidney Viability Testing in Newcastle-upon-Tyne

#### 1.5.3.1 Early Problems

Hypothermic machine perfusion was introduced into the Newcastle NHBD protocol in phase III of the program in 1998. The consensus view at the time was that the required flow rate was in the region of $0.5ml^{-1}$ gram of $kidney^{-1}$ $minute^{-1}$ It was also thought that a safe maximum for perfusion pressure was 60mmHg (Balupuri *et al*., 2000b, Balupuri *et al*., 2000c). A locally-constructed system was initially employed, based on a haemodialysis pump (capable of delivering a much higher pressure than 60mmHg). It was found that achieving the desired flow rate in marginal NHBD kidneys was often difficult. Subsequently, it became common practice to increase the perfusion pressure to >60mmHg, especially in the early phase, in order to 'open up' the renal vasculature. Clearly this contravened the consensus regarding maximal pressure (Grundmann *et al*., 1975). In addition, it had been shown that maximal resistance to flow was expected at the beginning of machine perfusion, and that it would fall significantly during the first hour. This fall in resistance was assumed to represent the absence of intra-vascular thrombosis, and was cited as an indicator of organ viability (Talbot *et al*., 2003). However, a resistive drop may be seen in organs with both very good and very poor initial flows. It was also possible to unintentionally make a highly damaged kidney appear less damaged by using an excessive perfusion pressure. The process responsible

involved the opening of arterio-venous shunts (Last, 1978 p.319) which caused a drop in resistance. Also, with the advent of perfusate enzyme measurement these arterio-venous shunts assumed further importance as they effectively cause 'bypassing' of the renal cortex. Thus the enzymatic markers of ischaemia, released by damaged tubular cells, are not encountered and washed out into the perfusate. The levels measured in the effluent would therefore appear much lower than if the cortex of the kidney had been perfused (Talbot *et al*., 2003). Again, a highly damaged organ may appear less damaged. Another effect of excessive perfusion pressure is excessive organ weight gain. This phenomenon is seen where fluid is forced out into the interstitium by hydrostatic pressure. As such, it was seen equally frequently in less damaged kidneys, and was exacerbated by the low oncotic pressures associated with early (starch-free) solutions.

#### 1.5.3.2 The Newcastle-upon-Tyne Pressure Flow Index (PFI)

In an effort to overcome these issues, the Newcastle group devised an index which incorporated factors of systolic pressure as well as pure flow or resistance. This was termed the Pressure Flow Index (PFI), and was calculated from the flow per 100g renal mass divided by the systolic pressure of machine perfusion ($ml^{-1}$ $min^{-1}$ $100g^{-1}$ $mmHg^{-1}$). Retrospective analysis of machine perfusion and dichotomous (successful/unsuccessful) outcome data, identified a PFI viability threshold of 0.4 $ml^{-1}$ $min^{-1}$ $100g^{-1}$ $mmHg^{-1}$ (Balupuri *et al*., 2000b). The maximum pressure utilised for machine perfusion was reduced to 40 then 30mmHg. By using pressure as a denominator operators were less likely to increase perfusion pressures in order to achieve minimum requirements set for flow rate; a practice which had the potential to cause injury. Furthermore, with the change from the locally-constructed device to the Organ Recovery Systems Lifeport® machine such practices were no longer possible as the Lifeport limits perfusion pressures.

#### 1.5.3.3 Perfusate Analysis for Renal Viability Assessment

An ideal viability marker for renal NHBD transplantation could be defined as an easily measurable, accurate indicator of ischaemic injury irreversibility. Physiological markers of ischaemia commonly used clinically have disadvantages in this context; pH is affected by physiological and perfusion fluid buffering systems and is hence of limited value. Perfusate lactate levels may indicate the degree of cellular acidosis but do not denote reversibility i.e. the potential for recovery. This has led transplantation clinicians to examine the release of certain intracellular enzymes into the effluent. Intra-cellular enzymes should only be released following membrane disruption, when a cell dies. They should therefore confer an indication of irreversible injury.

#### 1.5.3.4 Glutathione-S-Transferase (GST)

GST (or ligandin) was originally isolated as an abundant intracellular enzyme in the proximal tubules of Sprague Dawley rats. Subsequently, GST has been found to be present in various cell types; liver, heart, ovary, testes, small intestine and adrenal. Isometric differences exist between cell types. In the kidney GST isomers are involved in detoxification via conjugation reactions. Proximal tubular cells express predominantly α-GST and hence this is the isomer released following injury. Distal tubular cells express π-GST which therefore can be measured in urine. That said, various isomers may be released from both renal cell types and as the machine perfusion system only involves one organ it could be argued that determining isometric levels of the enzymes is unnecessary. The total GST enzyme level is sufficient for the purposes of assessment of viability for a single kidney in a circuit. Total GST levels can be determined by a relatively simple spectrophotometric assay. This assay is described in detail within the methods chapter. It is based

upon the rate of change of photon absorbance occurring at 340nm when the substrate 1-chloro-2, 4-dinitrobenzene is conjugated with glutathione (Habig and Jakoby, 1981).

GST has been used to assess injury in NHBD kidneys for some time. Daemen *et al* established a viability threshold perfusate level of 200 IU/L by retrospective analysis of outcome data (Daemen *et al*., 1997a), based on a perfusate circuit volume of 0.5L. This upper limit was adopted in Newcastle (Balupuri *et al*., 2000d), although it was noted that levels above 200IU were more commonly seen in young donors (presumably with more nephrons per kidney). In such situations, where other factors in the donation were considered favourable, some discretion regarding marginal breaches of the 200IU threshold were employed. Following introduction of the Lifeport system in Newcastle, the circuit volume required was increased to 1L. The GST viability threshold was therefore also altered, and has been set at 100IU/L since 2003. The current criteria used for viability testing in Newcastle are summarised in Table 1.2.

**Table 1.2**

**Newcastle-Upon-Tyne NHBD Machine Perfusion Viability Parameters**

| | |
|---|---|
| **Perfusion pressure (mmHg)** | < 30 mmHg |
| **Flow** | > 12ml/100g |
| **PFI (ml/min/100g/mmHg)** | >0.4 |
| **Temperature (°C)** | < 12˚C (surface temperature)<br>(five time points) |
| **GST** | ≤ 100 IU/100g |
| **Wt increase (%)** | < 25 % (relative contraindication) |
| **Perfusion fluid** | Machine Perfusion Solution<br>KPS (Belzer II, Belzer MPS) |
| **Donor pre-treatment** | Heparin and Streptokinase |

### 1.5.4 Problems with Viability Testing

For a viability assessment protocol to be deemed 100% successful, the program PNF rate would necessarily be 0%. This is a utopian aim and in reality, when dealing with marginal donor situations, viability assessment can be judged a success if PNF rates are maintained below the accepted limit of 10% (BTS guidelines 2008). In Newcastle, the use of PFI and GST-based tests have been reasonably successful at identifying heavily damaged NHBD organs and hence PNF levels have been uniformly kept below the 10% yearly limit (Gok *et al*., 2002a, Gok *et al*., 2004a). However, as above, every case of PNF represents a failure of viability assessment.

Deficiencies associated with the markers used in the Newcastle protocol are likely to represent deficiencies in our understanding of the extreme complexity of ischaemia reperfusion

injury and the transplantation process. It is also likely that any viability assessment protocol capable of 100% sensitivity would suffer significantly in terms of specificity. In other words, the non-use rate would be very high. Organ non-use is extremely resource expensive and damaging to NHBD programs.

Ethics determine that the PFI and GST viability protocol in Newcastle must remain unvalidated. However if the effects of increasing ischaemic duration on PFI and GST could be demonstrated prospectively, the case for such assessment would be strengthened. The efficacy of current protocols in the determination of transition between states of reversible and irreversible injury should also be examined. Lastly, given the program-damaging effects of organ non-use further consideration should be given to the fate of organs on the borderlines of viability. If such organs could be used, possibly through improved preservation or by novel transplantation techniques, organ resources could be maximised.

## 1.6 Renal Cryopreservation

In his famous 'Regional Renal Hypothermia' paper of 1967, Wickham reviewed evidence from human and mammalian histological, physiological and transplantation studies in order to better elucidate the tolerated period of renal ischaemia (Wickham *et al*., 1967). He suggested that to avoid permanent loss of renal function any warm ischaemic period should not exceed 30 minutes. To avoid even minimum depression of function renal ischaemia should not exceed 10 minutes.

Cooling of an organ results in decreased metabolic requirements and hence ischaemic protection. Wickham went on to describe his own renal cooling experiments with rabbits in relation to the body of the literature available at the time. Levy and Semb's $O_2$ consumption studies (Semb, 1959a; Semb, 1959b) had shown almost full suspension of renal metabolic activity at 15-20$^{o}$C, and

Bickford had shown complete suspension of tubular transport function at 17$^{o}$C (Bickford and Winton, 1937). Wickham himself showed that in rabbits, kidneys cooled to 20$^{o}$C could sustain up to three hours of renal ischaemia without appreciable deterioration in renal function, as measured by serum creatinine, or development of histological evidence of damage. The conclusions were that to achieve short-term hypothermic functional preservation with human kidneys, moderate parenchymal cooling to temperatures of approximately 15-25$^{o}$C is required.

### 1.6.1 Cryopreservation in Urological Surgery

The field of renal cryopreservation also has clinical applications outside of organ transplantation. The second major area of interest is that of urological surgery, in particular the procedure of partial nephrectomy, where it is necessary to subject kidneys to an ischaemic period, prior to reperfusion.

The surgical management of renal cell carcinoma has traditionally meant patients proceeding to radical nephrectomy. This leads to approximately 50% reduction in nephron mass and consequently a similar loss of renal function to the patient. However partial nephrectomy is now considered a safe alternative for small localised renal cell tumours (Fergany *et al*., 2000, Gilbert *et al*., 2003). Due to the nephron-sparing capacity of the procedure, partial nephrectomy is considered the preferred form of treatment for T1a tumours (<4cm) in patients with small or solitary kidneys (Adkins *et al*., 2003, Ghavamian *et al*., 2002) and those with small bilateral tumours (Bernardo and Gill, 2002).

The role of laparoscopic radical nephrectomy for management of T1-2 renal cell carcinoma is universally accepted (Abbou *et al*., 1999). However, laparoscopic partial nephrectomy (LPN) has been slow to gain universal acceptance, despite data suggesting that LPN can produce equivalent

outcomes to open partial nephrectomy (El-Ghoneimi *et al*., 2003, Gill *et al*., 2003b). LPN follows the same principles as the open procedure; the renal blood supply is occluded by soft clamping of the renal vasculature prior to incision. Renal blood flow can then be re-established once the tumour has been excised and the edges are closed. During this period, the remaining kidney mass is exposed to the effects of warm ischaemic injury. It is difficult for a surgeon, especially whilst learning the procedure, to complete LPN within the prescribed 30 minute safe period (Wickham *et al*., 1967). Therefore where a urological surgeon anticipates the necessity of a significant period of renal ischaemia, there is a need to ensure renal hypothermia, in order to afford the organ sufficient ischaemic protection.

During open partial nephrectomy, topical cooling is applied to the relevant kidney during renal pedicle clamping, usually via the introduction of ice slush to the region. Similarly in laparoscopic renal surgery attempts have been made to reduce the warm ischaemic damage occurring during renal pedicle clamping by use of a cooling jacket (Herrell *et al*., 1998), placement of ice slush (Ames *et al*., 2005, Gill *et al*., 2003a, Laven *et al*., 2007), irrigation with cold fluid (Webster *et al*., 2005, Weld *et al*., 2007), intra-arterial cold perfusion (Janetschek *et al*., 2004), and by retrograde ureteral perfusion of the kidney with cold saline (Crain *et al*., 2004, Landman *et al*., 2003).

Vascular cooling can be achieved through trans-arterial cold perfusion and effective performance has been reported using this approach (Janetschek *et al*., 2004). Despite this, many surgeons would prefer alternative techniques due to the unavoidable risks of vascular injury and thrombosis, as well as issues of technical complexity.

Ureteral cooling is undertaken by retrograde perfusion with cold saline via a ureteral access sheath. This method requires post-operative stenting, and provides less effective cortical cooling than surface techniques (Crain *et al*., 2004, Landman *et al*., 2003).

Surface cooling may be achieved in a number of ways; Herrel *et al* described a renal cooling device consisting of a rectangular double-layered plastic envelope through which cold fluid was circulated. The kidney to be cooled was placed into the envelope via an open side. Data was presented from cooling trials in four pigs. In 3 pigs the device was deployed via an open incision, and in one via an 18mm laparoscopic port. This limits assessment of the manoeuvrability and ease of application of the Herrel device in a purely laparoscopic setting. The Herrel device was not developed further.

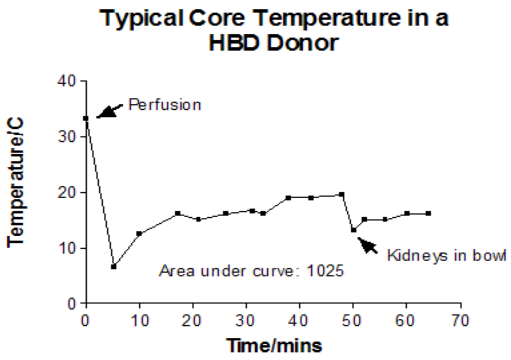
More recently, techniques involving placement of ice slush in close proximity to the kidney have been employed and demonstrate effective renal cooling (Ames *et al*., 2005, Gill *et al*., 2003a, Laven *et al*., 2007). However, critics point to the potential for whole body cooling and the limitation of access. Also, techniques involving direct application of coolant have the potential for causing injury through tissue freezing. Irrigation/suction systems using chilled normal saline (Webster *et al*., 2005, Weld *et al*., 2007) avoid access and freezing issues, but remain subject to issues of potential contamination and core temperature reduction. A device which could demonstrate effective laparoscopic renal cooling and ischaemic protection, whilst avoiding the weaknesses associated with previous methods, would be of great interest to urological surgeons.

### 1.6.2 Cryopreservation in renal transplantation

In heart beating donation, normal perfusion with warm blood is followed immediately by cold perfusion before the organs are removed and placed on ice i.e. there is no primary warm

ischaemic period. As cryopreservation greatly attenuates the effects of ischaemia, organs retrieved in this way can be assumed to suffer minimal injury (see Fig 1.2).

**Figure 1.2 – Typical Core Temperature in a Controlled Heart-Beating donor**




Measurement of intra-abdominal organ temperature during retrieval from a Brain-Stem Dead donor. Note that organs are well-perfused with warm oxygenated blood until the marked point of *in-situ* cold perfusion and introduction of ice. Relatively rapid cooling is then achieved (Jennings, 2001).

In the case of NHB donation, a primary warm ischaemic period is unavoidable. In this situation warm kidneys with high metabolic rates are denied oxygen and metabolic substrates. The length of this period and therefore the severity of ischaemic injury resulting injury varies with the Maastricht categories.

NHBD renal transplantation is made possible only because of interventions which ameliorate the effects of warm ischaemia. In the controlled situation (cat. III and IV) kidneys may be subjected to injury during the period of potential sub-optimal or hypoperfusion known as the ‘agonal time’.

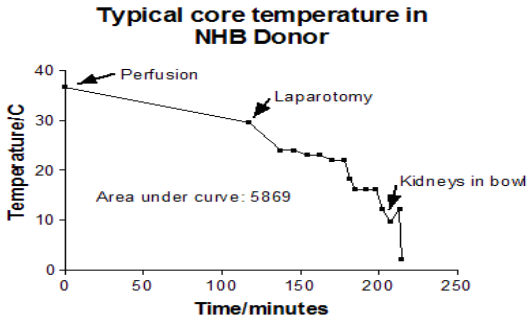
This is defined as the period between withdrawal of life-supporting treatment and cardiac arrest. Most centres place a limit on the maximum agonal period accepted. However, the controlled situation allows prior mobilisation of personnel and resource, and following a 'no touch' period after cardiac standstill, the retrieval team is able to act immediately. Techniques of DBTL femoral cannulation (in the ITU setting) and/or rapid laparotomy and aortic cannulation (in theatre), prior to cold *in-situ* perfusion and the introduction of ice to the abdomen, allow rapid establishment of organ cryopreservation interventions.

The uncontrolled (cat. II) NHBD situation requires powerful organ preservation techniques. The French have recently instituted an uncontrolled NHBD program with the majority of donors subject to a procurement/preservation protocol based on aortic placement of a DBTL catheter (Figure 1) and cold in-situ perfusion as soon as possible after declaration of death (Antoine *et al*., 2008). The recently established 'condition T' protocol in Pittsburgh employs similar techniques (International NHBD Meeting, London, May 2008). The European approach to the uncontrolled donor was developed through the experiences of groups such as Maastricht (Daemen *et al*., 1994). Newcastle-upon-Tyne has been retrieving kidneys from uncontrolled donors since 1998, using a modified Maastricht approach which includes an initial streptokinase flush. Although the protocol has been subject to evolution and improvement over time, techniques are focused on the timely introduction of cold *in-situ* perfusion to provide sufficient renal cooling and preservation until the donor can be taken to theatre; often as long as two hours after declaration of death (Balupuri *et al*., 2000c, Gok *et al*., 2002d).

Unfortunately, Newcastle has found that these techniques have a limited effect on core body and hence renal temperature. A previous study used an infra-red laser thermometer to measure intra-abdominal temperature (left lobe of liver surface temperature at laparotomy) on retrieval of category

II kidneys with a mean of 29.5°C after 120 minutes of perfusion (Jennings, 2001) (Figure 1.3). For this period renal cells would have remained metabolically active and continued to consume significant quantities of ATP. Adequate cooling and reduction of the effects of warm ischaemia cannot currently occur until laparotomy is performed and abdominal ice slush introduced. Because of the logistical difficulties and legal formalities associated with uncontrolled NHBDs two hours of in-situ perfusion may pass before the donor reaches theatre for laparotomy.

**Figure 1.3 – Typical Core Temperature in an uncontrolled NHBD.**




Measurement of intra-abdominal organ temperature during retrieval from a Category II NHBD. Note that effective cooling is not achieved until laparotomy and the introduction of ice, approximately two hours after death (Jennings, 2001).

At present, during part of the warm ischaemic period of the NHBD, *in-situ* perfusion and external cooling will improve the level of ischaemic injury. This is achieved by a small cooling effect and via the benefits of the preservation solution. However the temperatures achieved suggest

that the increased PNF and DGF rates associated with NHBDs are likely to be related to inadequate preservation methods.

### 1.6.3 Peritoneal Cooling in NHBDs

The peritoneal cavity is large and easily accessible percutaneously, while the surface area of the peritoneum itself is extensive enough to permit effective dialysis. These properties would suggest that the peritoneum may offer an excellent route for core body cooling, and indeed the use of peritoneal cooling for systemic hypothermia has been described since the 1950s. The technique has also been described for cooling in heat stroke and malignant hyperpyrexia (Gjessing *et al*., 1976) and resuscitative hypothermia to limit neurological injury in stroke and cardiac arrest (Horowitz, 1989). In contrast, the intra-peritoneal administration of warm fluids is an established method of treating severe hypothermia, and can achieve re-warming rates of 5-10°C/hour.

Peritoneal cooling has been shown to be effective at reducing core temperature in animal studies. Xiao *et al* showed in a canine cardiac arrest model that peritoneal cooling reduced core temperature measured in the pulmonary artery by 0.6°C/min, four times the cooling rate achieved by surface cooling with ice bags (Xiao *et al*., 1995). Takahashi *et al*, using an *ex vivo* canine model with ATP measurements and biochemical markers of ischaemia, demonstrated that peritoneal cooling gave equivalent results to cooling by cardiopulmonary bypass, and better results than *in-situ* perfusion alone, in heart, liver and kidney transplantation (Takahashi *et al*., 1996). Yadav *et al* compared *in situ* perfusion alone with a combination of in situ perfusion and peritoneal cooling in a porcine model of warm ischaemia, and found the addition of peritoneal cooling to significantly reduce renal core temperatures (Yadav *et al*., 1997).

In Human donors, the Washington Hospital Center group reported data from their Rapid Organ Recovery Program (RORP) for uncontrolled DCDs which was implemented in 1994. RORP used both intra-vascular cooling and peritoneal cooling with two laparoscopic trocars inserted into the lower abdomen with a closed refrigerated circuit of iced normal saline continuously recirculated throughout the peritoneal cavity. The fully refined system was reportedly capable of achieving peritoneal temperatures of 10°C in 8 minutes, with IVC temperature falling to 15°C in 30 minutes and 10°C by 60 minutes (Light *et al*., 1997, Light *et al*., 2000a, Light *et al*., 2000b). However, the donors described were younger than commonly seen in Europe, and were mainly trauma victims at the MedSTAR trauma unit. Furthermore, no control group approximating the Newcastle / European protocols of *in-situ* cold perfusion alone were described. It is therefore not possible to draw firm conclusions regarding any potential benefit over that achieved by the Maastricht approach-based protocols.

The aims of this thesis flow from the broad areas covered within this introduction, namely; ischaemic injury assessment, laparoscopic renal cryopreservation, and peritoneal cooling for NHBDs.

# Aims

## 1.7 Aims

### Ischaemic injury Assessment

To assess the efficacy and potential of ischaemic injury assessment or viability testing within a renal Non Heart Beating Donor (NHBD) transplantation program by:

a) Prospective assessment of the effects of warm ischaemia on retrospectively established measures of organ viability; machine perfusion and perfusate enzyme analysis in a large animal model.

b) Assessment of outcomes of NHBD kidneys with low severity pre-arrest acute renal failure selected using machine perfusion viability testing

c) Assessment of outcomes from dual renal transplantation of 'marginal kidneys' selected using machine perfusion viability testing

### Laparoscopic Renal Cryopreservation

d) To develop a device for laparoscopic renal cooling

e) To develop a large animal model for laparoscopic renal cooling

f) To assess the efficacy of a laparoscopic renal cooling device using transplantation organ viability assessment

### Peritoneal Cooling

g) To develop a large animal model of the uncontrolled NHBD for assessment of the efficacy of additional peritoneal cooling versus current protocols.

h) To establish a human trial of uncontrolled NHBD peritoneal cooling.

# Chapter 2

# Methods

## Methods

### 2.1 Organ Recovery Systems Lifeport ® Kidney Transporter

This is the most commonly used kidney hypothermic machine perfusion device in transplantation today. The unit is self-contained within a plastic shell, which incorporates a lockable lid (Figure 2.1).

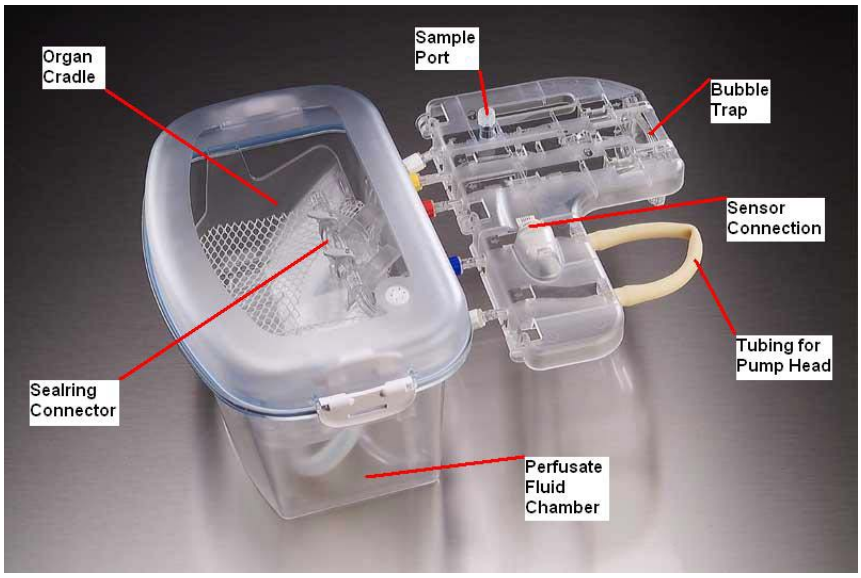
**Figure 2.1**

**Organ Recovery Systems Inc. Lifeport ® Kidney Transporter**

The kidney is contained within disposable sterile plastic inserts, which include an organ cradle and lidded fluid chamber. The insert also incorporates the fluidics system, which involves sterile tubing, a filter, and a bubble-trap (Figure 2.2).

**Figure 2.2**

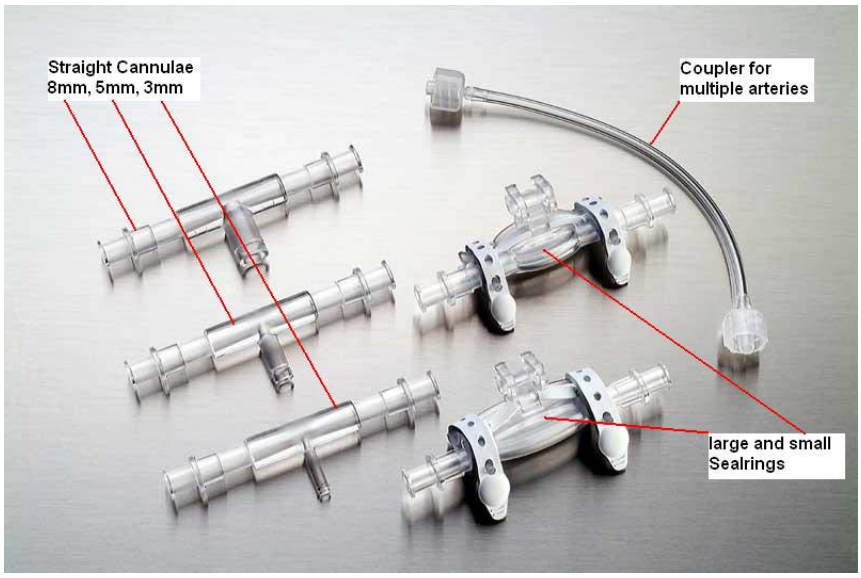
**Lifeport Sterile Insert; Organ Cradle sitting within the Perfusate Fluid Chamber, Fluidics System and Sensor Connector.**




Once situated within the insert, the renal artery is connected to the perfusion circuit using specialized connection devices. Two methods exist; the Sealring and straight cannula (Figure 2.3).

**Figure 2.3**

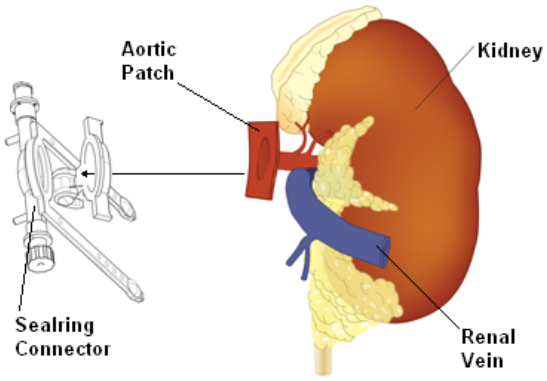
**Lifeport Components for Connection between Renal Artery and the Machine Perfusion Circuit**




For Sealring connection an adequate aortic (Carrel) patch is required. This method represents the ideal technique for connection to the machine as the renal artery is untouched and hence undamaged, with the seal formed at the edges of the patch (which may be used safely as trimming invariably occurs prior to transplantation). The Carrel patch is gently passed into the Sealring as shown in Figure 2.4. The patch is then carefully positioned so as on hinge-closure the arterial wall of the patch is sandwiched circumferentially between the upper and lower seals. The rubber securing bands can then be tightened to complete the connection.

**Figure 2.4**

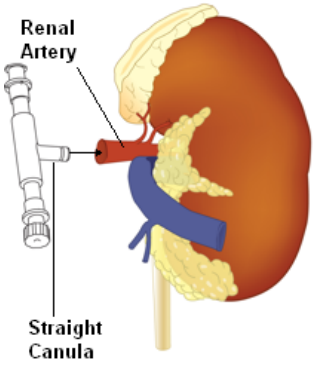
**Connection of Lifeport® Sealring to Renal Arterial Aortic Patch**




If the patch is absent or too small, a straight cannula may be used by direct placement into the arterial lumen, before securing with a tie (Figure 2.5). This method is effective, but risks damage to the arterial intima which may necessitate shortening of the artery prior to transplantation. This may cause technical difficulties and increases the risk of vascular thrombosis.

**Figure 2.5**

**Connection of Lifeport® Straight Cannula to Renal Artery**



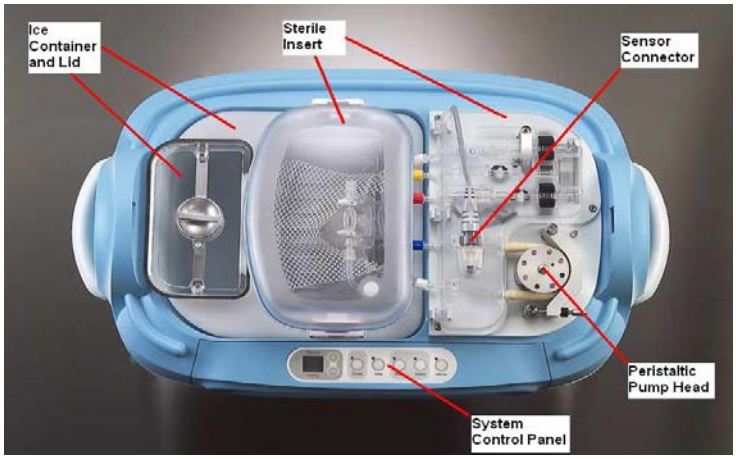

In the case of multiple patches or cut vessels, as a result of non-standard anatomy and additional polar renal arteries, combinations of two sealrings and/or straight cannulae may be connected using the coupler tubing shown in Figure 2.3. This allows both arterial circulations to be perfused on the single circuit.

The insert's fluid chamber (inside which sits the cradle and organ) is placed into the ice container, so as to surround the chamber with ice to effect cooling. The insert / ice container then fits into the Lifeport casing. An electronic link connects the parametric sensors, within the insert fluidics system, to the Lifeport displays and computer / memory systems. In addition a flexible portion of the insert tubing is designed to fit around a peristaltic pump head (Figure 2.6). Once all of this is in place the lid of the perfusate chamber is removed under aseptic conditions, and cold perfusion/preservation solution (1L Kidney Preservation Solution, KPS®) is poured, using a no-touch technique, into the chamber so as to fill it and surround the kidney. At this point the unit's power is activated and

instructed to ‘wash’. This activates the roller pump and commences an initial fluidic system prime and bubble purge. Fluid is drawn from the bottom of the chamber, through the filter system, into the bubble trap, and back to the chamber. Once completed the system is set to ‘prime’, which sends fluid from the bubble trap to the perfusion portion of the circuit. This tubing can then be connected to the seal ring or straight cannula. On completion of the circuit, the pressure rise is detected by the system and the pump stopped. At this point, the operator instructs the Lifeport to ‘perfuse’. The pump is activated and will adjust its speed in order to achieve the pressure instructed (usually 30mmHg). This pressure drives the preservation solution into the renal artery and the kidney is perfused. The solution then leaves the kidney via the renal vein back into the fluid chamber reservoir.

**Figure 2.6**

**Lifeport® with Sterile Insert installed**



Machine perfusion at high pressures is potentially damaging. The Lifeport therefore prevents the operator from using excessively high pressures (40mmHg max.). As mentioned above, the pump will drive fluid until the selected pressure is achieved down-stream. It then stops rotating before resuming when the pressure falls below the target.

Data available to the operator is: flow rate, resistance, pressure setting, perfusate temperature, and ice container temperature. These parameters are involved in viability assessment. The flow rate is estimated from pump speed, which has been calibrated and set in the factory using different size apertures and by measurement of the volume circulating per minute. This is an accepted and accurate method, used for flow estimation in all clinical dialysis pump systems.

## 2.2. Machine Perfusion Viability Testing

### 2.2.1 Pressure Flow Index

The protocol is detailed in the Introduction, Chapter 1.5.3.2.

### 2.2.2 Measurement of Perfusate Glutathione-S-Transferase (GST)

Total GST (i.e., including alpha-, mu-, pi-, and other GST isoforms) is used for viability assessment in Newcastle. The method employed is a GST Colorimetric Activity Assay (Gok *et al*., 2003b) and is based upon the GST-catalyzed reaction between glutathione (GSH) and the GST substrate, CDNB (1-chloro-2,4-dinitrobenzene, which has the broadest range of isozyme detectability). The GST-catalyzed formation of CDNB-GSH produces a dinitrophenyl thioether which can be detected by a spectrophotometer at 340 nm. One international unit of GST is defined as the amount of enzyme producing 1 mmol of CDNB-GSH conjugate/minute under the conditions of

the assay. All GST assays were kindly performed by the Freeman Hospital department of biochemistry under the auspices of Dr. Robert Peaston.

## 2.3 Microdialysis

Microdialysis is a technique to monitor the chemistry of the extracellular space in living tissue. When a physiological salt solution is slowly pumped through a microdialysis probe the solution equilibrates with the surrounding extracellular tissue fluid. After a period of time it will contain a representative proportion of the tissue fluid's molecules (Figure 2.7).

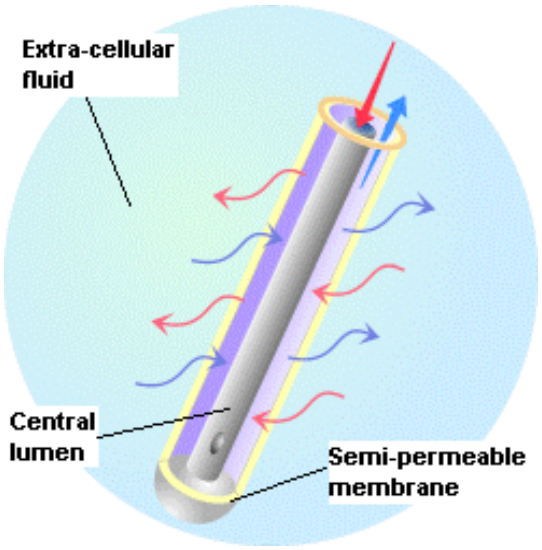
Microdialysate is then extracted and can be analysed immediately. This study employs the validated markers of glucose, pyruvate, lactate and glycerol for assessment of the degree of renal ischaemia and cell injury within a tissue (Keller *et al*., 2008, Weld *et al*., 2008). In well-perfused, oxygenated renal tissue extra-cellular fluid (ECF) glucose levels will remain within normal levels. Glycolysis produces relatively static concentrations of pyruvate, measurable prior to convertion into acetyl-CoA which enters the Kreb's cycle. In ischaemic tissues glucose concentrations fall as perfusion decreases and available glucose is exhausted. As the degree or duration of ischaemia increases, anaerobic conditions will force the conversion of pyruvate to lactate. Thus in early or partial renal ischaemia pyruvate/lactate ratios may reflect aerobic/anaerobic metabolic balance. With complete ischaemia of longer durations, only anaerobic metabolic activity is possible due to absolute absence of oxygen. Therefore pyruvate levels will fall as cellular glucose is exhausted, and all available pyruvate will be converted to lactate. Subsequently, lactate levels will rise, and plateau as pyruvate is consumed in an effort to raise ATP.

Glycerol can be used as a marker of cell injury or death. In this context, the role of glycerol is as a major constituent of the plasma membrane (Keller *et al*., 2008, Weld *et al*., 2008). As ATP

levels fall the ATPase pump transporters in the cell membrane fail, leading to cellular influx of sodium and calcium and efflux of potassium. This results in osmotic cell swelling, loss of plasma membrane integrity, glycerol release, and cell death.

**Figure 2.7**

**Schematic representation of the microdialysis catheter tip.**




The bold red arrow demonstrates the flow of microdialysis fluid inside the central lumen of the catheter, before exiting at the tip and passing alongside the semi-permeable membrane to form microdialysate (following equilibration with the ECF). The bold blue arrow then indicates passage of microdialysate into a collection vial.

### 2.3.1 CMA 600 Microdialysis Analyzer ®

The CMA 600 Microdialysis Analyzer is a small clinical chemistry analyzer, designed and manufactured by CMA Microdialysis Ltd, Stockholm, Sweden (Figure 2.8). It allows analysis of the very small volumes of microdialysate obtained from microdialysis catheters or probes. Reagents are available for glucose, lactate, glycerol, urea, pyruvate and glutamate.

The CMA 600® incorporates a single-beam photometer system for colorimetric measurement of the differential optical absorbance of a sample with respect to a reference. The sample and the reference are held in a two compartment container. A rotating transport wheel driven by a stepping motor is utilized to move the container so as to pass the sample and the reference through a light beam in succession. The intensity of the light transmitted through the sample and the reference is measured by a photocell. The output from the photocell is fed into an electronic system which produces an output signal corresponding to the differential optical absorbance of the sample with respect to the reference. This allows for production of a calibrated measurement.

The sample tray has 9 positions for direct analysis and 24 positions for batch analysis. There are positions for four reagents and one calibrator. The CMA 600 needs about 1 µL of sample plus the amount required for the different assays. Sample and reagent volumes are controlled by a syringe pump equipped with a precision glass syringe of 500 µL. Absorbance measurements are made with the single-beam filter photometer described above, equipped with a LED and wavelength filters for 375 and 546 nm.

Four different analytes can be measured per sample and data may be displayed as trend curves on a computer screen. It is possible to monitor nine catheters or probes simultaneously. The

analysis takes approximately 1.5 minutes per analyte and the results are stored immediately hard disk to minimize the risk of data loss.

**Figure 2.8**

**CMA 600 ® Microdialysis Analyzer**

### 2.3.2. Assays

The CMA microdialysis assays for measurement of biochemical markers of ischaemia utilize a final common product; quinonamine is a red / violet coloured molecule which absorbs light at a wavelength of 546nm. The CMA 600 is consequently set up to detect absorbance at this wavelength allowing quantitative assessment of the proportional analyte sample concentration.

#### 2.3.2.1 Lactate

Microdialysate lactate concentration was determined using a quantitative colorimetric method. Lactate is ezymatically oxidized by lactate oxidase (LOD). Hydrogen perosxide is produced

reacts with 4-chlorophenol and 4-aminoantipyrine, catalysed by peroxidase (POD). This produces the red-violet coloured quinonamine. The rate of formation of quinonamine is proportional to the lactate concentration as measured photometrically by the CMA 600 microdialysis analyzer at a wavelength of 546nm (Figure 2.9).

**Figure 2.9**

**Colorimetric assay for quantitative determination of Lactate concentration**

$$\text{Lactate} + O_2 + H_2O \xrightarrow{\text{LOD}} \text{Pyruvate} + H_2O_2$$

$$H_2O_2 + \text{4-Chlorophenol} + \text{4-Aminoantipyrine} \xrightarrow{\text{POD}} \text{Quinonamine} + 2\,H_20 + HCl$$

### 2.3.2.2 Pyruvate

Pyruvate is enzymatically oxidized by pyruvate oxidase (PyrOx). The hydrogen peroxide formed reacts with 4-aminoantipyrine and N-ethyl-N-(2-hydroxy-3-sulfopropyl)-m-toluidine (TOOS). This reaction is catalysed by peroxidase (POD) and produces quinonamine to allow colorimetric measurement of pyruvate concentration.

**Figure 2.10**

**Colorimetric assay for quantitative determination of Pyruvate concentration**

$$\text{Pyruvate} + \text{Inorganic Phosphate} + O_2 \xrightarrow{\text{PyrOx}} \text{Acetylphosphate} + CO_2 + H_2O_2$$

$$H_2O_2 + \text{4-Aminoantipyrine} + \text{TOOS} \xrightarrow{\text{POD}} \text{Quinonamine} + 4\,H_20$$

### 2.3.2.3 Glucose

Microdialysate glucose is oxidized by glucose oxidase (GOX). Again hydrogen peroxide is formed which reacts with phenol and 4-aminoantipyrine, catalysed by peroxidase, yielding quinonamine.

**Figure 2.11**

**Colorimetric assay for quantitative determination of Glucose concentration**

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOX}} \text{Gluconic acid} + H_2O_2$$

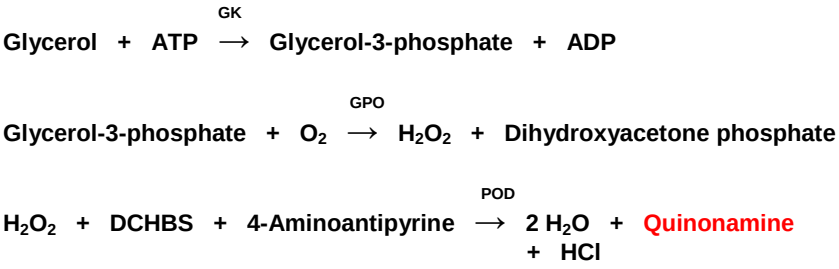
$$2\,H_2O_2 + \text{4-Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinonamine} + 4\,H_2O$$

### 2.3.2.4 Glycerol

Microdialysate glycerol is phosphorylated by adenosine triphosphate (ATP) and glycerol kinase (GK) to glycerol-3-phosphate, which is subsequently oxidized in the presence of glycerol-3-phophate oxidase (GPO). Hydrogen peroxide is formed and reacts with 3, 5-dichloro-2-hydroxybenzene sulphonic acid (DCHBS) and 4-aminoantipyrine. This reaction is catalysed by peroxidase and again produces the red-violet quinonamine for colorimetric assessment.

**Figure 2.12**

**Colorimetric assay for quantitative determination of Glycerol concentration**



# Chapter 3

# Renal Ischaemic Injury Assessment

# Renal Ischaemic Injury Assessment

## 3.1 Introduction

Kidneys transplanted from Non Heart Beating Donors (NHBDs) are generally regarded as 'marginal' grafts due to their association with warm ischaemic injury. The degree of warm ischaemia sustained by NHBD grafts is related to the Maastricht criteria, with the most prolonged periods occurring in the Category II (uncontrolled) donor. Prolonged warm ischaemia results at best in delayed graft function (DGF) and at worst in primary non function (PNF).

In the UK the demand for kidneys continues unabated, prompting interest in the use of 'marginal' organs in order to increase the donor pool (Gagandeep *et al*., 2006, Gok *et al*., 2002b, Marks *et al*., 2006, Whiting *et al*., 2006). Concerns exist regarding the outcomes of such grafts, with several groups reporting increased rates of DGF and PNF (Brook *et al*., 2003, Cooper *et al*., 2004, Keizer *et al*., 2005). However, many series have shown encouraging short and medium term functional and graft survival outcomes from these donors (Gagandeep *et al*., 2006, Gok *et al*., 2002c, Marks *et al*., 2006, Whiting *et al*., 2006). In addition, it has been shown that transplantation of marginal kidneys is associated with a significant survival benefit over maintenance on dialysis (Ojo *et al*., 1999).

Kidneys from NHBDs have often sustained a significant degree of ischaemic injury. As a result it is necessary to assess the extent of this injury prior to transplantation, in order to determine whether thresholds of viability have been breached (Balupuri *et al*., 2000b, Daemen *et al*., 1995, Daemen *et al*., 1997c, Light, 2000). The Newcastle-upon-Tyne group has shown that viability testing can be used to avoid transplantation of organs that are likely to have PNF (Gok *et al*., 2002b).

### 3.2 Machine Perfusion Viability Assessment

In Newcastle-upon-Tyne NHBD renal transplantation relies upon a rapid-response retrieval team which allows institution of early organ preservation interventions in the controlled and uncontrolled donor situation. Following organ retrieval, all NHBD kidneys undergo hypothermic machine perfusion which allows assessment of viability. This involves calculation of the Pressure Flow Index (PFI), defined as the flow per 100g renal mass divided by the systolic pressure (Balupuri *et al*., 2000b, Gok *et al*., 2002b), and measurement of perfusate glutathione-S-transferase (GST), an enzymatic marker of ischaemic injury (Balupuri *et al*., 2000b, Daemen *et al*., 1995, Kievit *et al*., 1997, Nyberg *et al*., 2005). The Newcastle viability protocol for single NHBD renal transplants requires a PFI of ≥0.4 ml/min/100g/mmHg, and a GST level of less than 100 IU/L/100g renal mass (Gok *et al*., 2002b).

Machine perfusion viability assessment has been in existence for some time. However, controversy remains regarding the un-validated nature of current assessment protocols. The research described in this chapter seeks to address this controversy; the first study described is the first to prospectively assess the Newcastle viability assessment criteria at known warm ischaemic time points. This has the potential to confirm or refute a genuine and predictable relationship between ischaemic duration and said criteria.

It shall be seen that with evidence of reliability of current assessment protocols it may be possible to utilise this information so as to allow maximisation of organ resources within an active renal transplantation program. To achieve this, novel ways of utilising viability assessment techniques must be investigated. The second study focuses on the thesis that in terms of organ resource management, assessment of potentially damaged organs after retrieval may be more

efficient than flat refusal of donors exhibiting certain negative characteristics (in this case, evidence of acute renal failure).

Another area with the potential to improve donor organ utilisation lies around the 'borderline' viability assessments. An organ which just passes viability assessment and is subsequently transplanted may be of similar quality to one which just fails and is subsequently discarded. The third study seeks to assess the suitability of dual transplantation for such organs, with the aim of increasing the effectiveness of function within recipients and also to decrease the numbers of organs discarded.

### 3.3 Viability Testing using Machine Perfusion and Perfusate Enzyme Analysis:

### Prospective assessment of the effects of warm ischaemia on retrospectively established measures of organ viability

The viability criteria described above were determined retrospectively through analysis of outcomes during the evolution of the Newcastle NHBD program (Gok *et al*., 2002b), much as other viability testing centres have established their respective criteria. In a sense all such criteria are destined to remain unvalidated, as it would be unethical to transplant organs for which a strong suspicion of non-viability exists. In this way it could never be proven that an organ which fails a viability assessment would indeed have resulted in PNF if transplanted (Chang, 1995). Although the opportunity for true validation of a retrospectively produced viability test is lacking, the case for such assessment would be strengthened by demonstration of the relationship between injury and the marker seeking to reflect it. Clearly it is impossible to prospectively validate the effect of increasing duration of warm ischaemia within a human subject. In order to better elucidate the relationship an animal model is required.

### 3.3.1 Materials and Methods

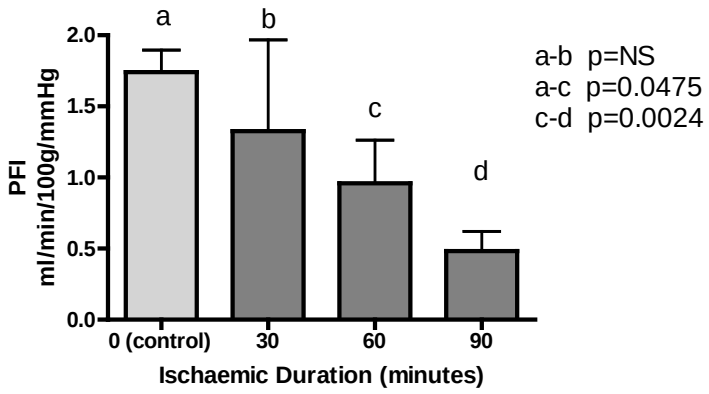
Ten anaesthetised juvenile landrace pigs underwent laparoscopic renal mobilisation. In 9 pigs, one kidney was then subjected to a period of ischaemia of 30, 60 or 90 minutes (giving 3 kidneys per ischaemic period group). The contra-lateral kidney was used for another study. In the $10^{th}$ pig, both kidneys were used as controls, with normal perfusion prior to retrieval. After rapid laparotomy the kidneys were placed on ice and prepared for machine perfusion according to local NHBD viability testing protocols (Gok *et al*., 2002b). PFI and peak GST levels were then determined during 4 hours of machine perfusion.

Statistical analysis was performed using Prism version 4.0 for Windows. Data are expressed as mean ± SD. Normality of distribution was confirmed using the D'Agostino and Pearson omnibus normality test, prior to unpaired t-test comparison. Non-parametric analysis of continuous data was performed using the Mann-Whitney U-test. Categorical variables were compared using Fisher's exact test. Statistical significance was defined as $p < 0.05$.

### 3.3.2 Results

Peak PFI ($ml.min^{-1}\ 100g^{-1}\ mmHg^{-1}$) at 4 hours (usually the T3 or T4 value) is the primary viability assessment criterion in Newcastle. Mean peak PFI was 1.74 ± 0.16 in the control group (normal perfusion prior to retrieval). In the 30 minutes warm ischaemic group mean PFI was similar to that seen in controls study groups, 1.33 ± 0.64 ($p = 0.456$). Mean PFI then decreased significantly with increasing ischaemic time *versus* controls. At 60 minutes it was 0.96 ± 0.30 (p 0.0475), and at 90 minutes 0.48 ± 0.14 (p 0.0024) (Figure3.1). A linear relationship between PFI and ischaemic duration was demonstrated ($r^2 = 0.9976$) (Appendix 1).

**Figure 3.1**



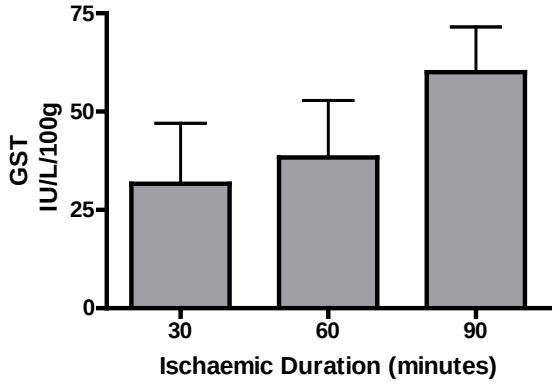

**Pressure Flow Index (PFI) according to ischaemic duration**

Column demonstrates mean data with error bars denoting SD. In group a (control) n=2 kidneys. In groups b-d n = 3. Mann-Whitney U-test statistical comparison between groups is shown.

GST perfusate concentration adjusted for renal mass (IU/L/100g) showed a clear rising trend with increasing ischaemia (Figure 3.3), although statistical significance was not achieved. GST was not detected in control samples (the assay was not capable of detecting levels <14 IU/L). At 30, 60, and 90 minutes, mean peak total GST levels were 31.67 ± 15.37, 38.33 ± 14.50, and 60.00 ± 11.53 IU/L/100g respectively.

**Figure 3.3**

**Mean peak Glutathione-S-Transferase (GST) concentration per 100g renal mass according to ischaemic duration.**




### 3.3.3 Discussion

The Newcastle PFI was seen to accurately reflect *in-situ* warm ischaemic time and therefore the potential degree of ischaemic injury prospectively in a large animal model. This builds on the suggestion of previous retrospectively-produced studies that the PFI *(Balupuri et al., 2000b, Gok et al*., 2004b, Gok *et al*., 2002b), and other protocols which employ resistive indexes (Daemen *et al*., 1995, Light, 2000), are able to accurately reflect ischaemic exposure and hence injury.

Peak GST levels during machine perfusion showed a rising trend with increased ischaemic duration, which mirror the retrospective data from earlier work in Maastricht and Newcastle (Daemen *et al*., 1997c, Gok *et al*., 2003b).

This study is the first to provide prospective large animal model validation to further support the use of combined PFI and GST enzyme viability assessment in NHBD renal transplantation.

## 3.4 Pre-Arrest Acute Renal Failure in NHBD transplantation

Kidneys from controlled NHBDs (cat. III and IV) are retrieved from donors subject to withdrawal of prognostically futile life-support (Gok *et al*., 2004b). As mentioned elsewhere, they generally have a shorter and more predictable warm ischaemic time than those from Maastricht category I and II uncontrolled donors, and are thus predisposed to a more favourable outcome after transplantation (Daemen *et al*., 1996, Gok *et al*., 2002b).

These donors are usually admitted, treated and investigated in Intensive Care or Therapy Units (ITU). Some show evidence of Acute Renal Failure (ARF) through a raised serum creatinine (SCr) prior to withdrawal of treatment, without any previous history of renal impairment. This apparent deterioration in renal function is invariably caused by the pathological processes responsible for situation of medical futility. Many transplant specialists would consider ARF in a NHBD as an absolute contraindication for renal transplantation, and hence would halt the donation pathway at this point. This view ignores the dynamic nature of renal function in the donor context and fails to appreciate the potential reversibility of early ARF. In other words, the threat of transplanting damaged organs which might result in PNF is enough to justify abandoning the retrieval process. However, the organ viability assessment protocols used in Newcastle-upon-Tyne allow testing of the potential reversibility of renal ischaemic injury (Balupuri *et al*., 2000b, Balupuri *et al*., 2001). Judicious use of such assessments may allow a proportion of individuals with evidence of pre-arrest ARF or impairment to be used as renal donors.

This approach has been followed in Newcastle-upon-Tyne, and in order to address the understandable concerns of poor outcome of grafts from category III NHB donors with evidence of ARF prior to organ recovery, graft function from these donors was examined, with comparison to

kidneys transplanted from a control group of category III NHBDs with normal pre-arrest renal function.

### 3.4.1 Methods

All renal transplant recipients from category III donors between 1998 and 2005 were identified. Category III NHB referrals come predominantly from the Intensive Care Units (ICU). The NHBD retrieval protocol described elsewhere (Gok *et al*., 2002b) was employed for all donors. Viability assessment using PFI machine perfusion (Balupuri *et al*., 2001), and perfusate GST criteria (Daemen *et al*., 1995, Gok *et al*., 2003a) was undertaken to determine the suitability of individual organs for transplant.

The RIFLE criteria (acronym indicating Risk of renal dysfunction; Injury to the kidney; Failure of kidney function, Loss of kidney function and End-stage kidney disease), can be used to classify the severity of acute renal failure (Table 3.1) (Bellomo *et al*., 2004a, Bellomo *et al*., 2004b).

**Table 3.1**

**RIFLE Criteria for Acute Renal Failure**

The RIFLE criteria were designed to stratify the severity of renal injury and failure according to clinical parameters.

| Category | GFR Criteria | Urine Output U/O Criteria | |
|---|---|---|---|
| **Risk** | Increased creatinine x1.5 or GFR decrease >25% | UO < 0.5ml/kg/h x 6 hrs | High Sensitivity |
| **Injury** | Increased creatinine x 2 or GFR decrease >50% | UO < 0.5ml/kg/h x 12 hrs | |
| **Failure** | Increase creatinine x 3 or GFR decrease > 75% or S-Cr > 4mg/dl | UO < 0.3ml/kg/h x 24 hr or Anuria x 12 hrs | High Specificity |
| **Loss** | Persistent ARF = complete loss of kidney function > 4 weeks | | |
| **ESKD** | End Stage Kidney Disease (> 3 months) | | |

Recipients were divided into study (ARF) and control groups. Allocation to the ARF group was made on the basis of changes in serum-creatinine (SCr), as this data is routinely available in category III NHBDs. The study therefore included grafts recovered from donors with ARF of any severity according to the RIFLE criteria. RIFLE status was determined through comparison of SCr, 24 hrs before cardiac arrest and immediately prior to withdrawal of treatment. For instance, if SCr 24 hours prior to cardiac death was 100 $mmol.L^{-1}$, but at withdrawal had risen to 200 $mmol.L^{-1}$, this donor would qualify for the 'Injury' RIFLE category, and recipients of organs from such a donor would enter the ARF study group. Where no qualifying changes in SCr were identified in the donor, recipients entered the control group. In other words, control group donors had no evidence of deterioration in renal function prior to cardiac arrest.

The primary endpoints of this study were recipients' Glomerular Filtration Rate (GFR) calculated by Modification of Diet in Renal Disease (MDRD) formula at 3 and 12 months post-transplant. The secondary endpoints were PNF rate, duration of DGF and rejection rate in the first year.

PNF was defined as permanent loss of graft without evidence of function at any point after the transplant, and DGF was defined as graft function sufficiently impaired for the recipient to require dialysis.

Statistical analysis was performed using SPSS version 11.0 for Windows. Continuous variables were compared using Mann Whitney U for non-parametric data. Categorical variables were compared using the chi-square test or Fisher's exact test if the underlying assumptions of asymptotic methods could not be met.

### 3.4.2 Results

From the beginning of phase III of the NHBD program in Newcastle-upon-Tyne (1998) until the end of 2005, 49 single kidney recipients from category III NHBDs were identified. As mentioned, all kidneys had undergone hypothermic machine perfusion and viability testing prior to transplantation.

9 recipients received kidneys from 5 donors with ARF according to the RIFLE criteria (ARF group). In the control group, there were 40 recipients from 25 donors with normal SCr prior to cardiac arrest.

There were no statistically significant differences between the groups for potential confounding factors including donor and recipient age and sex, ischaemic times, HLA mismatches, incidence of acute rejection, duration of DGF and PNF rates. (Tables 3.2 and 3.3)

**Table 3.2**

**Statistical Comparison of Donor-related Factors between Study Groups**

| | ARF Donor N=5 | Normal RF Donor N=25 | P |
|---|---|---|---|
| **Age Donor (Mean ± SE)** | 45.2±7.3 | 38.5±3.6 | 0.41* |
| **Sex Donor M:F** | 3:2 | 3:2 | |
| **Ischaemic times (min)** | | | |
| **1st WIT** | 19.33±2.6 | 19.68±1.1 | 0.94* |
| **2nd WIT** | 33.89±4.4 | 36.53±1.3 | 0.56* |
| **CIT** | 1608±128 | 1358±52 | 0.096* |
| **Total IT** | 1662±127.2 | 1414±52 | 0.095* |
| **Donor Cause of Death** | | | |
| **HI** | 1 | 6 | |
| **POD** | 1 | 1 | |
| **ICB** | 1 | 3 | |
| **SAH** | 2 | 4 | |
| **MI** | | 1 | |
| **CVA** | | 3 | |
| **SDH** | | 3 | |
| **Other** | | 4 | |
| **HLA Mismatch (Median)** | | | |
| **A** | 1 | 1 | 0.2† |
| **B** | 1 | 1 | 0.52† |
| **DR** | 1 | 1 | 0.06† |
| **S-Cr micromol/l** | | | |
| **24 Hrs pre-arrest** | 88.0±13.2 | 79.2±6.16 | 0.91* |
| **Pre-arrest** | 175±35.2 | 89.8±6.16 | 0.004* |

** Mann Whitney U test*

*† Chi-square test*

*HI Head injury, POD Paracetamol over Dose, ICB Intracranial Bleed, SAH Sub-Arachnoid Haemorrhage, MI Myocardial Infarction, CVA Cerebrovascular Accident, SDH Sub-Dural Haemorrhage, WIT Warm Ischaemic Time, CIT Cold Ischaemic Time*

**Table 3.3**

**Statistical Comparison of Recipient Related Demographic Factors and Outcomes**

| | ARF Donor N=9 | Normal Renal Function Donor N=40 | P |
|---|---|---|---|
| **Age Recipient (Mean ± SE)** | 57.1±2.4 | 49.3±2.0 | 0.09* |
| **Sex Recipient M:F** | 5:3 | 2:1 | |
| **Immediate Function %** | 75 | 52.5 | 0.43† |
| **DGF %** | 25 | 47.5 | 0.43† |
| **DGF (days)** | 18.0 ±1.1 | 7.5 ±1.7 | 0.15* |
| **N = Primary Non-Function** | 1 | 0 | 0.18† |
| **Rejection %** | 25 | 27.5 | 1† |
| **eGFR (MDRD)** | | | |
| **3 months** | 45.36± 6.07 | 43.78 ±2.23 | 0.966* |
| **12 months** | 42.18± 6.28 | 44.68± 2.89 | 0.805* |

** Mann Whitney U test*
*‡ Fisher Exact test*

There was a statistically significant difference in mean pre-arrest SCr of the donor between two groups (175 µmol.$L^{-1}$ ± 35.2 and 89.8 ± 6.16 for ARF and control groups respectively, p 0.004, Mann Whitney U test).

One transplant in the ARF group resulted in PNF (11.1%). Of the remainder (n=8) the mean GFR was 45.36 ml. $^{-1}$ min $^{-1}$ 1.73m $^{-2}$ ± 6.07 after 3 months. In the control group after 3 months, the mean GFR was 43.78 ± 2.23. Mean GFR after 12 months was 42.18 ± 6.28 and 44.68 ± 2.89 in ARF and control groups respectively There was no significant difference between the groups at 3 months (p 0.966, Mann Whitney U test) (Figure 3.4), nor at 12 months (p 0.805, Mann Whitney U test) (Figure 3.5)

**Figure 3.4 – Recipients' GFR at 3 months**

Category III NHBD kidney recipients Glomerular Filtration Rate at 3 months post transplantation; Acute renal failure study group *versus* normal function controls (p 0.966, Mann Whitney U test)

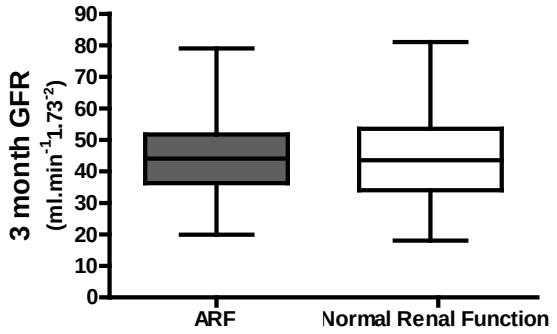
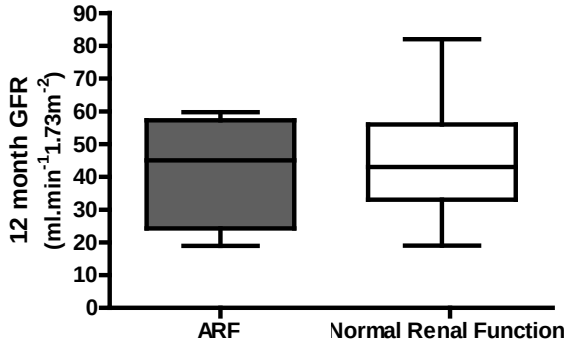



**Figure 3.5 – Recipients' GFR at 12 months**

Category III NHBD kidney recipients Glomerular Filtration Rate at 12 months post transplantation (p 0.805, Mann Whitney U test).



### 3.4.3 Discussion

Most clinicians view ARF as a potentially reversible condition. It has multiple aetiologies often resulting in renal hypo-perfusion, and in many cases normal renal function resumes following adequate resuscitation (Bellomo *et al*., 2004a, Bellomo *et al*., 2004b). In NHBDs, kidneys may show evidence of ARF prior to arrest, demonstrated by deterioration in SCr. The extent of this damage can be assessed by machine perfusion and viability testing, and where the thresholds of viability have been breached, the organs are not used. However, organs with pre-arrest evidence of ARF which pass viability testing may retain the potential for functional improvement.

The Newcastle-Upon-Tyne group uses viability testing to avoid transplantation of organs that are likely to have PNF (Balupuri *et al*., 2000c, Balupuri *et al*., 2001). Machine perfusion has enabled selection of viable kidneys based on physical (flow, pressure, resistance, temperature) (Balupuri *et al*., 2001, Light, 2000) and biochemical parameters (glutathione S-transferase) (Daemen *et al*., 1997c, Gok *et al*., 2003a, Kievit *et al*., 1997). Non-viable kidneys are discarded.

Of 9 kidneys from donors with pre-arrest evidence of ARF, one resulted in PNF. In this case, the donor renal artery was damaged during retrieval preventing machine perfusion and viability testing. The second kidney of the pair passed viability testing, and on this basis, both kidneys were transplanted. Unfortunately, the un-perfused kidney represents the one case of PNF.

Those kidneys which passed viability testing and were subsequently transplanted show similar results to the Category III donors with normal renal function at the time of donation. The data suggests that the Newcastle protocols of ischaemic injury assessment are sufficiently robust as to allow determination of the potential reversibility of early renal damage. If other centres pursued a similar approach, additional organs from NHB situations (which would at present never progress to

donation) could contribute to the donor pool. In the short term results are certainly favourable. However, long term results of using such donor kidneys remains to be determined.

## 3.5 Dual renal transplantation for kidneys from 'Marginal' NHBDs

Several groups have shown that viability testing can be used to avoid transplantation of organs that are likely to have PNF (Balupuri *et al*., 2000b, Daemen *et al*., 1995, Light, 2000). However, in Newcastle-upon-Tyne the parameters assessed in the course of viability testing appear to identify a second group of kidneys, which although unsuitable for single transplantation may be considered for dual transplantation. The group is best considered as 'marginal failures' against original viability testing thresholds. The pressure on renal transplant waiting lists is well-emphasised in the introduction (section 3.1).These pressures push retrieval centres to seek options for decreasing the rate of discard for retrieved kidneys, in order to maximise resources. One obvious group for assessment are the 'marginal failure' organs, which lie just beyond the accepted thresholds of viability.

An argument could be made that all organs which fail viability testing, even marginally, should be discarded without further thought. In this way, recipients are better protected against PNF. However, another view is that although kidneys of this group would be unlikely to produce a sufficient glomerular filtration rate (GFR) to support the recipient as a solitary transplant, when used together as a dual transplant they could potentially produce sufficient renal function for one patient.

In order to maximise the number of transplants from NHBD retrievals a program of dual kidney transplantation was commenced in Newcastle in 2003. The basic parametric viability thresholds used are detailed in the Introduction chapter (section 1.5.3) *(Balupuri et al., 2000b, Gok et al*., 2002b). They involve calculation of the Pressure Flow Index (PFI), defined as the flow per 100g

renal mass divided by the systolic pressure, and measurement of perfusate glutathione-S-transferase (GST), an enzymatic marker of ischaemic injury.

The Newcastle viability protocol for single NHBD (or all prior to 2003) renal transplants requires a PFI of ≥0.4 ml/min/100g/mmHg, and a GST level of less than 100 IU/L/100g renal mass. All organs with a PFI of <0.4 are discarded, as retrospective analysis suggests irreversible injury is likely to have occurred beyond this point (Talbot, D. data on file, 2003). However, if the GST measurements are above the original threshold for single organ transplantation (or occasionally due to other factors such as donor co-morbidity or cold ischaemic time) Newcastle surgeons may opt for a dual transplant.

The majority of the dual transplant organs in this study were selected on the basis of viability assessment. Therefore they had invariably breached the viability criteria deemed safe for single organ transplantation in our centre. This precluded randomisation of such organs to single transplantation in the context of a randomised control trial. Subsequently, in order to determine whether the dual transplants achieved appropriate early functional outcomes, a retrospective study was performed.

### 3.5.1 Materials and Methods

Dual renal transplantation is performed as an ipsilateral procedure in our centre, allowing preservation of one iliac fossa for future use as required. In an ipsilateral dual transplant, both kidneys from a single donor are implanted into the right iliac fossa with anastomoses to the common (right kidney) and external iliac (left kidney) arterial circulations. The result is a “double-decker” configuration, with the right kidney above the right (Figure 3.6). The ureters are implanted separately as two ureteroneocystostomies. The procedure therefore necessitates a longer ureter for

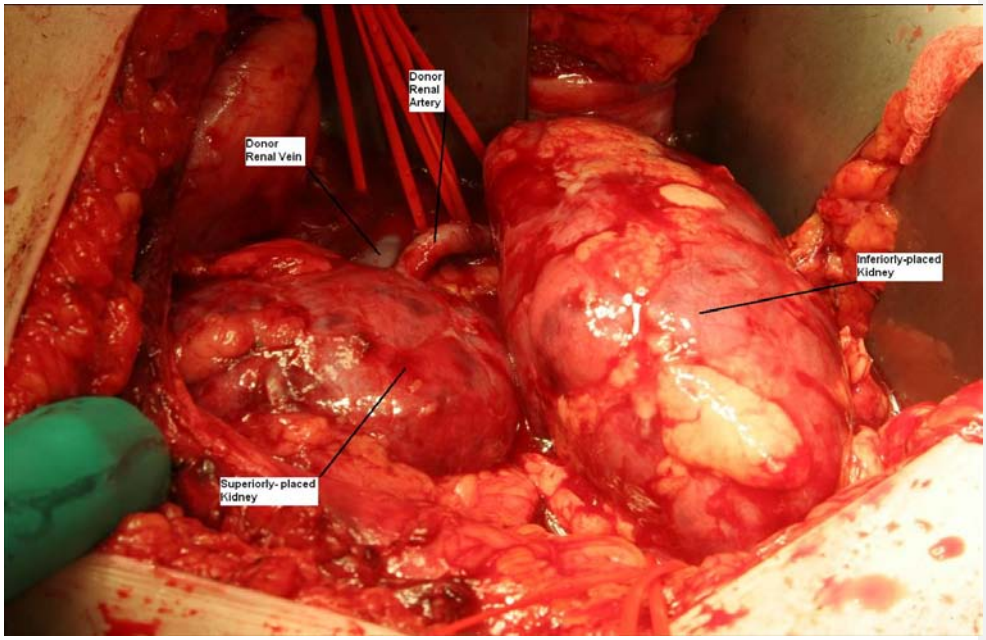
the cephalad kidney, as this ureter must exceed the length of the caudal kidney, and allow a tension-free ureteroneocystostomy.

The Newcastle unit performed its first dual NHBD renal transplant in December 2003. From December 2003 - 2007, 23 dual NHBD renal transplants were identified. Using post-transplant recipient GFR at 3 and 12 months as primary endpoints, the dual transplant group was compared with a series of 115 single NHBD transplants. Function was assessed using GFR ($ml^{-1}$ $min^{-1}$ $1.73m^{-2}$), estimated from patient age, sex, ethnicity, serum creatinine, urea, and albumin using the extended MDRD formula (Levey *et al*., 1999).

Secondary endpoints were incidence of DGF, PNF and organ discard rate. DGF was defined as insufficient initial graft function requiring dialysis, and PNF as permanent graft loss without evidence of function at any point.

**Figure 3.6**

**Ipsilateral Dual NHBD Renal Transplant following reperfusion and prior to closure in Newcastle**




Statistical analysis was performed using Prism version 4.0 for Windows. Data are expressed as mean ± SD. Normality of distribution was confirmed using the D'Agostino and Pearson omnibus normality test, prior to unpaired t-test comparison. Non-parametric analysis of continuous data was performed using the Mann-Whitney U-test. Categorical variables were compared using Fisher's exact test. Statistical significance was defined as $p<0.05$.

### 3.5.2 Results

From December 2003 to December 2007 23 dual renal transplants were performed (17 Category II, 6 Category III). The majority (n=18) were selected using viable PFIs (≥0.4 ml/min/100g/mmHg) with high GSTs (>100 IU/100g) in both organs. The remainder were selected using viable PFIs with high GSTs plus evidence of poor in-situ perfusion (n=1), poor in-situ perfusion alone (n=1), donor co-morbidity alone (n=2), and long CIT alone (n=1). The remainder of NHBD transplants for the same period; 115 single NHBD transplants (48 Category II, 67 Category III, 1998-2006) were identified for comparison.

The dual group contained a greater proportion of Category II donors compared to the single group according to the NHBD Category II: III ratio; 17:6 (n=23) versus 48:67 (n=115) (p 0.009). Otherwise the dual and single groups were well-matched with no significant differences found with respect to donor and recipient age, ischaemic times, HLA mismatch, CMV mismatch, and recipient cardiovascular risk score. (Table 3.4)

One dual transplant recipient died 8 months post-transplantation with a functioning graft. There was no significant difference in rates of PNF between the groups. Two dual transplants resulted in PNF (8.7%), compared with 11 single transplants (9.6%;Table 3.5). The incidence of DGF in the dual transplant group was 81.0%, significantly higher than in the single group where the incidence of DGF was 59.2% (p 0.049). However no difference was found between the dual group and the single Category II donor transplants, where DGF incidence was 88.9%.

Of the 21 functioning dual transplants the mean GFR after 3 months was 46.2 ± 17.3. In the single transplant group (n=104) the 3 month mean GFR was 40.7 ± 13.7. In the dual group 16 recipients have reached 12 months post-transplant. The mean GFR at this point was 44.6 ± 17.2

versus 43.0 ± 15.7 in the single group (n=81). No significant differences in graft function were observed at 3 (p 0.21) or 12 months (p 0.72) post-transplant (Table 3.6, Figure 3.7).

**Table 3.4**

**Dual and Single Groups; Matching for Potential Confounding Factors**

| | DUAL (N=23) | SINGLE (N=115) | P |
|---|---|---|---|
| **Donor Age (yr)** | 49.9 +/-11.6 | 42.3 +/-15.3 | NS |
| **Recipient Age (yr)** | 52.8 +/-11.4 | 49.9 +/-13.1 | NS |
| **Ischaemic Times** | | | |
| $1^{o}$ WIT (mins) | 26.5 +/-6.7 | 21.5 +/- 7.3 | NS |
| $2^{o}$ WIT (mins) | 33.1 +/-6.1 | 38.5 +/- 8.0 | NS |
| CIT (hr) | 21.7 +/- 1.8 | 23.7 +/- 4.7 | NS |
| **HLA Mismatch (median)** | | | |
| A | 1 | 1 | NS |
| B | 1 | 1 | NS |
| DR | 1 | 1 | NS |
| **Maastricht Cat. II:III** | 15:8 | 48:67 | 0.009 |
| **CMV Mismatch N (%)** | 4 (23.5%) | 13 (11.3%) | NS |
| **Recipient Cardiovascular Score (median)** | 7 | 6 | NS |

**Table 3.5**

**Complications in the Dual Transplant Group**

| Complication | Number of Cases (%) |
|---|---|
| Primary Non-Function | 2 (8.7%) |
| Delayed Graft Function | 17 (81.0%) |
| Acute Rejection | 6 (28.6%) |
| Vascular Thrombosis | 0 |
| Ureteric complications / Urine leak | 2 (9.52%) |
| Wound dehiscense | 1 (4.76%) |

**Table 3.6**

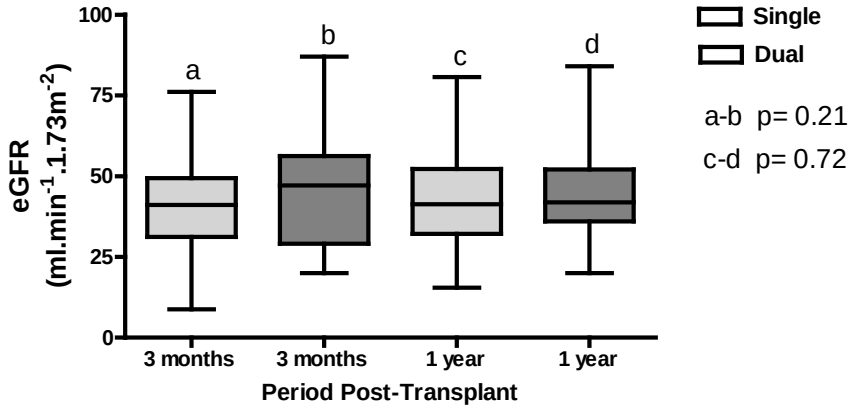
**Early Outcomes in NHBD Dual Transplants**

| RECIPIENT | MAASTRICHT CATEGORY | REASON FOR DUAL | PNF | DGF | GFR 3/12 | GFR 12/12 |
|---|---|---|---|---|---|---|
| 1 | II | Long CIT | - | + | 45 | 67 |
| 2 | II | High GST | - | + | 73 | 84 |
| 3 | III | Poor in-situ perfusion | - | + | 24 | 33 |
| 4 | II | High GST | - | - | 30 | 39 |
| 5 | II | Diabetic, renal impairment | - | + | 20 | 20 |
| 6 | II | High GST | + | NA | - | - |
| 7 | II | High GST | - | + | 87 | 22 |
| 8 | II | High GST | - | + | 52 | 48 |
| 9 | III | Diabetic, renal impairment | - | + | 40 | 49 |
| 10 | II | High GST | - | - | 56 | 52 |
| 11 | II | High GST | - | + | 49 | 52 |
| 12 | II | Poor in-situ perfusion High GST | - | + | 28 | 41 |
| 13 | II | High GST | - | + | 47 | Died |
| 14 | III | High GST | - | + | 27 | 20 |
| 15 | III | High GST | - | + | 58 | 62 |
| 16 | II | High GST | - | + | 53 | 41 |
| 17 | II | High GST | - | + | 40 | 43 |
| 18 | III | High GST | - | + | 50 | 39 |
| 19 | II | High GST | - | + | 56 | - |
| 20 | III | High GST | - | + | 41 | - |
| 21 | III | High GST | + | NA | - | - |
| 22 | II | High GST | - | - | 67 | - |
| 23 | III | High GST | - | - | 25 | - |

**Figure 3.7**

**Functional eGFR outcomes in dual and single NHBD grafts at 3 and 12 months post-transplantation**.

The median, inter-quartile range and spread of the data is presented with un-paired t-test statistical significance.




The organ discard rate for each year of Newcastle NHBD program is shown in Table 3.7. When examining these, it is useful to consider our NHBD program in three eras; 1998-2001 (basic protocol) 2001-2003 (introduction of streptokinase flush during in-situ perfusion), and 2004-2006 (Dual transplantation established). As previously reported, the introduction of streptokinase produced a significant reduction in the non-use rate from 49.4 ± 10.0 %to 28.8 ± 2.9% between the first and second eras respectively (p 0.0125). The introduction of the dual renal transplantation option has also resulted in a reduction in the non-use rate to 19.1 ± 4.3%, although this did not reach statistical significance. (Figure 3.8)

**Table 3.7**

**Discard Rates for NHBD Kidneys; 1998-2006**

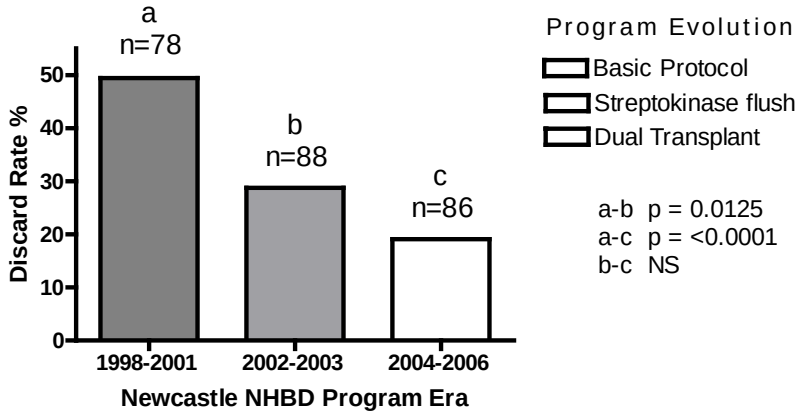
Discarded kidneys are those which fail viability assessment and are therefore not transplanted following retrieval.

| | NHBD PROGRAM ERA | CAT. II-IV DISCARDS (N/TOTAL) | CAT. II-IV DISCARD RATE (%) | CATEGORY II DISCARDS (N/TOTAL) | CATEGORY II DISCARD RATE (%) | DUAL TRANSPLANTS (N) |
|---|---|---|---|---|---|---|
| **1998** | Basic | 7/16 | 43.8 | 4/10 | 66.7 | 0 |
| **1999** | Basic | 13/32 | 40.6 | 9/20 | 45.0 | 0 |
| **2000** | Basic | 19/30 | 63.3 | 16/24 | 66.7 | 0 |
| **2001** | Streptokinase | 16/32 | 50.0 | 12/18 | 66.7 | 0 |
| **2002** | Streptokinase | 8/26 | 30.8 | 7/14 | 50.0 | 0 |
| **2003** | Streptokinase | 8/30 | 26.7 | 5/12 | 41.7 | 1 |
| **2004** | Dual Transplant | 9/34 | 26.5 | 6/16 | 37.5 | 4 |
| **2005** | Dual Transplant | 5/26 | 19.2 | 2/12 | 16.7 | 5 |
| **2006** | Dual Transplant | 3/26 | 11.5 | 2/14 | 14.3 | 7 |

**Figure 3.8**

**Percentage Kidney Discard Rate according to NHBD program evolution**

Statistical analysis performed using Fisher's exact test.



### 3.5.3 Discussion

The concept of 'nephron dosing' suggests that for successful transplantation a critical mass of renal tissue is required to satisfy the metabolic demands of the recipient (Nicholson *et al*., 2000). It could be argued that organs which have sustained acute or chronic injury may, if transplanted, produce less function per unit mass than might otherwise be expected. As the severity of injury increases, a point is reached where the use of a single organ graft will result in transplantation of insufficient renal mass to support the recipient. The only option available to increase the mass of renal tissue received from such a donor is to transplant both kidneys into a single recipient, effectively doubling the 'nephron dose'.

Dual renal transplantation has been previously reported for 'expanded criteria donors' (ECDs) in the USA, with excellent functional recipient outcomes (Alfrey *et al*., 1997a, Alfrey *et al*., 1997b, Lu *et al*., 2000). Alfrey *et al* retrospectively reviewed the outcomes of 52 transplants from ECD donors which had been turned down by other centres. These included 15 dual transplants. In the dual transplants, the majority of which were from donors aged ≥59 years with a GFR <90 ml. $min^{-1}$ $1.73m^{-2}$, the mean sCr at one year was 1.6mg.$dl^{-1}$ (141 µmol.$l^{-1}$) ± 1.5, compared to 2.8mg.$dl^{-1}$ (248 µmol.$l^{-1}$) ± 1.5 in single organ transplants. The authors therefore suggested that organs retrieved from ECDs aged ≥59 years, with a GFR <90 ml.$min^{-1}$ $1.73m^{-2}$ would be best utilised as dual grafts.

Employing different criteria for age (≥54 years vs. <54 years) the same group reported later outcome data confirming the encouraging performance of 121 dual ECD grafts. At one year dual graft recipients mean sCr was 1.7 ± 0.7 mg.$dl^{-1}$ (150 ± 62 µmol.$l^{-1}$) compared with the single organ group where sCr was 2.2 ± 1.0 (194 ±88 µmol.$l^{-1}$). In a 50 year old Caucasian male, these sCr values would represent GFRs of approximately 46 and 34 ml.$min^{-1}$ $1.73m^{-2}$ in the dual and single groups

respectively. This suggests that both the dual and single ECD transplant groups reported by Alfrey *et al* and Lu *et al* were providing roughly appropriate levels of recipient renal function.

ECDs might generally be expected to perform inferiorly to 'standard criteria donors' (SCDs) on viability assessment, as they have had a greater exposure to chronic disease processes. Indeed, when the Dual and Single transplant groups are assessed according to UNOS ECD/SCD status, ECD donors can be seen to make up approximately half of the Dual group, compared to less than 20% of the Single group. However, early functional graft performance is unaffected by ECD/SCD status, with no significant differences observed between the groups.

**Table 3.8**

**Dual and Single Study Groups according to Expanded (ECD) and Standard (SCD) Criteria Donor Status; 12 month Functional Outcomes.**

| | Dual ECD | Single ECD | Dual SCD | Single SCD |
|---|---|---|---|---|
| **Number (% study group)** | 11 (47.8%) | 20 (17.4%) | 12 (52.2%) | 95 (82.6%) |
| **GFR at 12 months** | 41.7 ± 14.0 | 42.2 ± 11.0 | 48.3 ±21.2 | 43.2 ± 16.7 |

A greater proportion of ECDs were present in the Dual group (47.8% vs. 17.4%). No significant differences were observed between any of the 4 groups below following unpaired t-test analysis.

Transplanting two kidneys into one recipient which then produce an excellent GFR is a waste of resources. In this situation, patients on the waiting list would have been better served by two single organ transplants producing two significantly lower, but sufficient and appropriate recipient GFRs. Equally, a dual transplant which produces insufficient function or results in PNF is extremely unwelcome. However, consideration must be given to any transplant procedure which has the potential to provide an appropriate level of recipient renal function, and also may prevent

unnecessary organ non-use following retrieval. Thus the decision to utilise the dual transplant option is a crucial one.

In this series kidneys suitable for dual transplantation have been identified by several factors including prolonged cold ischaemic time after non heart beating donation and where donor stable renal function was known to be sub-optimal. However, the majority were selected on the basis of viability tests, namely a viable PFI combined with high perfusate GST levels. Currently PFI is used purely to decide when an organ must be discarded. Examination of the possibility of using different PFI thresholds for single and dual organ transplantation is planned.

In retrospect two pairs of kidneys (Table 3.6, recipients 2 and 7) probably had an insufficient flush prior to machine perfusion producing an artificially high GST, and therefore could have been transplanted singly. Patient 7 after excellent initial progress developed problems with immunosuppressant medication concordance, resulting in deterioration of graft function.

Outcomes in the single group were broadly in line with previous publications from this group (Gok et al., 2002b), and the GFRs resulting from the dual transplants from 'marginal' NHBDs correlate well with these. The data demonstrates that pairs of NHBD kidneys with individually viable PFIs, but high GST levels, may be transplanted together successfully as dual grafts. Such kidneys, which do not satisfy the criteria for single organ transplantation, can then go on to produce early recipient renal function statistically comparable to that of their single organ counterparts.

Therefore, by utilising the factors outlined above to identify NHBD kidneys suitable for dual transplantation, unnecessary organ non-use is avoided and organ resources are maximised. Early functional outcomes suggest that dual NHBD renal transplants can reliably generate appropriate graft function for recipients.

## 3.6 Conclusions

The aims of studies detailed in this chapter were designed to assess the three main aspects of the current viability assessment debate. The first issue centres on the arguments of validity; an organ which fails a viability test is not transplanted, and therefore the opportunity to prove that the correct decision was made (PNF of the graft) is lost. This would be possible in an animal model but unfortunately was beyond the resources of the investigator. However, with a clear and predictable relationship established between the primary criterion (the PFI) and ischaemic duration in porcine model, further weight is added to viability assessment argument.

When seeking to maximise an organ resource, two further areas for study are suggested; contraindications for donation (or a broadening of the donor pool to include more potentially marginal grafts) and organs with 'borderline' viability assessment profiles. For the former, one doubtful exclusion criteria common to many institutions was highlighted for analysis. As discussed, the dynamic and often reversible nature of renal injury suggests that blanket contraindications to donation resulting from clinical evidence of such injury may be unwise. The second study demonstrates more than acceptable outcomes from donors which other centres would have declined. The proviso is that such organs are transplanted only where machine perfusion viability assessment requirements are met.

Organs which fail viability assessment are discarded. This is clearly disastrous for transplantation programs and must be minimised where possible. One way to achieve this is by ensuring all routes to effective utilisation are explored. The novel technique of dual renal transplantation was identified as exhibiting such potential, and the third study describes excellent outcomes from organs which would have previously been discarded. Another route to effective

utilisation is the optimisation of the retrieval and organ preservation process. This is addressed later in the thesis (Chapters 5 and 6).

The validation of the Newcastle machine perfusion renal viability assessment protocols with clarification and further delineation of the relationships involved, prompted consideration of the use of such techniques in the assessment of renal ischaemic injury as it pertains to other areas of local research. An opportunity arose to assess the effects of renal cooling, the current mainstay of renal preservation, on viability tests. The next chapter focuses on the assessment of the efficacy of a laparoscopic renal cooling device, designed to provide ischaemic protection in the context of a partial nephrectomy procedure, using the viability assessments described above.

# Chapter 4

# Laparoscopic Renal Cryopreservation

# Laparoscopic Renal Cryopreservation

## 4.1 Introduction

Renal surgery often necessitates clamping of the renal artery in order to control blood loss from what is an extremely vascular organ. Once surgery is completed, and the cut edges of the kidney closed, renal arterial supply can be re-established. Unfortunately, this means ischaemic exposure for the renal tissue required to regain normal function following completion of surgery. Urologists have long followed the rules set down by Wickham in the late 1960s (Wickham *et al*., 1967). The most important of which was to limit renal ischaemic to a maximum of 30 minutes. He also suggested that organs should be cooled to limit damage. Consequently, accepted urological practice requires effective renal cooling during ischaemic periods. Prior to the advent of laparoscopic surgical techniques, the generous exposure of operative sites meant introduction of ice could be achieved simply and easily. Although this approach was open to complications of total body hypothermia, it was successfully employed for many years.

The rise of laparoscopic surgery, with general benefits including reduced infection, pain, hospital stay etc, has brought new technical challenges in many surgical specialties. One such challenge is renal hypothermia for laparoscopic partial nephrectomy. In section 1.6 of the Introduction chapter, previous attempts to achieve an effective device are described. The emerging picture is one of a ‘work in progress’ with no single method or device proving effective without significant flaws in design, issues of technical complexity, or the potential for complications for the patient. To this end, the prototype Newcastle-Upon-Tyne laparoscopic renal cooling device was produced.

### 4.2 Design of the Laparoscopic Renal Cooling Device

The laparoscopic kidney cooling device consists of two parts: the laparoscopic cooling bag and the cooling circuit. The principle behind the design of the system is to achieve hypothermia in the renal tissue by circulating coolant, maintained at a temperature close to 2°C, across the surface of the kidney.

The laparoscopic cooling bag has a two-layer construction. The coolant circulates between these layers. The bag incorporates three catheters, two allow coolant inflow and the other outflow. The catheters connect directly into the cooling circuit. The cooling bag opens distally with a drawstring running around the open edge. Thus, when the drawstring is pulled from the proximal end of the device, the bag closes. 'Manoeuvring tags' on the open edge of the bag allow easy points for laparoscopic grasper application during deployment (Figure 4.1). Prior to use of the device the kidney to be cooled is fully mobilized. The device is then introduced into the abdomen through an 18mm port. Using graspers on the manoeuvring tags, the open edges of the bag are pulled down over the kidney. Once the kidney is positioned inside the bag the drawstring is pulled, allowing the bag to be secured around the hilum whilst completely enveloping the kidney. To begin cooling, the bag is primed with coolant and the circuit activated (Figures 4.1 and 4.2).

**Figure 4.1**

**Schematic representation and photograph of the prototype Newcastle-Upon-Tyne Laparoscopic Renal Cooling Device**

Catheters are connected to the fluidic cooling circuit, providing both in- and outflow of coolant.

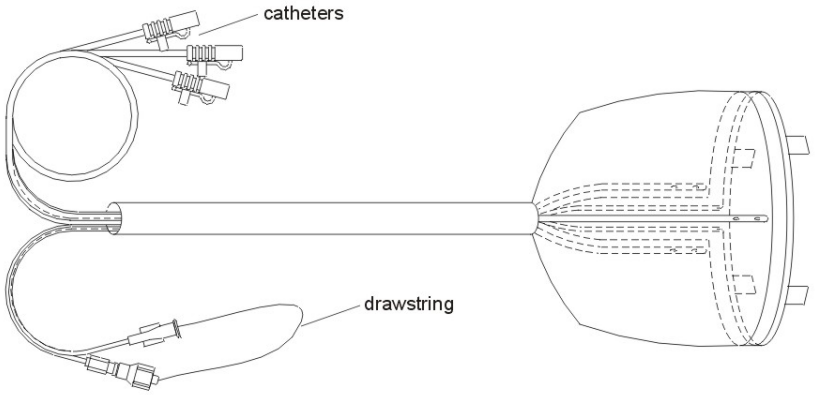



**Figure 4.2**

**Schematic representation of the device in full deployment, with the drawstring secured around the renal hilum.**

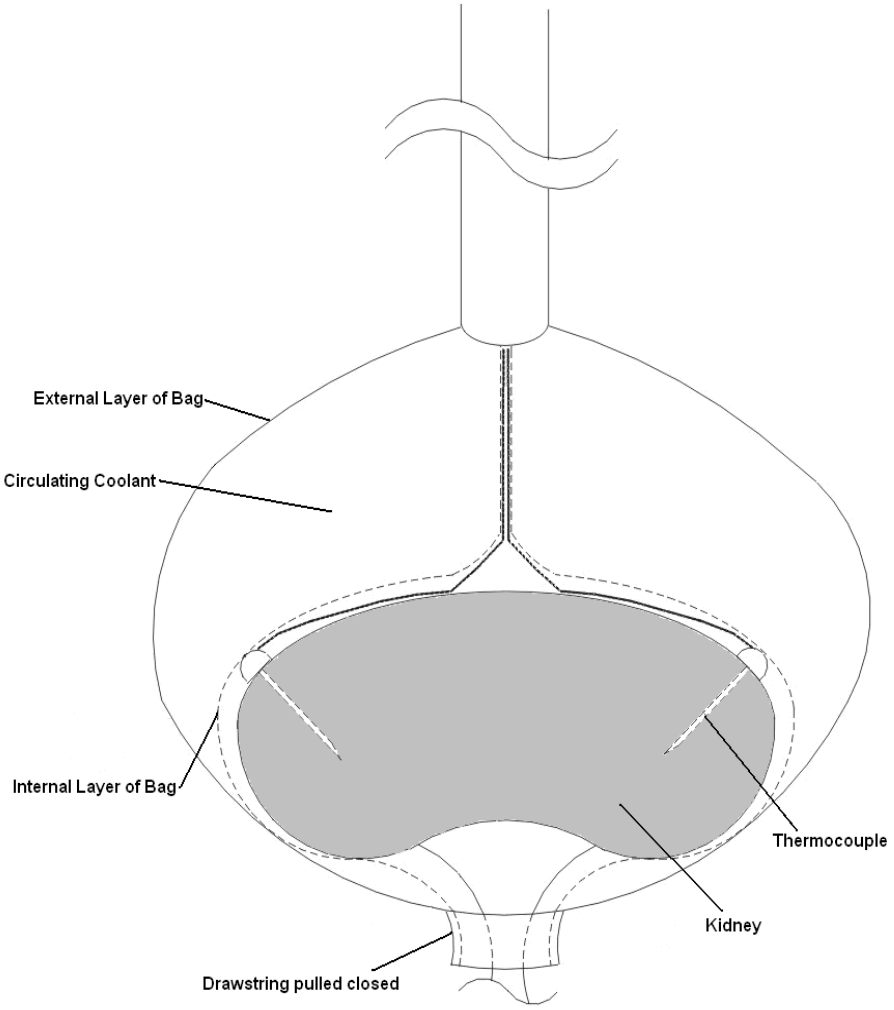
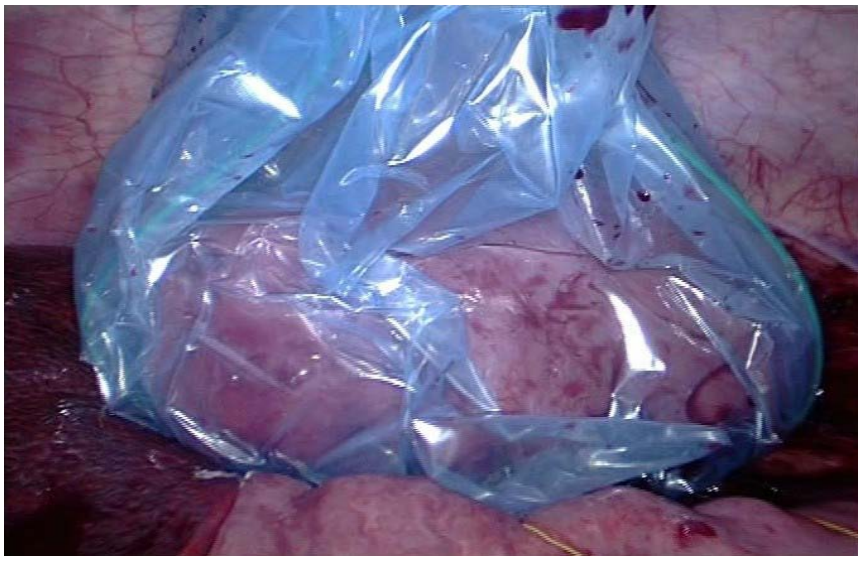


**Figure 4.3**

**Photograph of the device in full deployment, with the drawstring secured around the renal hilum.**

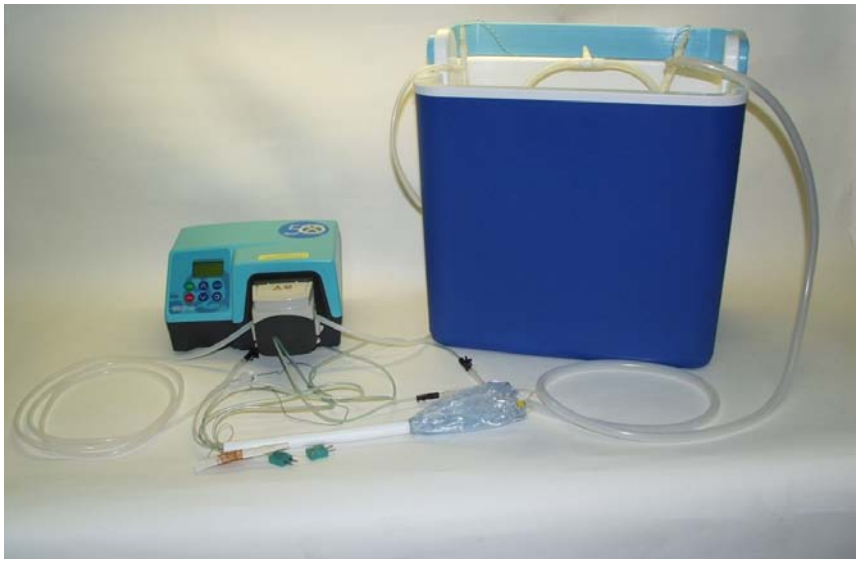


The cooling circuit consists of a heat exchanger and peristaltic pump (323 U/D pump, Watson-Marlow Bredel Pump Ltd, UK). The heat exchanger is constructed from two stainless steel coils in series, packed in ice in a thermally insulated container. When in use the circuit is closed and a fixed volume of coolant (0.9% saline) is circulated at a constant rate of 250ml.min$^{-1}$ controlled by the peristaltic pump. Coolant leak can be detected visually or through changes in flow parameters within the circuit.

The device is intended as a prototype to achieve proof of concept in a non-recovery animal model, and is therefore not sterile. As the device is developed towards human clinical viability, it is planned to reduce the calibre to allow use via a standard 10/12mm access port.

**Figure 4.4**

**The laparoscopic renal cooling system incorporating the device, heat exchange tank containing ice slush and steel coils, peristaltic pump and tubing.**



## 4.3 Evaluation of the ischaemic protection efficacy of the Newcastle Laparoscopic Renal Cooling Device using renal transplantation viability assessment criteria in a porcine model

The aim of regional renal hypothermia in laparoscopic surgery is to limit ischaemic injury and extend safe operating time. A renal cooling device capable of achieving these aims should also be capable of demonstrating functional preservation. To date, many studies which have attempted to address the issue of renal injury after warm and cold ischaemia in animal models and have employed serum creatinine as a surrogate marker of renal function. However it is increasingly acknowledged that serum creatinine is not an accurate indicator of renal damage (Webster *et al*., 2005).

In a non-recovery animal model it is not possible to demonstrate functional preservation directly. However the relationship between renal hypothermia, attenuation of ischaemic injury, and the preservation of renal function following reperfusion is universally accepted. Therefore a device which can demonstrate both effective renal cooling and attenuation of ischaemic injury should also preserve function.

A reliable model for the assessment of ischaemic injury exists in the field of renal transplantation. As described in detail elsewhere, kidneys from NHBDs are expected to sustain a significant degree of ischaemic injury prior to organ retrieval. As a result many transplant centres require assessment of the extent of this injury prior to transplantation, in order to determine whether thresholds of viability have been breached (Daemen *et al*., 1995, Gok *et al*., 2002b, Light, 2000). Viability testing thus demonstrates a spectrum of ischaemic injury, which varies with increasing ischaemic duration and efficacy of organ preservation interventions (chapter 3, section 3.3). These techniques, applied to an animal model of laparoscopic renal ischaemia, allow a reliable means of assessing the efficacy of a cryopreservation device.

### 4.3.1 Materials and Methods

The study was designed to achieve a power of 80%. Using an alpha of 0.02 to detect a pressure/flow index difference of 0.75 $ml.ml^{-1}$ $100g^{-1}$ $mmHg^{-1}$, three kidneys in each group produces a power of 82.2%. Two kidneys in each group would have produced a power of 62.8%. The requirement, therefore, was for 18 study kidneys with the addition of 2 controls.

In ten anaesthetised juvenile landrace pigs a 10/12mm infra-umbilical laparoscopic port was placed using Hassan's technique prior to formation of pneumo-peritoneum at 12 mmHg. Two further 5mm ports were placed as necessary under vision. Laparoscopic renal mobilisation was then achieved bilaterally. Once fully mobilised with adequate control of the renal pedicle, vessel loops were passed twice around the renal vessels. Each end of the loop was then passed through a 15cm plastic sleeve. The sleeve was positioned such that one end lay in contact with the renal vasculature, it then passed through a port wound so as the other end lay externally. By clamping the vessel loop within the sleeve the tension applied could be adjusted at will, in order to effect and relieve renal ischaemia.

In the ten pigs, individual kidneys were subjected to periods of renal ischaemia of 30, 60 and 90 minutes, with or without device *in-situ* cooling. This produced one control and six study groups. In the control group two kidneys were left untouched with normal perfusion prior to retrieval. Each study group therefore included three kidneys. The device was introduced via an 18mm port above the location of the kidney. With the kidney completely mobilised save the hilum, the open edge of the 'bag' portion of the device was pulled down over the kidney using graspers. The open edge of the bag was then closed and secured around the hilum using the drawstring, so as the cooling apparatus completely enveloped the kidney. Renal parenchymal and cooling circuit temperature monitoring was undertaken throughout each trial using thermocouples and recorded using a data

logger (Pico Technology Ltd, UK). Three thermocouples were placed in the heat exchanger to measure the temperature of the coolant and two thermocouples were incorporated in the cooling bag for monitoring the renal temperature. These thermocouples were specially constructed in the tip of a syringe needle and inserted to a fixed depth of 10mm at each pole. The depth of 10mm was chosen to allow measurement of parenchymal rather than surface temperature, whilst limiting the risk of damaging the collecting system. This depth and position of thermocouple placement reflects methods of core renal cooling assessment used previously (Ames *et al*., 2005, Gill *et al*., 2003a, Herrell *et al*., 1998, Laven *et al*., 2007, Weld *et al*., 2007). Correct placement was monitored visually and function assessed prior to cooling; the measured renal parenchymal temperature was expected to approximate oesophageal core temperature.

With the device in place, the vessel loops were then tightened to achieve renal artery occlusion and the cooling circuit activated. In order to fully assess the cooling efficacy of the device, the circuit was activated for 30 minutes in each case. Where the ischaemic study period exceeded this time, the circuit was stopped and re-warm temperature data collected. The cooling device was not re-activated beyond the initial 30 minute cool. The warm ischaemic period effected in the contra-lateral kidney was timed so as the end of both study periods would coincide exactly. At the end of the study period rapid laparotomy was performed and the kidneys placed on ice.

Following retrieval each kidney received an initial flush of approximately 200ml cold Marshall's solution. The renal artery was then cannulated and placed onto Lifeport® hypothermic machine perfusion. Each kidney was then machine perfused with 1L KPS® solution using local NHBD protocols (Balupuri *et al*., 2000b, Gok *et al*., 2002b).

The PFI was calculated after four hours machine perfusion. The PFI is defined as flow per 100g renal mass divided by the mean systolic pressure of machine perfusion. A 10ml sample of

perfusate was removed after each hour, before immediate snap freezing in liquid nitrogen. This was later analysed for GST using a spectrophotometric assay (Balupuri *et al*., 2000b, Gok *et al*., 2002b). Peak GST during four hours machine perfusion forms the second major viability criteria in Newcastle.

Statistical analysis was performed using Prism version 4.0 for Windows. Data are expressed as mean ± SD, unless otherwise stated. Normality of distribution was confirmed using the D'Agostino and Pearson omnibus normality test, prior to unpaired t-test comparison. Non-parametric analysis of continuous data was performed using the Mann-Whitney U-test. Statistical significance was defined as $p<0.05$.

### 4.3.2 Results

#### 4.3.2.1 Cooling Efficacy

The mean performance of the device is shown in Table 4.1 and Figure 4.5 (Appendix 2). Core renal temperature measurements presented for any given time point are the mean of the two thermocouple readings. In Pig 5, data logger malfunction led to presumably erroneous thermocouple readings (Figure 4.6). This data was therefore removed from temperature efficacy analysis. However, as the measurement reliability improved during the latter portion of the cooling phase, it was noted that effective cooling appeared to have occurred. Data from Pig 5 was therefore included in final cooling temperature and later viability assessment analysis.

The best performance of the device achieved a renal parenchymal temperature of 15 $^{o}$C in 11.2 minutes (Pig 4).

**Table 4.1**

**Temperature Data for the Newcastle Laparoscopic Cooling Device (n=9)**

| | Renal Parenchymal Temperature after 30 minutes Cooling ($^{o}$C**)** | Time taken to reach 15$^{o}$C (minutes) | Re-warm Time 15-25$^{o}$C (minutes) |
|---|---|---|---|
| Mean ± SD | **12.86** ± 4.999 | **21.35** ± 8.420 | **22.73** ± 6.470 |

**Figure 4.5**

**Mean performance of the Newcastle Laparoscopic Renal Cooling Device**

**Core renal temperature data is presented according to duration of cooling. Error bars denote SD. n=9.**

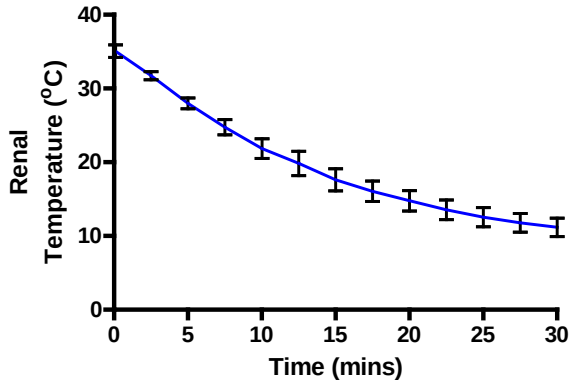
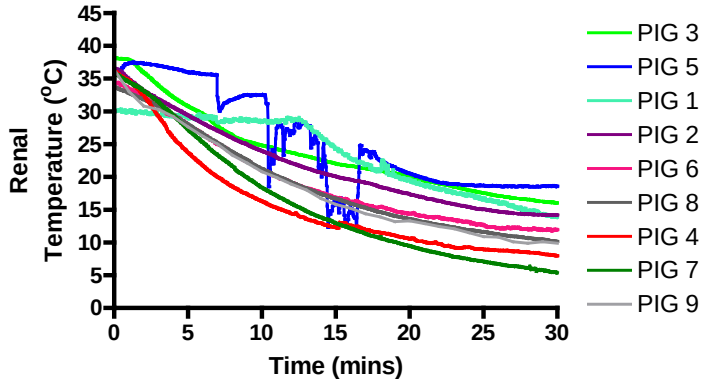



**Figure 3.6**

**Individual cooling curves produced using the Laparoscopic Renal cooling Device according to study animal.**



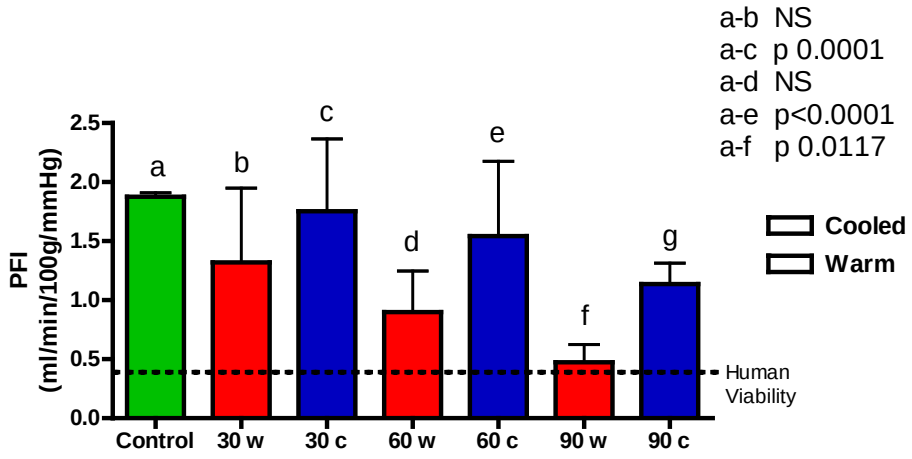
#### 4.3.2.2 Assessment of Ischaemic Injury Severity (Viability Testing).

##### 4.3.2.2.1 Pressure Flow Index (PFI)

In all groups PFI decreased significantly with increasing ischaemic time. Cooling was associated with a significantly higher PFI for all ischaemic periods (Figure 4.7). In the warm ischaemia groups, significant deterioration of PFI compared to controls occurred by 60 minutes; mean PFI 0.90 ± 0.52 vs. 1.88 ± 0.357 (p 0.0001). However in the cooled kidneys at 60 minutes mean PFI was not significantly different from controls; 1.54 ± 0.633 vs. 1.88 ± 0.357 (p=NS).

**Figure 4.7**

**Laparoscopic Renal Cooling demonstrates a significant cryopreservation effect according to Pressure Flow Index (PFI) Viability Assessment.**

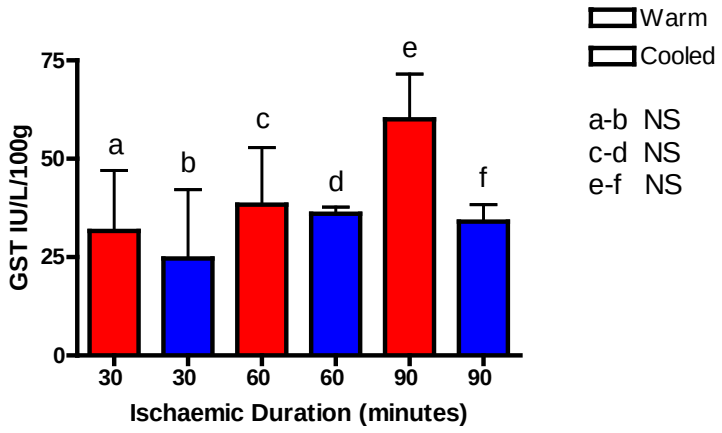


#### 4.3.2.2.2 Glutathione-S-Transferase (GST)

Perfusate GST was not detected in the control samples. The assay is unable to detect levels <14 IU/L. For each ischaemic period greater mean GST measurements were associated with the warm ischaemic groups in comparison with the cooled groups. GST data showed a clear rising trend with increasing warm ischaemia, although statistical significance was not achieved (Figure 4.8). In the Cooled groups a plateau was reached after 60 min, with the 60 and 90 min groups demonstrating similar mean perfusate GST levels (36.0 ± 1.732 vs. 34.0 ± 4.36 IU/L/100g).

**Figure 4.8**

**Laparoscopic Renal Cooling demonstrates a trend suggesting limitation of perfusate GST/100g concentration**



### 4.3.3 Discussion

LPN is considered an excellent treatment for T1a renal cell carcinomas. It has also been performed without cooling by expert surgeons with excellent results (Gill *et al*., 2003b). However, arguably the worldwide uptake of LPN has been slower than expected because laparoscopic urologists, in the early phase of their experience, do not feel confident that they can perform the operation safely within the 30 minute accepted limit for warm ischaemia. This study has demonstrated that with the use of a laparoscopic cooling device, safe operating times could be extended. This might allow more complex partial laparoscopic resections to be performed, and training opportunities for future laparoscopic surgeons would be enhanced.

The Newcastle laparoscopic renal cooling device is based on a non-invasive concept, with potential for rapid application, removal, and re-application during laparoscopic renal dissection. The best performance of this prototype device demonstrated an ability to cool a porcine kidney to 15°C in just over 11 minutes, which is in line with the requirements of LPN. The mean performance of the device was comparable with many previously published techniques, including methods based on principles of vascular (Janetschek *et al*., 2004), ureteral (Crain *et al*., 2004, Landman *et al*., 2003), and surface cooling (Ames *et al*., 2005, Gill *et al*., 2003a, Herrell *et al*., 1998, Webster *et al*., 2005, Weld *et al*., 2007).

Vascular cooling can be achieved through trans-arterial cold perfusion and effective performance has been reported using this approach (Janetschek *et al*., 2004). Despite this, many surgeons would prefer alternative techniques due to the unavoidable risks of vascular injury and thrombosis, as well as issues of technical complexity.

Ureteral cooling is undertaken by retrograde perfusion with cold saline via a ureteral access sheath. This method requires post-operative stenting, and provides less effective cortical cooling than surface techniques (Crain *et al*., 2004, Landman *et al*., 2003).

Surface cooling may be achieved in a number of ways; Herrel *et al* described a renal cooling device based on a concept similar to our own. It consisted of a rectangular double-layered plastic envelope through which cold fluid was circulated. The kidney to be cooled was placed into the envelope via an open side. Data was presented from cooling trials in four pigs. In 3 pigs the device was deployed via an open incision, and in one via an 18mm laparoscopic port. This limits assessment of the manoeuvrability and ease of application of the Herrel device in a purely laparoscopic setting. The Newcastle device possesses practical design advantages. The 'bag and drawstring' design is likely to allow greater manoeuvrability and ease of deployment within confined spaces when compared to a relatively inflexible, rectangular device. The Herrel device cooled to 25ºC within 5 minutes (Herrell *et al*., 1998), which is comparable to the performance of the Newcastle device (mean temperature following 5 minutes of cooling 28.04ºC ± 2.23). Pointedly, the Herrel device was not developed further.

More recently, techniques involving placement of ice slush in close proximity to the kidney have been employed and demonstrate effective renal cooling (Ames *et al*., 2005, Gill *et al*., 2003a, Laven *et al*., 2007), as do irrigation/suction systems using chilled normal saline (Webster *et al*., 2005, Weld *et al*., 2007). The Newcastle device works on similar physical principles, but unlike these systems there is no direct contact between the cooling agent and the organ surface. This reduces the likelihood of organ surface damage, contamination or excessive cooling. Also the potential problems with core temperature reduction associated with irrigation systems are reduced.

The time necessary for cooling forms part of both the ischaemic and operative times, both of which must be minimized. In other words, the benefit of cooling must outweigh the effects of an increase in the total length of the procedure. If the best 15$^{o}$C cooling time of 11.2 minutes can be reliably reproduced or improved upon in human studies, LPN could be interrupted, once cortical temperature has reached 25 $^{o}$C (mean 22.7 minutes in this study), for a short second period of cooling prior to completion. This would provide approximately 60 minutes of protected ischaemia with 45 minutes available for operating. Given the potential for development and improvement of the Newcastle device, this might prove to be a conservative estimation of benefit to both the surgeon and the patient.

Assessment of benefit assumes that cooling and maintaining kidneys between 15 and 25$^{o}$C provides ischaemic protection and functional preservation. Previous studies have been unable to demonstrate this in non-recovery animal models. Furthermore, in expensive and time-consuming animal recovery studies it is difficult to directly assess individual renal function and functional preservation. Techniques, such as individual renal vein serum creatinine sampling may not demonstrate differences between cooled kidneys and those left to the effects of warm ischaemia, despite previous demonstration of excellent cooling (Webster *et al*., 2005).

The Newcastle renal NHBD program relies on accurate techniques of viability assessment in order to identify a spectrum of ischaemic injury. The application of thresholds of viability to individual measures of machine perfusion pressure/flow data (PFI) and enzymatic markers of ischaemic injury (GST), allow identification of retrieved organs which will not function if transplanted (Balupuri *et al*., 2000b, Gok *et al*., 2002b). When applied in an animal model of renal ischaemia, these viability tests demonstrate a clear laparoscopic cryopreservation effect with use of the cooling device.

## 4.4 Conclusions

This study has reinforced the efficacy of topical renal cooling in the laparoscopic setting. It is the first to use device-assessment techniques capable of accurate quantitative measurement of renal tissue injury in a large animal model. The Newcastle cooling device is currently undergoing further development to enhance its efficiency. Availability of such a device would increase the number of urologists able to undertake LPN safely, removing the pressure of completing the procedure within 30 minutes.

With regard to NHBD renal transplantation, the results of this study are highly relevant to both the viability assessment, and organ preservation debates. Further to the degree of validation accorded to the Newcastle viability assessment protocols by the studies described in Chapter 3, the cooling achieved by the laparoscopic device was uniformly and predictably reflected by viability assessment. By demonstrating a clear relationship with effective cryopreservation, as well as the relationship with warm ischaemic duration established previously, greater credibility has been added to the argument that viability assessment is capable of accurate reflection of renal injury.

The debate pertaining to NHBD organ preservation is also informed by this study; the PFIs achieved (albeit in porcine tissue) in the cooling arm of the study were better than those commonly seen in kidneys retrieved from uncontrolled NHBDs (Talbot, 2008, data on file). Effective cryopreservation relies on rapid cooling as until sufficiently low temperatures are reached, ischaemic damage will continue to be accrued. It is likely that the impressive organ viability suggested by the PFIs achieved after 90 minutes of cooled ischaemia can be explained by the relative rapidity of cooling, and subsequent maintenance of efficaciously protective core renal temperatures. As suggested above, current techniques of cryopreservation for uncontrolled NHBDs do not appear to

be similarly effective. Therefore the focus of the next Chapter is the optimisation of the cryopreservation approach to kidney retrieval in the uncontrolled NHBD.

# Chapter 5

# Peritoneal Cooling in NHBD Renal Transplantation

## Peritoneal cooling in NHBD renal transplantation

### 5.1 Introduction

NHBD kidneys are associated with greater exposure to the deleterious effects of ischaemic injury compared to conventional Heart-beating (HB) or Living Related Donor (LRD) organs. Variations in warm ischaemic duration are classified according to the Maastricht criteria, with the longest periods associated with the uncontrolled (category I and II) donor. Prolonged warm ischaemia results in the clinical entities of delayed graft function (DGF) and the much-feared primary non function (PNF).

The inexorable demand for kidneys has continued unabated in Europe and the USA in recent years. This has led to the consideration of marginal donor groups in order to increase the donor pool. Given the ischaemic challenges outlined above, especially pertaining to uncontrolled NHBDs, many have expressed concerns with the outcome potential of such organs (Cooper *et al*., 2004, Keizer *et al*., 2005). However, there is a considerable body of evidence which would suggest that despite increased rates of DGF, following recovery, NHBD kidneys can go on to function comparably to their HB counterparts (Farney *et al*., 2008, Gagandeep *et al*., 2006, Gok *et al*., 2002b, Whiting *et al*., 2006). In the case of uncontrolled NHBD renal transplantation specifically, some European countries are legally prevented from utilising controlled NHBDs due to issues of treatment withdrawal. Hence they must concentrate on uncontrolled donors. However, many are simply mindful of the potential numerical superiority of uncontrolled NHBDs over other donor groups.

Uncontrolled NHBD renal transplantation is made possible only because of interventions which ameliorate the effects of warm ischaemia. The French have recently instituted an uncontrolled NHBD program (Antoine *et al*., 2008) with the majority of donors subject to a procurement/preservation protocol based on aortic placement of a DBTL catheter and cold in-situ

perfusion as soon as possible after declaration of death. The recently established 'condition T' protocol in Pittsburgh also employs similar techniques (International NHBD meeting presentation, London, May 2008). The European approach to the uncontrolled donor was developed through the experiences of groups such as Maastricht (Booster *et al*., 1993a, Booster *et al*., 1993b). Newcastle-upon-Tyne has been retrieving kidneys from uncontrolled (cat. II) donors since 1998, using a modified Maastricht approach which includes an initial streptokinase flush (Asher *et al*., 2004, Balupuri *et al*., 2001, Gok *et al*., 2003c). Although the protocol has been subject to evolution and improvement over time, techniques are focused on the timely introduction of cold *in-situ* perfusion to provide sufficient renal cooling and preservation until the donor can be taken to theatre; often as long as two hours after declaration of death. However, in category II donors the mean renal temperatures at laparotomy are 29.5$^{o}$C (Jennings N, Navarro A, Talbot D. Data on file. 2007). This would indicate that significant improvements in cryopreservation may be possible.

The literature would suggest that techniques of peritoneal cooling have the potential to provide sufficient organ cooling to achieve effective cryopreservation (section 1.6.3). In the previous chapter we have also seen evidence of renal ischaemic preservation achieved through rapid cooling (section 4.3.2.2). However, in order to assess any potential benefit for human uncontrolled NHBD programs a porcine model of the uncontrolled NHBD, comparing current in-situ perfusion (ISP) techniques with additional peritoneal cooling is required. This study involves a'human-ready' peritoneal cooling circuit, real-time direct measurement of biochemical markers of ischaemia within the kidneys using microdialysis, core renal temperature measurement, and post-retrieval machine perfusion viability testing.

## 5.2 Materials and Methods

10 juvenile landrace pigs were used with two providing pure controls, each subjected to 2 hours of warm ischaemia. The remainder formed the 2 study groups of 4 pigs each; the In-Situ Perfusion (ISP) group modelled our current uncontrolled NHBD protocol (section 1.5). The peritoneal cooling group (PC) modelled current protocols with the addition of peritoneal cooling.

All pigs were anaesthetised prior to laparotomy and renal dissection (Figure 5.1). Two thermocouples and one microdialysis catheter were then placed into each kidney (Figure 5.2). In the study groups distal aortic cannulation was achieved with a primed and clamped DBTL catheter (balloons deflated). A primed and clamped inferior vena cava venting catheter was also placed (Figure 5.3). Renal inspection was then carried out to ensure normal perfusion. The abdomen was then closed, and baseline microdialysate samples collected. In the PC group a 10/12mm laparoscopic port was included in the abdominal closure, as this is the intended method for gaining access to the peritoneal cavity in the human donor situation (Figure 5.4).

**Figure 5.1**

Renal Dissection.

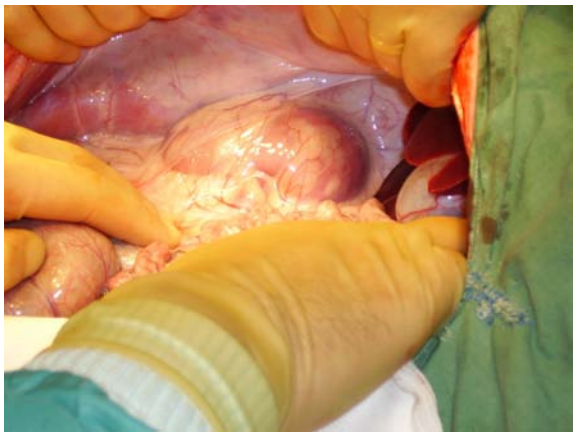


**Figure 5.2**

Placement of renal monitoring devices. Thermocouples for temperature measurement and Microdialysis catheter for biochemical analysis

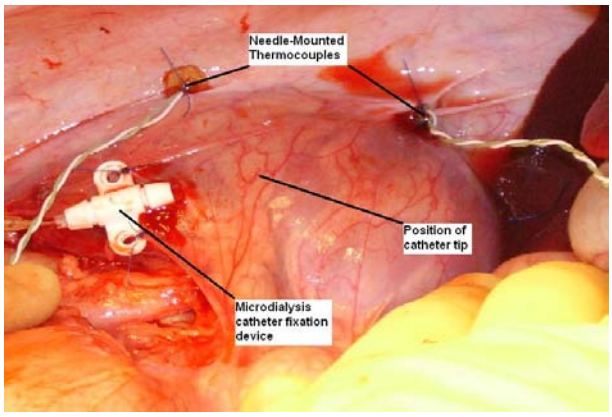


**Figure 5.3**

Preparation for *in-situ* perfusion (ISP) in the operative phase involving distal aortic placement of a clamped and primed DBTL catheter. It was ensured that the renal arteries were positioned between the two balloons. A venous venting catheter allows outflow of blood/ perfusate when ISP begins.

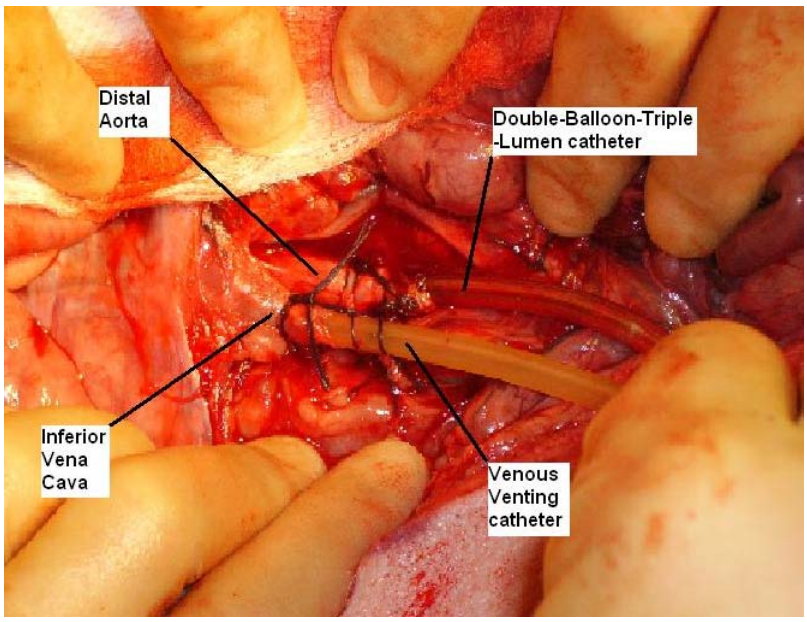
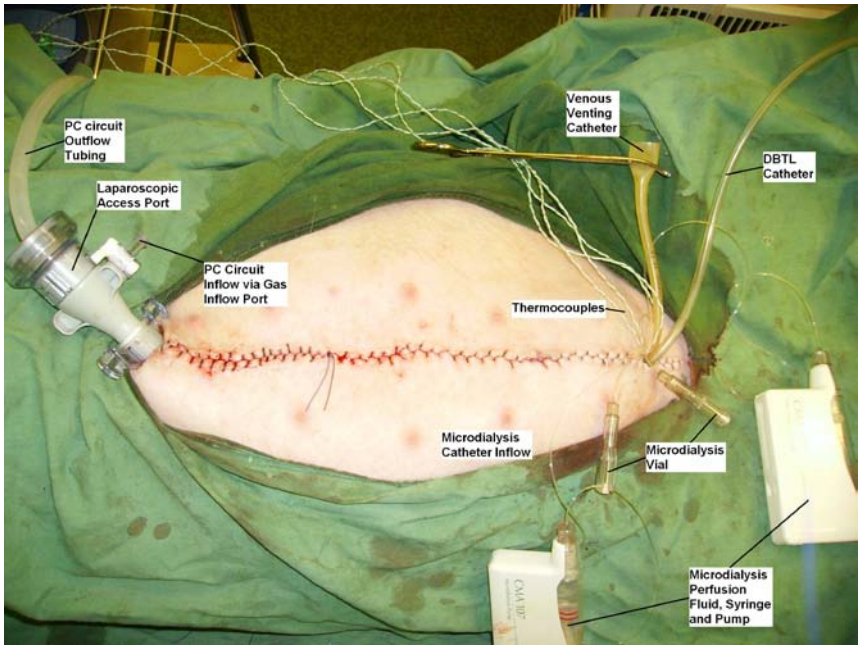


**Figure 5.4**

**Abdominal closure at completion of the operative phase of the study protocol**

Annotated photograph from the peritoneal cooling group, during the warm ischaemic period. Included are; the laparoscopic port (for peritoneal access), outflow tubing, primed and clamped *in-situ*-perfusion catheters (DBTL and venous vent), thermocouples and microdialysis monitoring equipment.



The pig was then killed and the kidneys subjected to a 30 minute warm ischaemic period, as this is the limit for uncontrolled NHBD warm ischaemic duration in Newcastle. After 30 mins the DBTL balloons were inflated to isolate the renal arterial circulation. *In-Situ* Perfusion was then commenced with cold HTK® solution ($4^{o}$C). Local NHBD protocol drug doses were adjusted for pig weight (streptokinase flush 0.5 million IU, phentolamine 5 mg, heparin 10,000 IU). In the PC group only, the cooling circuit was then assembled (Figure 5.5, section 5.2.1) the abdomen filled with cold peritoneal dialysis (PD) fluid and the circuit activated. Temperature and microdialysis monitoring of the renal tissue occurred throughout the ischaemic period.

After 2 hours rapid re-laparotomy and kidney retrieval was performed. The kidneys were then placed onto ice, given a Hartmann's fluid flush, before machine perfusion and viability testing.

### 5.2.1 Peritoneal Cooling Circuit

Simple thermodynamic estimations suggest the required base load required for effective peritoneal cooling is 7.29kW. Packaged air cooled chillers of sufficient power are available commercially. Acrol Ltd. UK assisted with the conversion of such a unit to function within the peritoneal cooling circuit. In order to accommodate the base load, the flow rate required was in the order of 0.1 $L.s^{-1}$. An appropriate Watson-Marlow® peristaltic pump was therefore incorporated into the system. (Figure 5.6)

**Figure 5.5**

**Schematic Representation of the Newcastle Peritoneal Cooling Unit**

Peritoneal dialysis (PD) fluid is introduced to the peritoneal cavity via the gas inflow on the laparoscopic port. A 2 meter length of tubing (8mm) is inserted into the lumen of the laparoscopic port and passed down into the pelvis. The free external end was connects to a stainless-steel heat exchange coil *(*Acrol® Engineering, UK*)*. A second 2 meter length of tubing then connects to the steel coil. The coil is placed into the heat-exchange tank, and one length of the tubing placed into the Watson Marlow® peristaltic pump. The circuit is then primed before completion of the circuit, by connection to the gas inflow of the laparoscopic port. On activation of the pump, warm fluid from the abdomen passes to the stainless steel coil (immersed in water at $4^{o}$C) where heat exchange occurs. The chilled fluid then passes back into the abdomen to effect organ cooling.

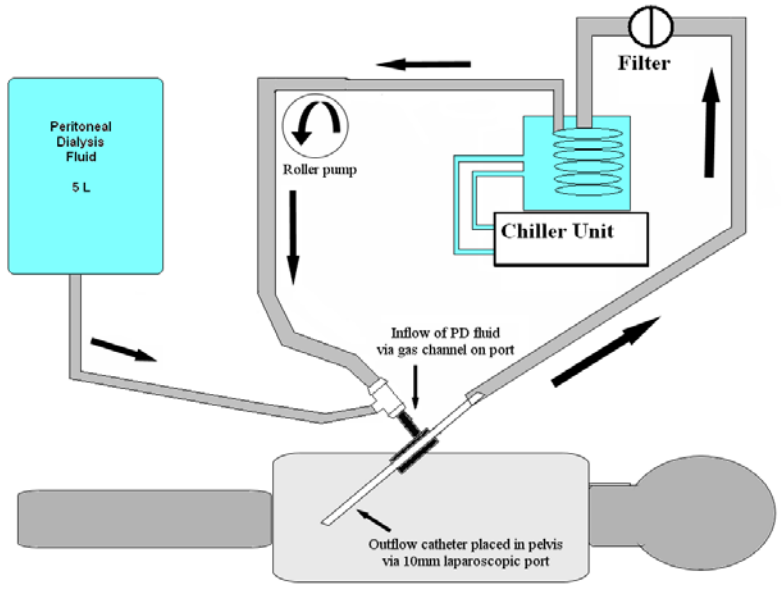
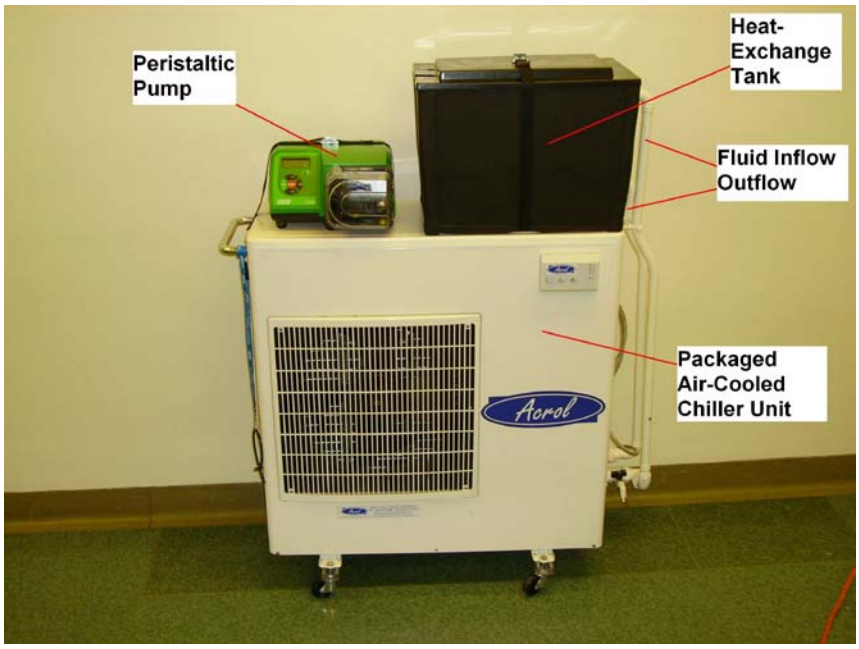


**Figure 5.6**

**Newcastle-upon-Tyne Peritoneal Cooling Unit**

Modified Acrol Ltd chilling unit with heat-exchange tank and peristaltic pump.




Peritoneal dialysis (PD) fluid was introduced to the peritoneal cavity via the gas inflow on the laparoscopic port. Once the peritoneal cavity had been filled, a 2 meter length of silastic tubing (8mm) was inserted into the lumen of the laparoscopic port before being passed down into the pelvis. The free external end was then connected to a stainless steel heat exchange coil. Another 2 metre length of tubing was then connected to the other end of the steel coil. The coil was placed into

the chilling unit's heat-exchange tank, and one length of the tubing placed into the Watson Marlow® peristaltic pump. The pump could then be activated at a slow rate to prime the circuit. Once primed, the pump was stopped before completing and closing the circuit by connection of the tubing to the gas inflow of the laparoscopic port. During assembly, care was taken to vent air from the system. Thus on activation of the pump, warm fluid from the abdomen passes to the stainless steel coil (immersed in water at $4^{o}$C) where heat exchange occurs. The chilled fluid then passes back into the abdomen to effect organ cooling.

### 5.2.2 Temperature monitoring

Type-K thermocouples were mounted at the tip of a 21G needle and placed at a depth of 1cm within the renal parenchyma. Thermocouples were connected to a Pico Technology Limited TC-08 USB® Data Logger, and recorded using Picolog recorder® software.

### 5.2.3 Microdialysis

Microdialysis is a technique to monitor the chemistry of the extracellular space in living tissue. Lactate, puruvate, glucose and glycerol are established markers of ischaemia in renal tissue (see Chapter 2 section 3.2). Microdialysis was performed using CMA Microdialysis Limited equipment; CMA-63® microdialysis catheters with relevant pumps and microvials for dialysate collection. Samples were analysed using the CMA-600® Analyser and reagent kit with Labpilot® software. Microdialysate samples were collected every 20 minutes. The first (Time 0) sample was collected over the 20 minute period prior to death of the animal and the beginning of the warm ischaemic period; samples therefore represent baseline values in well-perfused and oxygenated tissue.

### 5.2.4 Machine Perfusion and Viability Testing

In Newcastle NHBD kidneys undergo hypothermic machine perfusion using the Organ Recovery Systems Inc. Lifeport® machine, which allows assessment of viability. This involves calculation of the Pressure Flow Index (PFI), defined as the flow per 100g renal mass divided by the systolic pressure of machine perfusion (see Chapter 2 section 2). PFI is calculated at the start and then hourly during the first 4 hours of machine perfusion (T0-T4). PFI will generally rise during the first two hours of machine perfusion before reaching a relative plateau or peak. The peak PFI (usually T3 or T4) is used to determine viability. The Newcastle viability protocol for single NHBD renal transplants requires a peak PFI of $\geq$0.4 $ml.min^{-1}$ $100g^{-1}$ $mmHg^{-1}$ (Navarro *et al*., 2008b).

### 5.2.5 Statistical Analysis

Statistical analysis was performed using Prism version 4.0 for Windows. Data are expressed as mean ± SD. Normality of distribution was confirmed using the D'Agostino and Pearson omnibus normality test, prior to unpaired t-test comparison. Non-parametric analysis of continuous data was performed using the Mann-Whitney U-test. Statistical significance was defined as $p<0.05$.

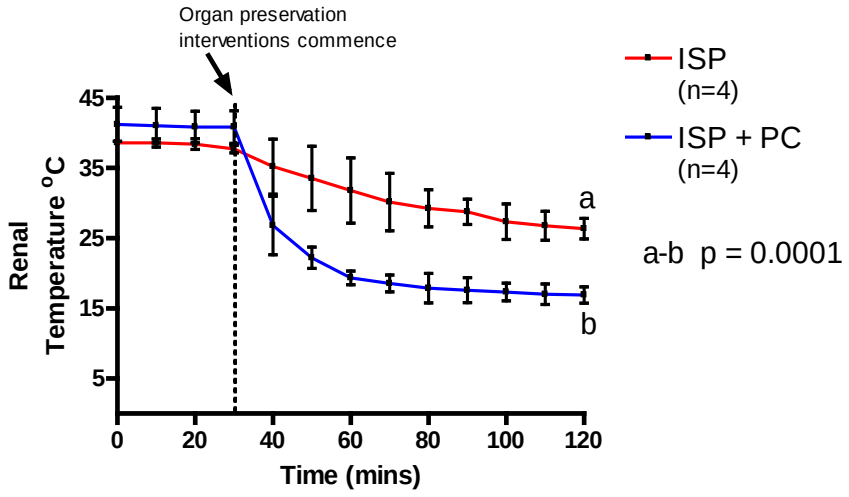
### 5.3 Results

(Appendix 3)

#### 5.3.1 Renal Cooling

In the ISP group only 1/4 cases reached a mean renal temperature of 25$^{o}$C. In the PC group the mean time taken to reach 25$^{o}$C was 12.0 ± 4.81 minutes. The final temperature 90 minutes after commencing organ preservation interventions was 26.3 ± 1.46$^{o}$C in the ISP group versus 16.9 ± 1.17 $^{o}$C in the PC group (p 0.0001) (Figure 5.7).

**Figure 5.7**




**Mean Core Renal Temperature**

Mean core renal temperature according to study group; *in-situ* perfusion (current protocols) *versus* peritoneal cooling (performed in addition to current protocols). Error bars denote SD.

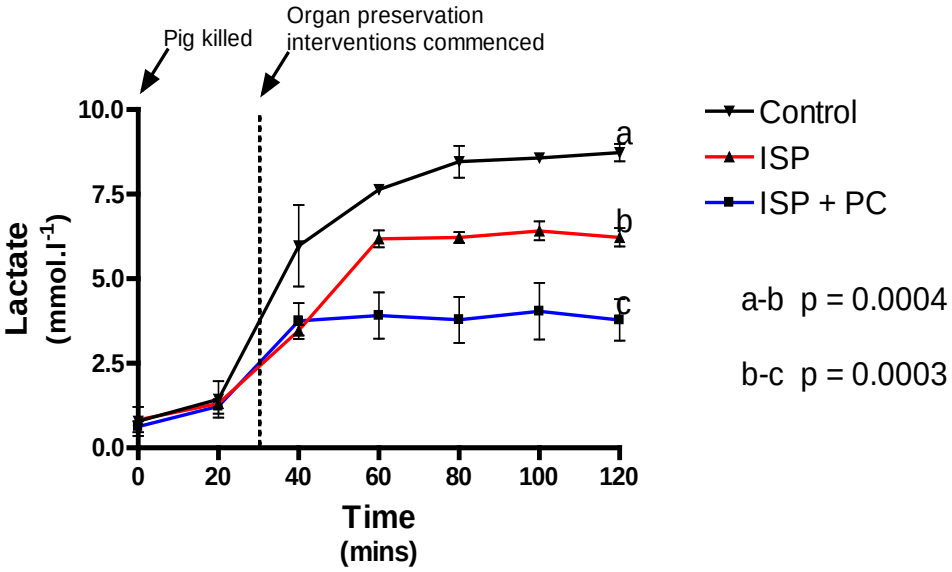
### 5.3.2 Microdialysis – Markers of Ischaemia

#### 5.3.2.1 Lactate

Lactate levels at time 0, representing values associated with well-perfused and oxygenated tissue, and at 20 minutes were not significantly different between the control and study groups. In the control group (2 hours warm ischaemia) lactate levels were seen to rise to a peak of 8.73 ± 0.26 $mmol.l^{-1}$ by 120 minutes. In the ISP group, a more modest increase in lactate levels of 1.31 to 3.45 $mmol.l^{-1}$ (vs. 1.44 to 5.98 $mmol.l^{-1}$ in the control group) was seen between 20 and 40 minutes (preservation interventions were commenced at 30 minutes). Microdialysate lactate reached a plateau after 60 minutes, presumably as the kidneys were cooled to effective cryopreservation temperatures. The peak level was significantly lower than controls at 6.42 ± 0.28 $mmol.l^{-1}$ (p 0.0004). With the addition of peritoneal cooling the lactate plateau was reached by 40 minutes (mean 3.75 ± 0.53 $mmol.l^{-1}$), with a peak at 120 minutes of 4.04 ± 0.84 $mmol.l^{-1}$. This was significantly lower than the current protocol ISP group (p 0.0003) (Figure 5.8).

**Figure 5.8**

**Mean Microdialysate Lactate**



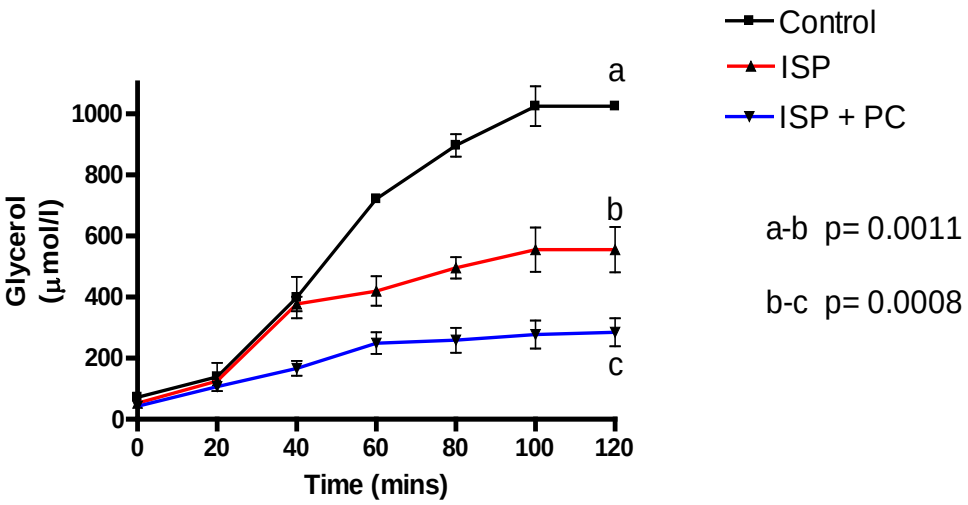

Mean renal parenchymal dialysate lactate concentrations according to study group. Mean renal parenchymal dialysate lactate concentrations (mmol.l$^{-1}$) give an indication of ischaemic severity and anaerobic metabolism. Time 0 samples represent dialysate collected prior to induction of ischaemia i.e. normal perfusion. Error bars denote SD. Statistical differences between peak-lactate concentrations in the ISP and PC study groups are also presented.

### 5.3.2.2 Glycerol

Microdialysate glycerol data is presented in Figure 5.9. Levels at time 0 and 20 minutes were not significantly different between the control and study groups. Significant differences were present by 40 minutes. Mean peak glycerol levels at 120 minutes were 1024.0 ± 65.1 µmol.l$^{-1}$ in the control group versus 555.3 ± 72.3 µmol.l$^{-1}$ in the ISP group (Control vs ISP, p 0.0011), and 284.5 ± 45.8 µmol.l$^{-1}$ in the PC group (ISP vs PC, p 0.0008).

**Figure 5.9**

**Mean Microdialysate Glycerol**




Mean renal parenchymal dialysate Glycerol concentrations (µmol/l) as an indicator of severe renal cell injury or death. Data presented by control and study groups. Error bars denote SD. Statistical differences between peak-glycerol concentrations in the ISP and PC study groups are also presented.

**Comment [h1]:** N = ?

#### 5.3.2.3 Pyruvate

Pyruvate was found to fall to undetectable levels by 80 minutes in all groups. No significant differences were seen between the control and study groups. This is most easily demonstrated in tabular form (Table 5.1).

**Table 5.1**

**Mean Microdialysate Pyruvate**

Mean renal parenchymal dialysate Pyruvate concentrations ($mmol.l^{-1}$) as an indicator of aerobic metabolism. Data presented by control and study group with statistical differences between the ISP and PC study groups shown.

| Sample Time (min) | Control | ISP | PC | ISP vs. PC Significance |
|---|---|---|---|---|
| **0 Normal Tissue** | 65.0 ± 12.7 | 113.3 ± 68.3 | 46.4 ± 16.3 | NS |
| **20** | 92.5± 20.5 | 77.5 ± 69.9 | 57.6 ± 39.5 | NS |
| **40** | 25.5 ± 2.1 | 22.5 ± 27.8 | 17.4 ± 5.18 | NS |
| **60** | 6.0 ± 8.5 | 0 | 0 | NS |
| **80** | 0 | 0 | 0 | NS |
| **100** | 0 | 0 | 0 | NS |
| **120** | 0 | 0 | 0 | NS |

#### 5.3.2.4 Glucose

Glucose was found to fall with increasing ischaemic duration in all groups. No significant differences were seen between the control and study groups. This is most easily demonstrated in tabular form (Table 5.2).

**Table 5.2**

**Mean Microdialysate Glucose**

Mean renal parenchymal dialysate glucose concentrations ($mmol.l^{-1}$) are expected to fall with deteriorating perfusion. Data presented by control and study group with statistical differences between the ISP and PC study groups shown.

| Sample Time (mins) | Control | ISP | PC | ISP vs PC Significance |
|---|---|---|---|---|
| **0 Normal Tissue** | 1.67 ± 1.10 | 2.73 ± 0.55 | 2.25 ± 0.95 | NS |
| **20** | 2.29 ± 0.89 | 3.15 ± 0.26 | 1.83 ± 0.6 | NS |
| **40** | 1.02 ± 1.26 | 1.91 ± 0.92 | 0.97 ± 0.92 | NS |
| **60** | 0.60 ± 0.84 | 0.72 ± 0.71 | 0.33 ± 0.30 | NS |
| **80** | 0.30 ± 0.42 | 0.28 ± 0.48 | 0.21 ± 0.20 | NS |
| **100** | 0.12 ± 0.17 | 0.19 ± 0.38 | 0.30 ± 0.42 | NS |
| **120** | 0 | 0.11 ± 0.23 | 0.17 ± 0.25 | NS |

### 5.3.3 Machine Perfusion Viability Testing

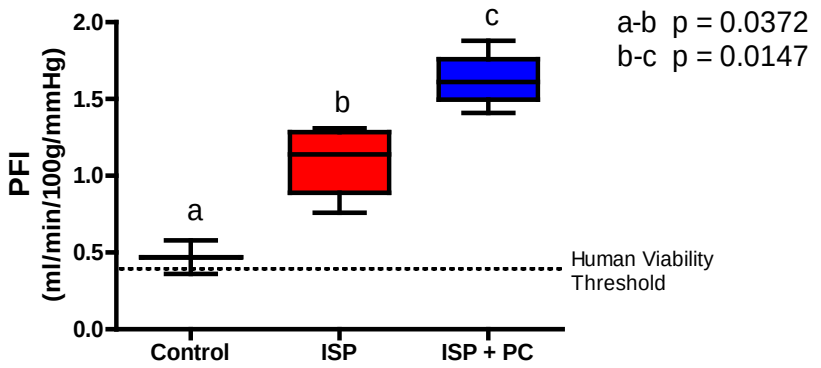
#### Perfusion Flow Index (PFI)

After fours hours of hypothermic machine perfusion the mean peak PFI in the pure control group (no preservation) was 0.47 ± 0.16 $ml.min^{-1}100g^{-1}$ $mmHg^{-1}$. This was approaching the PFI threshold set for human kidney viability of 0.4. In the study groups, the use of current protocol organ preservation interventions in the ISP group resulted in a significantly superior mean peak PFI of 1.09 ± 0.13, compared to controls (p 0.0372). An additional and significant cryopreservation benefit was associated with the addition of peritoneal cooling to current protocols in the PC group which demonstrated a mean peak PFI of 1.63 ± 0.19 (p 0.0147) (Figure 5.10).

#### Figure 5.10

**Peak Pressure Flow Index (PFI)**

Data according to control and study groups; box and whisker plot to demonstrate mean, SD, and spread of the data. The Newcastle-upon-Tyne PFI threshold for viability is shown (≥0.4 $ml.min^{-1}$ $100g^{-1}$ $mmHg^{-1}$).



## 5.4 Discussion

Peritoneal cooling has been shown to be effective at reducing core temperature in animal studies. Takahashi *et al*, using an *ex vivo* canine model with ATP measurements and biochemical markers of ischaemia, demonstrated that peritoneal cooling gave equivalent results to cooling by cardiopulmonary bypass, and better results than *in-situ* perfusion alone, in heart, liver and kidney transplantation (Takahashi *et al*., 1996). Yadav *et al* compared in situ perfusion alone with a combination of in situ perfusion and peritoneal cooling in a porcine model of warm ischaemia, and found the addition of peritoneal cooling to significantly reduce renal core temperatures (Yadav *et al*., 1997).

In Human donors, the Washington Hospital Center group reported data from their Rapid Organ Recovery Program (RORP) for uncontrolled NHBDs (Light *et al*., 1997). As discussed in chapter 1 section 6.3, these donors were younger than commonly seen in Europe, and were mainly trauma victims. Furthermore, no control group approximating European protocols of *in-situ* cold perfusion alone was described. The RORP system was reportedly capable of achieving peritoneal temperatures of 10°C in 8 minutes, with IVC temperature falling to 15°C in 30 minutes and 10°C by 60 minutes (Light *et al*., 2000a, Light *et al*., 2000b).

Despite this, many uncontrolled NHBD retrieval protocols (including some which have been very recently implemented) involve techniques of *in-situ* cold perfusion in the absence of peritoneal or other forms of effective supplemental cooling. Indeed the experience in Newcastle-Upon-Tyne is that *in-situ* perfusion alone does not achieve temperatures effective for renal cryopreservation within the timeframes we require. The data presented above, supported by the findings of previous studies, suggests that with effective supplemental cooling it might be possible to significantly improve uncontrolled NHBD cryopreservation in Newcastle, both in terms of the speed and final temperature

of cooling. Centres considering beginning or improving an uncontrolled NHBD retrieval protocol based on cryopreservation should consider the inclusion of peritoneal cooling.

This study has demonstrated a significant core renal temperature cooling benefit with the use of a novel 'human-ready' peritoneal cooling circuit. Evidence of improved limitation of anaerobic metabolic rate and cellular injury are demonstrated by means of renal parenchymal microdialysate lactate and glycerol levels respectively. Local NHBD viability testing protocols also demonstrate significantly improved parameters of viability which suggests improved protection from ischaemia. These findings translate to a potential for reduced rates of ischaemia-related clinical phenomena. In reaction to these findings, permissions were sought and granted to employ the peritoneal cooling system in uncontrolled NHBD retrieval in the Newcastle General Hospital, Accident and Emergency department.

# Chapter 6

# Discussion

# Discussion

## 6.1 Ischaemic injury Assessment

### 6.1.1 The Efficacy of NHBD Hypothermic Machine Perfusion Viability Testing

The NHBD is subject to a spectrum of ischaemic insults. The dichotomy of the controlled and uncontrolled NHBD is well described and reflects the large differences in the potential for injury seen between these groups. To those centres which confine their programs to controlled donors and produce acceptable outcomes for recipients, no clear need for additional assessment of organ quality is perceived (Chang, 1995). However, groups retrieving organs from uncontrolled donors with greater propensity for injury, have seen first-hand the consequences of a protocol lacking in objective viability assessment (Balupuri *et al*., 2000c). Such an approach also has the potential to suppress negative ischemia-related outcomes in controlled donors, by identification of often unexpectedly non-viable organs (Daemen *et al*., 1995, Gok *et al*., 2004b).

The first aim of this thesis was to assess the efficacy and potential of ischaemic injury assessment or viability testing within a renal NHBD transplantation program, by prospective assessment of the effects of warm ischaemia on retrospectively established measures of organ viability; machine perfusion and perfusate enzyme analysis in a large animal model. Despite small numbers, a clear linear relationship was demonstrated between the major viability criterion in Newcastle, the PFI, and increasing ischaemic duration, with kidneys exposed to 90 minutes of warm ischaemia demonstrating PFIs approaching human thresholds of non-viability. The linearity of this relationship adds weight to the argument that resistance-based machine perfusion indexes, such the Newcastle PFI, are indeed capable of accurately reflecting warm ischaemic duration. After all it is the duration of warm ischaemia which exposes organs to the injurious processes which ultimately result in irreversible damage.

The trends of increasing GST perfusate concentration seen with increasing ischaemic duration failed to reach statistical significance. This was possibly due to the main weakness of the studies described within this thesis; low numbers. However, with the statistically robust data for PFI assessments, it is also possible that the results reflect the weaknesses of GST as a perfusate injury marker. GST perfusate concentration is affected by flow (Daemen *et al*., 1997c, Gok *et al*., 2003b) and hence in very damaged organs the vascular destruction (responsible for poor flows, high resistance, and low PFI) results in less GST being washed out into the perfusate; effectively the assay will not 'see' the GST which remains inside the organ. This could explain the plateau in GST levels between 60 and 90 minutes of warm ischaemia. Conversely, situations have been described where high GST levels are associated with excellent flows and PFI on machine perfusion. Such situations often involve young organs, presumably with a large number of nephrons per unit mass.

The contribution of this study does not serve to refute prior criticisms of the accuracy of GST as an effective indicator of organ viability. The caveats, such as in young donors or low flow situations, which experienced clinicians apply to the interpretation of GST levels, highlight the weaknesses of the marker and justify its status as a secondary viability criterion (as it is used in Newcastle). However, in situations of moderate flow it can provide useful additional information. As cell membrane disruption (and inevitable death) is required for GST circulatory release, levels reflect the degree of irreversibility of damage; in effect the degree of nephron loss.

Critics of organ viability assessment have argued that validation of such tests is impossible as those organs which fail are not transplanted (Gerstenkorn *et al*., 2000). Such statements are strictly true but not particularly insightful or helpful. Faced with high rates of PNF, the program leader who introduces objective viability assessment will consider the tests 'validated' if the PNF rate drops significantly following introduction. This has been reported by several groups (Balupuri *et al*.,

2000b, Balupuri *et al*., 2000c, Daemen *et al*., 1995, Light, 2000). However, the retrospective manner in which such assessment protocols were established remains a continuous point of criticism. This study is the first to prospectively demonstrate the capability of hypothermic machine perfusion protocols to reflect warm ischaemic duration and hence severity.

#### 6.1.1.1 Weaknesses and Future Research

The statistical significance achieved in the PFI data refutes any significant type II error. However, the study can be criticised in its scope. Insufficient resources were available to allow recovery animal work, which might have included a transplantation model. Future studies should seek to evaluate whether the viability assessments described translate into the expected outcomes following transplantation. It would then be possible to transplant the kidneys which fail to reach thresholds set for human viability. In this way, the unethical and impossible validation of human viability assessment could be achieved in a porcine model.

### 6.1.2 Viability Assessment to Maximise Organ Resources

Objective viability assessments possess several advantages over subjective approaches. In the Introduction (section 1.3.5) the cautious response of the senior surgeon to bad experiences with PNF was described; stringent donor criteria are exerted in order to exclude situations where organ damage is more likely. Such approaches are effective in reducing PNF rates, but result in fewer transplants as centres decline opportunities to recover organs from sub-optimal donors or donor situations (Metcalfe and Nicholson, 2000).

The inefficiency of subjective NHBD assessments and non-recovery / discard decisions may ultimately threaten the continuation of NHBD procurement within a centre. This was seen in

Leicester in the late 1990's, where a high discard rate combined with a low organ yield attributed to 'next of kin' consent refusals (Elwell *et al*., 1997) resulted in eventual termination of the program. The Newcastle centre had observed dramatic reduction in PNF rates following the introduction of objective viability assessment (Balupuri *et al*., 2000c). However, the discard rate for uncontrolled donor kidneys was still as high as 50% in 2000.

The efficacy of hypothermic machine perfusion viability assessment protocols in the limitation of PNF rates is well established (Balupuri *et al*., 2000b, Daemen *et al*., 1995, Gok *et al*., 2003b, Light, 2000). However, as alluded to above, many deny an important role for such assessment in the controlled NHBD. This may be a justifiable position, but it ignores the potential 'net-widening' effect that judicious use of a 'recover and assess' approach can have, especially with sub-optimal donor situations. The second aim of the thesis set out to determine whether the value of objective viability assessment could be demonstrated in one such situation.

#### 6.1.2.1 Transplantation of Cat. III NHBD kidneys with evidence of pre-arrest Acute Renal Failure

Category III NHBDs may exhibit evidence of deterioration of donor renal function prior to death. For many centres, this is sufficient to justify declining the opportunity to recover kidneys. This was not the approach taken in Newcastle where objective machine perfusion viability assessments are relied upon to identify where thresholds of damage have been breached.

Clinically diagnosed ARF is characterised by deterioration of urine output and the accumulation of serum creatinine (SCr). The two are usually co-existent but data regarding SCr is more readily recorded and accessible. The RIFLE criteria for ARF (Bellomo *et al*., 2004a, Bellomo *et al*., 2004b) describes a familiar spectrum of injury as kidneys progressively approach irreversible injury and loss of function. The criteria for the 'Risk' and 'Injury' classifications suggest the

reversible part of this spectrum, but it should be remembered that such classifications would be sufficient for many to decline retrieval.

Examination of the data presented in section 3.4.2 suggests that Newcastle viability protocols effectively identify reversible injury situations, which result in acceptable early post-transplant function (Navarro *et al*., 2006a, Sohrabi *et al*., 2007). By allowing successful transplantation from one sub-optimal donor group, the implication is that viability testing may also allow other previously ignored donor sources to be considered. For instance, application of similar principles may be of interest in previously-well elderly donors. Prior to this, however, it would be wise to examine existing data more closely. Although acute injury appears to reflect predictably in Newcastle viability criteria, the effect of chronic pathological processes have yet to be established.

#### 6.1.2.2 Dual Renal Transplantation of 'Marginal Kidneys' selected using Machine Perfusion Viability Testing

NHBD transplantation is resource intensive and as such a high non-use rate is a cause of great concern for NHS programs. The first experimental chapter of this thesis is concerned with prospective assessment of the validity of local viability tests and assessment of potential applications for donor pool expansion. The third aim concentrates on an intervention aimed to increase the efficiency of the Newcastle NHBD program by utilising organs on the thresholds of viability.

Simple relaxation of previous viability thresholds will clearly reduce non-use rates, but equally certain would be increased rates of PNF. Therefore the only way to utilise 'borderline' organs is with a novel approach. The ideal solution would be to 'treat' the ischaemic injury, so improving the quality of the kidneys prior to transplantation. This was the aim of the final aspect of

this thesis, namely the optimisation of the cryopreservation approach. However, the very fact that kidneys are paired organs suggests a simple solution.

Newcastle NHBD program leaders were mindful of Professor Nicholson's concept of 'nephron-dosing' (Nicholson *et al*., 2000) when assessing the possibility of utilising acutely or chronically damaged organs as dual organ grafts. Other centres have used dual transplantation in order to allow utilisation of organs from older donors, or from kidneys with a known degree of functional impairment (GFR <90) (Alfrey *et al*., 1997a, Lu *et al*., 2000). The question for Newcastle was exactly how to select which organs to use in this way. The established viability assessment thresholds have been shown to produce satisfactory outcomes for single grafts (Gok *et al*., 2002b) and hence this situation was left unchanged. The PFI had proven itself a trusted indicator and as such no consideration was given to any form of transplantation from organs failing PFI criteria. The deficiencies of GST as an injury marker have been discussed above. However its usefulness in moderate flow situations lends itself to selecting moderately damaged organs suitable for dual transplantation. In the Newcastle pre-dual transplant era, viability test failures would often demonstrate GST levels greater than the threshold of 100 IU.100g $^{-1}$, but with comfortably viable PFIs. This suggested that a significant degree of nephron loss may have occurred, but without progression to the vascular destruction and total irreversible functional loss associated with a PFI fail.

The third study in this section demonstrated that selection and dual transplantation of organs with viable PFIs but high GST levels are capable of providing equivalent early graft function and levels of PNF to single NHBD grafts (Navarro *et al*., 2008b, Navarro *et al*., 2006b). Furthermore, the levels of renal function suggested by the eGFRs achieved in the dual transplantation group were also statistically similar to those of the single group. In other words the organs selected for dual

transplantation were shown to produce sufficient function only. Excessively high eGFRs or levels of function might suggest that two single transplants could have been successfully performed with the paired organs. This supports the criteria chosen for dual organ selection and, with the resulting low levels of organ non-use, represents evidence of maximisation of organ resources.

With a clearer picture of the benefits of viability assessment and its role in organ resource management and optimisation, attention is turned to focus on techniques capable of improving organ quality in kidneys exposed to the deleterious effects of ischaemia.

## 6.2 Novel Techniques for Clinical Renal Cryopreservation and Ischaemic Protection

### 6.2.1 Laparoscopic Renal Cryopreservation

The application of efficient cooling techniques to aid ischaemic protection is of relevance to many clinical disciplines. The procedure of laparoscopic partial nephrectomy (LPN) is one area where a clinical need for organ hypothermia has been identified but as yet no clearly superior technique for achieving it has been described. This premise is supported by the multiple reports of alternative methods of instituting laparoscopic renal cooling (Gill *et al*., 2003a, Herrell *et al*., 1998, Janetschek *et al*., 2004, Laven *et al*., 2007, Webster *et al*., 2005, Weld *et al*., 2007). Furthermore, difficulties in assessment of the efficacy of cryopreservation interventions stem from the weaknesses of clinical measures of renal function e.g. SCr. Chapter 4 describes the response to three of the original aims of the project; to develop a device for laparoscopic renal cooling, to develop a large animal model for laparoscopic renal cooling, and to assess the efficacy of a laparoscopic renal cooling device using transplantation organ viability assessment.

The prototype device produced was intended for 'proof of concept'. It therefore cannot be said to represent the final solution for laparoscopic renal hypothermia. The production of such a device would require significant further engineering and testing before a human-ready product could be made available to surgeons. However, the concept of a closed-system for topical cooling by re-circulation of chilled coolant across the surface of the organ clearly has merit; topical cooling ensures tissue invasion is minimised, and the separation of coolant from tissue, by a thin membrane of plastic, reduces the potential for contamination whilst allowing effective heat transfer. The various advantages over earlier systems have been well discussed in the relevant Chapter's conclusions.

The prototype device was shown to achieve satisfactory core renal temperature within the time-frames required for LPN. Furthermore, these temperatures translated into improved parameters of human renal transplantation-derived organ viability assessments when compared to un-cooled controls. This was taken as evidence of cryopreservation efficacy. However, although the PFI and GST machine perfusion viability tests are arguably reliable indicators of post-transplant (or in this context, post-reperfusion) function (Balupuri *et al*., 2000b, Daemen *et al*., 1995, Gok *et al*., 2004b), they remain 'indicators' only. The only way to truly demonstrate effective cryopreservation would be to perform recovery experiments in which LPN is completed with and without cooling, and the function of the isolated partially nephrectomised kidney measured directly. This could be achieved through a contra-lateral nephrectomy, and serial measurements of GFR using established clinical techniques during the recovery period.

Unfortunately limited resources precluded this comprehensive approach. However the results gained are of significant use in suggesting further research, both through the support of further

investment into device development, and in increasing specialty interest in the benefits of this form of laparoscopic renal hypothermia through publication (Navarro *et al*., 2008a).

### 6.2.2 Peritoneal Cooling for Uncontrolled NHBDs

In many ways the most challenging aspects of the project, and possibly for NHBD transplantation itself, lie in the approach to the uncontrolled NHBD. The cryopreservation approach in Newcastle is based on techniques of *in-situ* perfusion and has been shown to yield insufficiently low organ temperatures (Jennings, 2001). The project set out to develop a large animal model of the uncontrolled NHBD for assessment of the efficacy of additional peritoneal cooling versus current protocols, and ultimately to establish a human trial of uncontrolled NHBD peritoneal cooling.

The results from the porcine model of the uncontrolled NHBD demonstrate clear preservation benefits from the application of supplementary peritoneal cooling in addition to current *in-situ* perfusion protocols. Criteria of core renal temperature, the microdialysis ischaemic markers of lactate and glycerol, and hypothermic machine perfusion PFI and GST all suggest superior limitation of ischaemic injury compared with the current approach. The main strength of the conclusions drawn is the striking nature of the differences seen between study groups, and as such the feeling within the unit is that all future human donors should undergo supplementary peritoneal cooling. However, the results must be seen in the context of the study's limitations; these shall be discussed in more detail

#### 6.2.2.1 Limitations of the Porcine Study of supplementary Peritoneal Cooling

The most obvious limitation derives from the small sample size involved. Ten animals were suggested by original power calculations to reflect the expected differences in temperature and PFI

achievable by peritoneal cooling. However, the impressive statistical differences reported may be viewed sceptically due to the standard errors commonly associated with studies involving small numbers.

The viability criteria employed throughout the project have been shown to reflect ischaemic duration and injury and microdialysis markers of ischemia are well-validated (Keller *et al*., 2008, Weld *et al*., 2008). They therefore offer a means of assessing the likelihood of post-transplantation graft dysfunction. However, arguably the only completely reliable method is to transplant the organs. As with the LPN study, limited resources have precluded a transplantation model. Transplantation of kidneys preserved by *in-situ* perfusion ± peritoneal cooling, followed by close assessment of graft function, would undeniably be the best way of directly assessing the likely impact of the intervention on clinical NHBD transplantation.

Further practical points would include the effect of dissection of the kidneys prior to induction of preservation interventions. Partial dissection of the kidneys could potentially have improved cooling rates by increasing the surface area of kidney in contact with circulating coolant. Also, the pigs used were juvenile and as such possessed very little retroperitoneal fat. In uncontrolled NHBDs in Newcastle there is often a significant amount of fat present. The insulating effect of retroperitoneal peri-nephric fat could affect the efficacy of abdominal cooling, and this limits the strength of the study conclusions.

## 6.3 NHBD Organ Preservation - Directions for the future

Extracorporeal membrane oxygenation (ECMO) has the ability to provide near normal organ perfusion and oxygenation in the absence of cardiopulmonary function. It therefore possesses a theoretical advantage over preservation methods based on organ cooling; the organs are maintained

in physiologically near-normal conditions. This would suggest the potential to minimise injury and improve the subsequent viability of NHBD kidneys.

Systems designed to replace heart and lung function by providing a circulatory pump and means of oxygenating the blood have been in existence for over 50 years. In 1953 John Gibbon Junior invented the first successful heart-lung bypass machine (Gibbon, 1978). The following year, Dr Lillehei's group developed a cross-circulation technique using anesthetized adult volunteers as "live cardiopulmonary bypass machines" during the repair of congenital cardiac defects (Warden *et al*., 1954). By 1955, the Mayo Clinic reported improvements to Gibbon's device which they used in successful repair of atrial septal defects (Kirklin *et al*., 1955). With the establishment of cardiopulmonary bypass for elective cardiothoracic surgical applications, such techniques were adapted for use in paediatric cardiac and respiratory failure. The bubble oxygenator was described in this context in the mid-60s (Rashkind *et al*., 1965) but this was largely to be replaced by the membrane oxygenator (Baffes *et al*., 1970) found in modern systems. The term ECMO was coined at around this time, and differs from conventional cardiopulmonary bypass in that it is established through peripheral rather than central vascular cannulation. It is also focused on provision of oxygenation support over a longer period than associated with bypass. In essence, the aim is to support function and therefore aid recovery. Newer systems designed to provide more robust pump support, as well as oxygenation are often described as extracorporeal life support (ECLS) systems.

ECLS or ECMO devices are designed to perfuse essential organs with oxygenated blood at 37$^{o}$C. The loss of such an environment following death prompts the transplantation clinician to instigate interventions intended to limit the injury that follows. ECMO systems in many ways approach the 'ideal' preservation intervention (at least theoretically); optimal delivery of normothermic oxygenated blood can be restored at will, following declaration of death.

### 6.3.1 ECMO in Clinical Transplantation

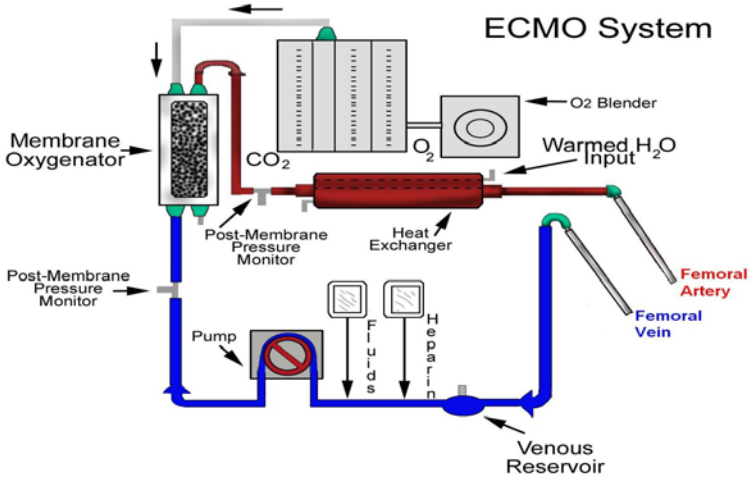
ECMO techniques have been applied in NHBD transplantation in several centres (D'Alessandro *et al*., 2004, Gravel *et al*., 2004, Ko *et al*., 2000, Magliocca *et al*., 2005). A schematic representation of the type of system employed is given in Figure 6.1.

The Michigan group implemented a controlled NHBD program involving post-mortem ECMO in 1999. ECMO cannulae are placed in the femoral artery and vein following consent to donate, but prior to withdrawal of support in the ITU. ECMO circulation is initiated immediately following declaration of death, eliminating the primary warm ischaemic time. The donor family may remain with the donor for a short time before transfer to theatre for organ recovery. 20 renal transplants from 13 ECMO-supported donors were performed between 2000 and 2003. One case of PNF secondary to surgical complications was reported. In the 19 functioning grafts, 2 (11%) resulted in DGF. The authors called for others to consider ECMO as a viable and effective organ preservation modality (Gravel *et al*., 2004).

In the University of Wisconsin, analysis of short-term outcomes from normothermic ECMO for NHB donation of abdominal organs was performed in 2005. Between 2000 and 2004, 20 patients entered the UW ECMO-supported NHBD protocol. Retrieval was performed in 15 cases; in 3 controlled NHBD situations the agonal time exceeded the local 60 minute threshold, and in 2 cases organs were deemed unsuitable for transplantation. 14 of the 20 original entrants were category II donors (70%). In comparison to results prior to the introduction of the ECMO protocol, the authors reported an increase in the donor pool of 33% and an increase of 24% in the number of kidneys

transplanted. Only 2 cases of DGF were encountered, with no PNF or deaths. Conclusions were that despite early results and small numbers the indications were that the use of ECMO in uncontrolled NHBDs could potentially provide outcomes at least comparable to brain-stem dead donors whilst increasing the donor pool (Magliocca *et al*., 2005).

**Figure 6.1**




Clearly, normothermic oxygenated organ preservation and retrieval systems, using techniques of ECMO, have been employed successfully in controlled and uncontrolled NHBD situations with excellent outcomes (D'Alessandro *et al*., 2004, Gravel *et al*., 2004, Light and Cecka, 2005, Magliocca *et al*., 2005). Both the raw numbers, in terms of transplants, and the numbers of centres utilising these expensive and complex techniques are currently small. However, the gross limitation of the ischaemia-related complications (DGF and PNF) to levels not previously seen in NHBD renal transplantation speaks for itself.

The small number of centres with expertise in this area may well be the pioneers of a new approach which all will eventually follow. However, for many established centres a change to an ECMO approach might be financially and logistically difficult. Such changes also often take time. In addition, interest in ECMO is growing in the field of emergency medicine; ECMO has been used successfully in the ITU setting for the resuscitation of respiratory arrest in children (Huang *et al*., 2008), and also in the Accident and Emergency setting for adult arrests (Chen *et al*., 2008). This introduces a dangerous problem regarding the role of ECMO in potential donor situations. ECMO may become increasingly attractive to both resuscitative emergency medicine and transplantation doctors alike. If ECMO demonstrates evidence of benefit to patients and improves in or out-of-hospital arrest survival then it must be implemented. However, this would represent the potential for significant difficulties for the donation process; the transition between a situation where ECMO is being used as treatment to preserve life, and where the aim is to preserve organs, will be fraught with ethical, legal and practical difficulty. At present the resuscitation team may reach a point where all feel that further resuscitation is unwarranted, the patient has died, and the decision is made to stop resuscitation interventions. At this point, after a no-touch period, separate interventions may be implemented to preserve the organs. With ECMO employed both pre- and post-declaration of death, the line becomes blurred; effectively the 'life support' machine is switched off and then switched back on again. It is difficult to predict the general perception or acceptance of this kind of eventuality.

One ethically ameliorating possibility would be to position an occlusive aortic balloon at the level of the diaphragm during preservative ECMO. This would prevent re-perfusion of the heart, lungs and brain and exclude the possibility of pseudo-resuscitation. However, such an action may

preclude heart or lung retrieval, the concept of which is growing in popularity within the cardiothoracic transplant community.

Perhaps lessons regarding the impact of such dilemmas will be learned from future experiences from the French NHBD protocol; although the majority of the uncontrolled French donors will be subject to a crypreservation protocol, in the small number of patients for whom ECMO resuscitation is instituted and fails, ECMO will be allowed to continue prior to organ recovery and transplantation (Antoine *et al*., 2008).

### 6.3.2 The Current Situation with Uncontrolled NHBD Organ Preservation

Regardless of the potential of ECMO-based approaches, the majority of transplant centres recovering organs from uncontrolled NHBDs continue to use protocols based on concepts of cryopreservation, often involving *in-situ* cold perfusion alone. Although ECMO-based protocols have excellent early results, cryopreservation approaches have yielded successful controlled NHBD kidney programs in many parts of the world. Furthermore, the deficiencies noted in results from uncontrolled NHBD transplantation programs, based on DBTL *in-situ* perfusion alone, may be attributable to the deficiencies in cooling efficacy demonstrated by studies within this thesis. The hope is that those centres aiming to optimise a cryopreservation approach will take note of the data presented herein and take steps to include techniques of supplementary cooling within their protocols.

## 6.4 Preservation for Uncontrolled NHBDs - Future Research

The final aim of the project was to establish a trial of human peritoneal cooling. The Newcastle peritoneal cooling unit described above is in place at Newcastle General Hospital's Accident and Emergency Department, and awaits the next uncontrolled NHBD donor. A comprehensive approach to data collection will be applied in these donors; information regarding renal surface temperature at laparotomy, machine perfusion viability testing, and all relevant outcome data may then be compared to data from phase III transplants, and the unit's HBD outcomes in retrospective comparisons.

It is arguable that the 'gold standard' approach to demonstration of the potential benefit of supplementary cooling requires a randomised controlled trial (RCT). The group gave careful consideration to this possibility but chose to follow a full-implementation approach for the following reason; the demonstration of the benefit of the addition of peritoneal was clear, despite small numbers. In terms of cooling efficacy, microdialysis markers of ischaemic severity, and organ viability assessment, a significant improvement in preservation was suggested. It was therefore decided that to withhold such an intervention for half of the donors presenting to Newcastle A&E would be ethically questionable. In this way uncontrolled NHBDs in Newcastle will all benefit from an improved cryopreservation protocol.

### 6.4.1 Optimised Cryopreservation vs. ECMO for NHBDs

The augmented cryopreservation protocol described herein, including supplementary peritoneal cooling must be compared to an ECMO-based approach before firm conclusions can be drawn as to the optimal preservation method. It is likely that in order to demonstrate differences in efficacy between two fundamentally different approaches, any animal model will need to assess

post-transplantation outcomes. This would require either a recovery transplantation model or a transplantation-simulation (possibly using a rig capable of warm oxygenated reperfusion with blood), where immediate or DGF can be identified, and renal function assessed through calculation of urine output and GFR.

The clear 'room for improvement' for conventional cryopreservation protocols, suggested by the parameters of viability seen in the peritoneal cooling arm of the uncontrolled NHBD study, might render the negative financial, practical and ethical implications of ECMO-based approaches less attractive to many NHBD programs.

## 6.5 Final Conclusions

This project has provided additional evidential support for the assessment of NHBD renal ischaemic injury using protocols of viability assessment based on hypothermic machine perfusion; a predictable relationship between warm ischaemic duration and renal viability criteria has been successfully demonstrated in a large animal model, and novel approaches to the use of such assessments have been explored in order to maximise organ resource opportunities and utilisation. These are important findings which have been received with interest by the transplant community at international meetings. However our understanding of the multitudinous factors contributing to renal NHBD transplantation success or failure remains incomplete. This is well-illustrated each time a transplanted organ which has passed viability assessment fails to function.

The recently published, large multi-centre European trial of hypothermic machine perfusion *versus* cold storage (Moers *et al*., 2009) has demonstrated the incontrovertible benefits of the former method of renal preservation. In this trial surgeons were blinded to any information regarding machine perfusion or perfusate enzyme parameters. In essence all organs were viability assessed in

the sense that both PFI and GST data was collected, but this information was not used to guide clinical decisions. Later sub-group analysis in controlled NHBDs suggested that no differences in PNF rates existed between organs above or below previously established thresholds of viability. The authors have since argued that no NHBD organs should be discarded on the basis of the viability assessment protocols described herein. This is not an unreasonable statement, but it must be remembered that only controlled NHBDs contributed to the data; these organs are generally found to be of acceptable quality and it is rare for PFI and GST parameters to be approaching or below viability thresholds. PNF in organs transplanted from these donors is likely to be due to an as yet unidentified confounding factor (or factors) not reflected by the established markers. This suggests incomplete understanding and the need for further research, but does not render current protocols irrelevant. Had the European study included uncontrolled NHBDs, the data within this thesis would suggest that with the considerably greater degrees of associated ischaemic injury, Newcastle viability assessment protocols may have provided clinically useful information regarding the risk of PNF.

Despite the evidence described for a role for machine perfusion-derived viability assessments the emerging picture is one of incompleteness. PFI and GST reflect only donor and initial organ preservation factors. Success or failure may also be influenced beyond this point; in recipient selection and preparation, the transplantation procedure itself, and in the post-operative period. The sheer complexity of renal ischaemia-reperfusion injury coupled with the equally complex effects of the surgical stress of transplantation and graft-recipient immunology would suggest that a viability assessment taking into account two relatively simple indicators is bound to be of limited accuracy. Future research should not aim to replace PFI and GST but instead to establish additional indicators, each aimed at reflecting different parts of the processes involved in the progress from organ

preservation to harvest, preservation and optimisation interventions, transplantation and post-operative management. In this way a more complex, but equally more robust method for accurately predicting organ viability may be achieved.

The project has made an important contribution in the approach to LPN and laparoscopic renal hypothermia. The studies involving the 'Newcastle Laparoscopic Renal Cooling Device' succeeded in achieving proof of concept with demonstration of effective renal cooling and preservation. The work has prompted further development of the device with a view to human studies in the near future.

The studies relating to preservation interventions in the porcine model of the uncontrolled NHBD have produced striking results. These results strongly suggest that uncontrolled NHBD centres employing cold in-situ perfusion approaches to preservation would be wise to consider supplementary techniques of organ cooling. Further avenues of research should assess the efficacy of peritoneal cooling in human donors, and seek comparison between ISP and ECMO based techniques of organ preservation.

This final study highlights the potential benefit of careful assessment of the efficacy of each part of the transplantation process and has suggested a change in practice capable of improving hypothermic organ preservation. Here the wider argument mirrors the call for a more comprehensive approach to viability assessment. In order to achieve the best possible outcomes from NHBDs, similar attention must be paid to every element of the transplantation process.

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# Appendix 1

# Appendix 1

## Chapter 3

## Renal Ischaemic Injury Assessment

### Prospective assessment of the effects of warm ischaemia on retrospectively established measures of organ viability

**Pressure Flow Index (PFI) according to warm ischaemic duration**

| Control | 30 mins | 60 mins | 90 mins |
|---|---|---|---|
| 1.63 | 0.80 | 0.86 | 0.64 |
| 1.85 | 2.04 | 1.30 | 0.38 |
| | 1.14 | 0.72 | 0.43 |

**Peak Glutathione-S-Transferase (GST) concentration per 100g renal mass according to ischaemic duration.**

| 30 mins | 60 mins | 90 mins |
|---|---|---|
| 42. | 24 | 59 |
| 39 | 53 | 49 |
| 14 | 38 | 72 |

## Pre-Arrest Acute Renal Failure in NHBD transplantation

### Recipients' GFR at 3 months

| ARF | Normal Function |
|---|---|
| 52.8 | 55 |
| 50.6 | 46 |
| 79 | 34 |
| 41 | 21 |
| 47.1 | 40 |
| 38.6 | 41 |
| 33.9 | 27 |
| 19.9 | 34 |
| | 28 |
| | 24 |
| | 40 |
| | 43 |
| | 46 |
| | 19 |
| | 34 |
| | 53 |
| | 44 |
| | 53 |
| | 63 |
| | 60 |
| | 74 |
| | 54 |
| | 42 |
| | 59 |
| | 47 |
| | 58 |
| | 38 |
| | 55 |
| | 81 |
| | 50 |
| | 33 |
| | 30 |
| | 41 |
| | 30 |
| | 39 |
| | 46 |
| | 18 |
| | 49 |
| | 57 |
| | 45 |

**Recipients' GFR at 12 months**

| ARF | Normal Function |
|---|---|
| 59.7 | 39 |
| 44 | 48 |
| 46 | 36 |
| 54.8 | 22 |
| 29.68 | 47 |
| 18.92 | 43 |
| | 35 |
| | 26 |
| | 33 |
| | 25 |
| | 25 |
| | 19 |
| | 51 |
| | 20 |
| | 35 |
| | 55 |
| | 51 |
| | 49 |
| | 68 |
| | 62 |
| | 75 |
| | 60 |
| | 33 |
| | 62 |
| | 40 |
| | 80 |
| | 43 |
| | 48 |
| | 82 |
| | 57 |
| | 48 |
| | 39 |
| | 22 |
| | 41 |

**Dual Group; Donor Demographics for 16 grafts reaching 12 months post transplantation**

| *Age* | *Weight* | *Sex* | *MC* | *1st WIT* | *2nd WIT* | *Total WIT* | *CIT hrs* | *A* | *B* | *DR* | *CMV mismatch* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 32 | 64.0 | M | 2 | 39 | 28 | 67 | 20 | 1 | 1 | 1 | 0 |
| 44 | 88.0 | M | 2 | 17 | 38 | 55 | 21 | 1 | 2 | 1 | 0 |
| 55 | 75.5 | M | 3 | 24 | 32 | 56 | 20 | 1 | 1 | 0 | 1 |
| 47 | 81.0 | M | 3 | 29 | 30 | 59 | 22 | 1 | 2 | 0 | 0 |
| 58 | 63.0 | F | 2 | 29 | 37 | 66 | 22 | 1 | 1 | 1 | 1 |
| 35 | 88.0 | M | 2 | 27 | 31 | 58 | 22 | 1 | 1 | 1 | 0 |
| 46 | 93.0 | M | 2 | 32 | 33 | 65 | 22 | 0 | 0 | 1 | 0 |
| 57 | 71.0 | M | 2 | 25 | 30 | 55 | 23 | 1 | 1 | 1 | 1 |
| 71 | 64.5 | M | 4 | 25 | 31 | 56 | 22 | 0 | 1 | 0 | 0 |
| 74 | 62.0 | F | 2 | 23 | 32 | 55 | 23 | 0 | 0 | 0 | 0 |
| 45 | 69.0 | F | 2 | 15 | 22 | 37 | 18 | 2 | 1 | 0 | 0 |
| 63 | 53.5 | F | 2 | 25 | 35 | 60 | 23 | 1 | 1 | 1 | 0 |
| 61 | 66.5 | M | 2 | 17 | 33 | 50 | 22 | 1 | 1 | 1 | 0 |
| 46 | 54.0 | M | 3 | 33 | 32 | 65 | 22 | 1 | 1 | 1 | 1 |
| 48 | 61.0 | M | 3 | 24 | 50 | 74 | 18 | 1 | 1 | 1 | 0 |
| 56 | 73.5 | M | 2 | 33 | 30 | 63 | 24 | 1 | 1 | 1 | 0 |

**Single Group; Donor Demographics for 115 single organ NHBD transplants**

| MC | Age | Weight | Sex | 1st WIT | 2ndWIT | Total WIT | CIT hrs | A | B | DR | CMV mismatch |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 3 | 63.0 0 | 79 | M | 20 | 35 | 55 | 28 | 1 | 1 | 1 | 1 |
| 3 | 63.0 0 | 75 | M | 20 | 42 | 62 | 21 | 1 | 1 | 1 | 0 |
| 2 | 47.0 0 | 86 | M | 31 | 60 | 91 | 27 | 1 | 1 | 0 | 1 |
| 2 | 52.0 0 | 66 | F | 27 | 36 | 63 | 21 | 0 | 0 | 0 | 0 |
| 3 | 64.0 0 | 59 | F | 21 | 35 | 56 | 18 | 1 | 2 | 1 | 0 |
| 3 | 37.0 0 | 58 | F | 20 | 38 | 58 | 22 | 1 | 0 | 0 | 1 |
| 3 | 51.0 0 | 62 | M | 31 | 37 | 68 | 37 | 1 | 1 | 1 | 0 |
| 3 | 53.0 0 | 53 | F | 20 | 38 | 58 | 18 | 1 | 1 | 1 | 0 |
| 2 | 65.0 0 | 74 | M | 15 | 32 | 47 | 24 | 2 | 1 | 0 | 0 |
| 3 | 42.0 0 | 63 | M | 15 | 26 | 41 | 26 | 0 | 1 | 0 | 0 |
| 3 | 42.0 0 | 30 | F | 15 | 37 | 52 | 33 | 1 | 1 | 1 | 0 |
| 2 | 47.0 0 | 83 | M | 26 | 22 | 48 | 19 | 1 | 1 | 2 | 0 |
| 3 | 38.0 0 | 75 | F | 31 | 44 | 75 | 20 | 1 | 1 | 1 | 0 |
| 3 | 59.0 0 | 85 | M | 30 | 37 | 67 | 29 | 2 | 2 | 0 | 0 |
| 3 | 71.0 0 | 61 | F | 18 | 35 | 53 | 25 | 1 | 2 | 0 | 0 |
| 2 | 47.0 0 | 70 | M | 24 | 35 | 59 | 27 | 2 | 0 | 0 | 1 |
| 2 | 52.0 0 | 66 | F | 17 | 42 | 59 | 32 | 1 | 1 | 0 | 0 |
| 2 | 52.0 0 | 68 | M | 20 | 40 | 60 | 28 | 0 | 1 | 0 | 0 |
| 2 | 59.0 0 | 52 | M | 25 | 54 | 79 | 23 | 1 | 2 | 1 | 1 |
| 3 | 33.0 0 | 51 | M | 20 | 45 | 65 | 22 | 2 | 2 | 0 | 0 |
| 3 | 33.0 0 | 65 | M | 20 | 31 | 51 | 24 | 1 | 2 | 2 | 0 |
| 3 | 49.0 0 | 140 | M | 25 | 46 | 71 | 22 | 1 | 1 | 1 | 0 |
| 2 | 52.0 0 | 79 | M | 34 | 32 | 66 | 30 | 1 | 0 | 1 | 0 |
| 3 | 47.0 0 | 92 | M | 16 | 31 | 47 | 24 | 2 | 1 | 1 | 0 |
| 3 | 47.0 0 | 85 | M | 16 | 41 | 57 | 27 | 2 | 1 | 1 | 0 |
| 3 | 63.0 | 73 | F | 20 | 37 | 57 | 22 | 1 | 0 | 1 | 1 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | | | | | | | | | | |
| | 52.0 | | | | | | | | | | |
| 2 | 0 | 75 | M | 24 | 38 | 62 | 23 | 2 | 0 | 0 | 0 |
| | 48.0 | | | | | | | | | | |
| 2 | 0 | 82 | M | 15 | 32 | 47 | 31 | 1 | 2 | 0 | 0 |
| | 59.0 | | | | | | | | | | |
| 3 | 0 | 75 | M | 30 | 41 | 71 | 20 | 1 | 2 | 0 | 0 |
| | 48.0 | | | | | | | | | | |
| 3 | 0 | 79 | M | 20 | 35 | 55 | 28 | 1 | 2 | 1 | 1 |
| | 48.0 | | | | | | | | | | |
| 3 | 0 | 73 | F | 20 | 32 | 52 | 34 | 2 | 1 | 1 | 1 |
| | 37.0 | | | | | | | | | | |
| 2 | 0 | 61 | F | 18 | 30 | 48 | 28 | 1 | 2 | 1 | 0 |
| | 51.0 | | | | | | | | | | |
| 3 | 0 | 61 | M | 10 | 40 | 50 | 24 | 0 | 0 | 1 | 0 |
| | 51.0 | | | | | | | | | | |
| 3 | 0 | 66 | F | 10 | 34 | 44 | 18 | 2 | 1 | 0 | 0 |
| | 50.0 | | | | | | | | | | |
| 3 | 0 | 70 | M | 24 | 35 | 59 | 18 | 1 | 0 | 1 | 1 |
| | 50.0 | | | | | | | | | | |
| 3 | 0 | 82 | M | 24 | 31 | 55 | 24 | 1 | 0 | 1 | 1 |
| | 51.0 | | | | | | | | | | |
| 2 | 0 | 58 | F | 14 | 40 | 54 | 24 | 0 | 1 | 1 | 0 |
| | 15.0 | | | | | | | | | | |
| 3 | 0 | 95 | M | 18 | 40 | 58 | 23 | 2 | 1 | 0 | 0 |
| | 15.0 | | | | | | | | | | |
| 3 | 0 | 91 | M | 18 | 45 | 63 | 18 | 1 | 0 | 1 | 0 |
| | 17.0 | | | | | | | | | | |
| 3 | 0 | 74 | M | 20 | 27 | 47 | 11 | 1 | 2 | 0 | 0 |
| | 17.0 | | | | | | | | | | |
| 3 | 0 | 71 | F | 20 | 34 | 54 | 21 | 1 | 1 | 1 | 0 |
| | 33.0 | | | | | | | | | | |
| 2 | 0 | 96 | M | 16 | 30 | 46 | 25 | 1 | 1 | 1 | 0 |
| | 33.0 | | | | | | | | | | |
| 2 | 0 | 92 | M | 16 | 45 | 61 | 13 | 1 | 1 | 0 | 0 |
| | 57.0 | | | | | | | | | | |
| 2 | 0 | 112 | M | 27 | 45 | 72 | 20 | 0 | 1 | 1 | 0 |
| | 52.0 | | | | | | | | | | |
| 2 | 0 | 57 | F | 35 | 54 | 89 | 30 | 1 | 2 | 0 | 0 |
| | 10.0 | | | | | | | | | | |
| 3 | 0 | 52 | M | 20 | 38 | 58 | 13 | 1 | 1 | 1 | 0 |
| | 10.0 | | | | | | | | | | |
| 3 | 0 | 79 | M | 20 | 39 | 59 | 17 | 1 | 1 | 1 | 0 |
| | 22.0 | | | | | | | | | | |
| 3 | 0 | 69 | M | 19 | 34 | 53 | 19 | 2 | 2 | 0 | 0 |
| | 22.0 | | | | | | | | | | |
| 3 | 0 | 52 | F | 19 | 51 | 70 | 20 | 0 | 1 | 1 | 0 |
| | 53.0 | | | | | | | | | | |
| 2 | 0 | 62 | M | 18 | 35 | 53 | 22 | 2 | 2 | 0 | 0 |
| | 64.0 | | | | | | | | | | |
| 3 | 0 | 98 | M | 13 | 42 | 55 | 21 | 0 | 2 | 0 | 0 |
| | 64.0 | | | | | | | | | | |
| 3 | 0 | 75 | F | 13 | 30 | 43 | 26 | 0 | 1 | 0 | 1 |
| | 20.0 | | | | | | | | | | |
| 3 | 0 | 65 | M | 10 | 33 | 43 | 18 | 1 | 2 | 1 | 0 |
| 3 | 20.0 | 58 | F | 10 | 46 | 56 | 25 | 1 | 0 | 0 | 0 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | | | | | | | | | | |
| | 40.0 | | | | | | | | | | |
| 3 | 0 | 54 | M | 35 | 92 | 127 | 27 | 1 | 2 | 1 | 0 |
| | 40.0 | | | | | | | | | | |
| 3 | 0 | 53 | F | 35 | 32 | 67 | 29 | 1 | 2 | 1 | 0 |
| | 46.0 | | | | | | | | | | |
| 2 | 0 | 68 | M | 10 | 40 | 50 | 21 | 2 | 1 | 1 | 0 |
| | 46.0 | | | | | | | | | | |
| 2 | 0 | 91 | M | 10 | 49 | 59 | 16 | 2 | 1 | 1 | 0 |
| | 43.0 | | | | | | | | | | |
| 2 | 0 | 55 | M | 19 | 45 | 64 | 22 | 1 | 2 | 1 | 0 |
| | 43.0 | | | | | | | | | | |
| 2 | 0 | 58 | F | 13 | 37 | 50 | 16 | 1 | 1 | 1 | 0 |
| | 24.0 | | | | | | | | | | |
| 3 | 0 | 66 | M | 14 | 38 | 52 | 17 | 1 | 1 | 0 | 1 |
| | 24.0 | | | | | | | | | | |
| 3 | 0 | 89 | M | 14 | 33 | 47 | 23 | 2 | 0 | 0 | 1 |
| | 55.0 | | | | | | | | | | |
| 2 | 0 | 56 | M | 24 | 45 | 69 | 24 | 1 | 1 | 1 | 0 |
| | 34.0 | | | | | | | | | | |
| 3 | 0 | 46 | F | 17 | 30 | 47 | 29 | 1 | 2 | 2 | 0 |
| | 34.0 | | | | | | | | | | |
| 3 | 0 | 93 | M | 15 | 22 | 37 | 24 | 2 | 1 | 0 | 0 |
| | 53.0 | | | | | | | | | | |
| 2 | 0 | 50 | M | 22 | 43 | 65 | 19 | 2 | 1 | 1 | 0 |
| | 51.0 | | | | | | | | | | |
| 2 | 0 | 73 | M | 22 | 50 | 72 | 24 | 2 | 2 | 0 | 0 |
| | 20.0 | | | | | | | | | | |
| 3 | 0 | 89 | F | 17 | 53 | 70 | 22 | 2 | 1 | 2 | 1 |
| | 20.0 | | | | | | | | | | |
| 3 | 0 | 104 | M | 17 | 69 | 86 | 23 | 2 | 1 | 0 | 1 |
| | 57.0 | | | | | | | | | | |
| 3 | 0 | 83 | M | 17 | 40 | 57 | 20 | 1 | 1 | 0 | 0 |
| | 57.0 | | | | | | | | | | |
| 3 | 0 | 83 | M | 17 | 40 | 57 | 25 | 1 | 0 | 0 | 0 |
| | 20.0 | | | | | | | | | | |
| 3 | 0 | 60 | F | 9 | 38 | 47 | 19 | 2 | 0 | 2 | 0 |
| | 20.0 | | | | | | | | | | |
| 3 | 0 | 55 | F | 9 | 30 | 39 | 23 | 2 | 1 | 1 | 0 |
| | 36.0 | | | | | | | | | | |
| 3 | 0 | 66 | M | 30 | 35 | 65 | 22 | 1 | 1 | 1 | 0 |
| | 61.0 | | | | | | | | | | |
| 2 | 0 | 53 | M | 20 | 30 | 50 | 25 | 1 | 1 | 1 | 1 |
| | 50.0 | | | | | | | | | | |
| 3 | 0 | 77 | M | 15 | 36 | 51 | 24 | 1 | 1 | 1 | 0 |
| | 43.0 | | | | | | | | | | |
| 2 | 0 | 83 | M | 24 | 29 | 53 | 25 | 0 | 1 | 1 | 1 |
| | 59.0 | | | | | | | | | | |
| 2 | 0 | 68 | M | 22 | 31 | 53 | 21 | 2 | 2 | 1 | 0 |
| | 54.0 | | | | | | | | | | |
| 3 | 0 | 80 | M | 22 | 16 | 38 | 33 | 1 | 1 | 1 | 0 |
| | 43.0 | | | | | | | | | | |
| 2 | 0 | 90 | M | 24 | 37 | 61 | 25 | 0 | 2 | 0 | 0 |
| | 26.0 | | | | | | | | | | |
| 3 | 0 | 56 | M | 21 | 28 | 49 | 21 | 1 | 1 | 1 | 1 |
| 3 | 26.0 | 73 | F | 21 | 31 | 52 | 26 | 1 | 1 | 1 | 1 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | | | | | | | | | | |
| | 50.0 | | | | | | | | | | |
| 3 | 0 | 70 | F | 17 | 45 | 62 | 24 | 1 | 1 | 1 | 0 |
| | 28.0 | | | | | | | | | | |
| 2 | 0 | 66 | M | 16 | 47 | 63 | 31 | 1 | 1 | 0 | 0 |
| | 45.0 | | | | | | | | | | |
| 2 | 0 | 61 | F | 18 | 32 | 50 | 26 | 1 | 1 | 1 | 0 |
| | 54.0 | | | | | | | | | | |
| 3 | 0 | 69 | M | 15 | 31 | 46 | 24 | 0 | 1 | 1 | 0 |
| | 65.0 | | | | | | | | | | |
| 3 | 0 | 64 | M | 21 | 35 | 56 | 35 | 1 | 2 | 1 | 0 |
| | 22.0 | | | | | | | | | | |
| 3 | 0 | 77 | M | 21 | 39 | 60 | 30 | 0 | 1 | 1 | 0 |
| | 49.0 | | | | | | | | | | |
| 2 | 0 | 75 | M | 35 | 35 | 70 | 24 | 0 | 0 | 0 | 1 |
| | 49.0 | | | | | | | | | | |
| 2 | 0 | 95 | M | 35 | 41 | 76 | 25 | 0 | 0 | 0 | 1 |
| | 38.0 | | | | | | | | | | |
| 2 | 0 | 86 | M | 14 | 52 | 66 | 20 | 1 | 1 | 1 | 0 |
| | 52.0 | | | | | | | | | | |
| 2 | 0 | 81 | F | 39 | 49 | 88 | 24 | 0 | 1 | 1 | 0 |
| | 38.0 | | | | | | | | | | |
| 2 | 0 | 74 | F | 20 | 47 | 67 | 25 | 1 | 1 | 1 | 0 |
| | 22.0 | | | | | | | | | | |
| 3 | 0 | 52 | F | 18 | 39 | 57 | 23 | 0 | 1 | 1 | 0 |
| | 50.0 | | | | | | | | | | |
| 3 | 0 | 59 | M | 15 | 42 | 57 | 19 | 1 | 1 | 1 | 0 |
| | 22.0 | | | | | | | | | | |
| 3 | 0 | 75 | M | 17 | 37 | 54 | 23 | 2 | 1 | 0 | 0 |
| | 57.0 | | | | | | | | | | |
| 3 | 0 | 75 | M | 27 | 40 | 67 | 21 | 0 | 1 | 1 | 0 |
| | 38.0 | | | | | | | | | | |
| 3 | 0 | 59 | M | 10 | 29 | 39 | 14 | 1 | 1 | 0 | 0 |
| | 45.0 | | | | | | | | | | |
| 2 | 0 | 72 | M | 33 | 33 | 66 | 14 | 1 | 1 | 1 | 0 |
| | 54.0 | | | | | | | | | | |
| 2 | 0 | 80 | M | 18 | 60 | 78 | 24 | 1 | 1 | 1 | 0 |
| 2 | 1.00 | 81 | M | 30 | 42 | 72 | 27 | 2 | 1 | 0 | 0 |
| | 39.0 | | | | | | | | | | |
| 2 | 0 | 76 | M | 20 | 40 | 60 | 19 | 2 | 2 | 1 | 0 |
| | 42.0 | | | | | | | | | | |
| 2 | 0 | 60 | F | 15 | 21 | 36 | 21 | 1 | 1 | 1 | 0 |
| | 61.0 | | | | | | | | | | |
| 2 | 0 | 70 | F | 12 | 50 | 62 | 20 | 1 | 1 | 1 | 0 |
| | 57.0 | | | | | | | | | | |
| 3 | 0 | 66 | M | 15 | 30 | 45 | 19 | 0 | 2 | 0 | 0 |
| | 54.0 | | | | | | | | | | |
| 2 | 0 | 86 | M | 40 | 34 | 74 | 26 | 1 | 1 | 1 | 0 |
| | 34.0 | | | | | | | | | | |
| 3 | 0 | 60 | F | 18 | 35 | 53 | 16 | 1 | 2 | 1 | 1 |
| | 54.0 | | | | | | | | | | |
| 2 | 0 | 70 | M | 40 | 36 | 76 | 21 | 1 | 2 | 1 | 0 |
| | 28.0 | | | | | | | | | | |
| 3 | 0 | 72 | M | 18 | 29 | 47 | 31 | 2 | 2 | 1 | 0 |
| | 28.0 | | | | | | | | | | |
| 2 | 0 | 64 | F | 18 | 32 | 50 | 23 | 2 | 2 | 0 | 0 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 57.00 | 77 | M | 34 | 38 | 72 | 28 | 1 | 1 | 0 | 0 |
| 2 | 28.00 | 62 | M | 30 | 34 | 64 | 27 | 1 | 1 | 1 | 0 |
| 2 | 58.00 | 61 | M | 19 | 29 | 48 | 27 | 1 | 1 | 0 | 0 |
| 3 | 16.00 | 82 | F | 32 | 41 | 73 | 19 | 1 | 1 | 1 | 0 |
| 3 | 16.00 | 56 | F | 32 | 43 | 75 | 26 | 1 | 0 | 0 | 0 |

**Functional GFR outcomes in dual and single NHBD grafts at 3 and 12 months post-transplantation**.

| Single 3/12 | Dual 3/12 | Single 1 year | Dual 1 year |
|---|---|---|---|
| 39.02 | 45 | 16.11 | 67 |
| 31.32 | 73 | 33.18 | 84 |
| 16.93 | 24 | 45.61 | 33 |
| 29.94 | 30 | 26.11 | 39 |
| 47.11 | 20 | 47.31 | 20 |
| 31.61 | 87 | 33.36 | 21.8 |
| 19.4 | 52 | 45.86 | 48.1 |
| 36.24 | 40 | 32.43 | 49.3 |
| 43.46 | 56.2 | 39.75 | 52 |
| 38.14 | 48.7 | 23.7 | 52.1 |
| 48.27 | 28.2 | 19.31 | 41 |
| 33.01 | 47.1 | 40.71 | 20.5 |
| 41.21 | 26.6 | 80.66 | 62.3 |
| 24.59 | 58.4 | 50.09 | 41 |
| 23.96 | 53.3 | 57.33 | 42.8 |
| 37.91 | 40.2 | 47.46 | 39.4 |
| 73.03 | 50.1 | 51.88 | |
| 64.29 | 56.3 | 15.43 | |
| 41.32 | 41 | 18.68 | |
| 36.69 | 67.4 | 39.21 | |
| 8.76 | 25.4 | 78.09 | |
| 61.73 | | 38.1 | |
| 9.07 | | 52.53 | |
| 21.26 | | 27.56 | |
| 45.66 | | 46.4 | |
| 53.14 | | 28.18 | |
| 31.18 | | 22.67 | |
| 46.6 | | 32.85 | |
| 26.9 | | 29.44 | |
| 30.11 | | 50.46 | |
| 33.89 | | 40.64 | |
| 26.3 | | 40.94 | |
| 45.77 | | 48.07 | |
| 30.84 | | 34.85 | |
| 27.26 | | 17.13 | |
| 43.79 | | 41.3 | |
| 45.74 | | 22.22 | |
| 30.69 | | 43.03 | |
| 41.08 | | 44.88 | |
| 39.75 | | 51.63 | |
| 20.22 | | 19.71 | |
| 32.93 | | 72.92 | |

| | |
|---|---|
| 17.13 | 52.67 |
| 38.70 | 53.33 |
| 30.24 | 31.80 |
| 37.76 | 69.83 |
| 32.52 | 57.06 |
| 44.16 | 46.99 |
| 43.21 | 55.89 |
| 57.11 | 51.17 |
| 30.00 | 69.8 |
| 39.21 | 41.06 |
| 34.67 | 40.46 |
| 32.21 | 63.61 |
| 55.34 | 29.65 |
| 49.31 | 58.32 |
| 48.45 | 48.69 |
| 31.62 | 61.65 |
| 57.58 | 48.33 |
| 49.36 | 63.25 |
| 45.52 | 40.85 |
| 54.71 | 60.21 |
| 46.66 | 33.91 |
| 60.93 | 77.1 |
| 52.75 | 62.8 |
| 42.64 | 22.78 |
| 60.43 | 33.89 |
| 31.53 | 69.21 |
| 39.43 | 49.53 |
| 51.11 | 56.19 |
| 33.02 | 35.1 |
| 60.84 | 19.87 |
| 55.31 | 49.82 |
| 60.31 | 26.1 |
| 47.13 | 25.28 |
| 54.69 | 35.88 |
| 42.81 | 25.83 |
| 76.07 | 35.83 |
| 61.25 | 44.15 |
| 21.80 | 40.46 |
| 29.17 | 49.44 |
| 64.24 | |
| 53.77 | |
| 53.55 | |
| 34.45 | |
| 19.27 | |
| 57.68 | |
| 34.91 | |
| 41.18 | |
| 45.15 | |
| 24.28 | |
| 27.95 | |

42.12
56.55
47.51

# Appendix 2

# Appendix 2

## Chapter 4

## Laparoscopic Renal Cryopreservation

**Evaluation of the ischaemic protection efficacy of the Newcastle Laparoscopic Renal Cooling Device using renal transplantation viability assessment criteria in a porcine model**

Schedule of Ischaemic Times
.

| PIG | COOLED KIDNEY (mins) | WARM KIDNEY (mins) |
|---|---|---|
| 1 | 60 | 30 |
| 2 | 30 | Control – not clamped |
| 3 | 60 | 90 |
| 4 | 90 | 60 |
| 5 | 60 | 60 |
| 6 | 30 | 30 |
| 7 | 30 | 30 |
| 8 | 90 | 90 |
| 9 | 90 | 90 |
| 10 | Control – not clamped | 60 |

**Cooling Efficacy over 30 minutes**

Pig 10 Control + warm organ
Pig 5 Thermocouple malfunction

| Time minutes | PIG 1 | PIG 2 | PIG 3 | PIG 4 | PIG 5 | PIG 6 | PIG 7 | PIG 8 | PIG 9 |
|---|---|---|---|---|---|---|---|---|---|
| 0.1 | 30.06 | 36.53 | 38.06 | 36.06 | Excluded | 34.4 | 35.66 | 33.68 | 36 |
| 2.5 | 29.75 | 32.48 | 34.93 | 30.84 | Excluded | 31.48 | 32.31 | 31.33 | 30.8 |
| 5 | 29.16 | 29.41 | 30.77 | 23.73 | Excluded | 27.93 | 27.25 | 28.02 | 27.6 |
| 7.5 | 28.8 | 26.49 | 27.01 | 19.23 | Excluded | 24.42 | 22.56 | 24.49 | 25 |
| 10 | 28.29 | 23.93 | 24.78 | 16.31 | Excluded | 21.16 | 18.39 | 21.23 | 20.8 |
| 12.5 | 28.65 | 21.7 | 23.39 | 14.04 | Excluded | 18.66 | 15.36 | 18.66 | 18.3 |
| 15 | 24.26 | 20.08 | 22.02 | 12.28 | Excluded | 16.85 | 12.98 | 16.71 | 15.8 |
| 17.5 | 21.07 | 18.86 | 21.1 | 11.69 | Excluded | 15.47 | 11.01 | 14.93 | 14.4 |
| 20 | 19.63 | 17.35 | 19.9 | 10.54 | Excluded | 14.38 | 9.47 | 13.59 | 13.3 |
| 22.5 | 17.69 | 16.08 | 18.77 | 9.45 | Excluded | 13.5 | 8.12 | 12.48 | 12.3 |
| 25 | 16.6 | 15.04 | 17.55 | 8.94 | Excluded | 12.57 | 7.07 | 11.65 | 10.9 |
| 27.5 | 15.36 | 14.41 | 16.67 | 8.55 | Excluded | 12.14 | 6.27 | 10.81 | 10 |
| 30 | 13.77 | 14.16 | 16.01 | 7.96 | 18.59 | 11.99 | 5.42 | 10.15 | 9.9 |

**Individual temperature data, including re-warm for total ischaemic periods exceeding 30 minutes cooling. (Pig 5 excluded – thermocouple malfunction)**

| Time minutes | Pig 1 | Pig 3 | Pig 4 | Pig 8 | Pig 9 |
|---|---|---|---|---|---|
| 0 | 30.17 | 38.6 | 36.06 | 33.68 | 36 |
| 5 | 29.16 | 30.77 | 23.73 | 28.02 | 27.6 |
| 10 | 26.29 | 24.78 | 16.31 | 21.23 | 20.8 |
| 15 | 24.04 | 22.02 | 12.28 | 16.71 | 15.8 |
| 20 | 19.36 | 19.9 | 10.54 | 13.59 | 13.3 |
| 25 | 16.6 | 17.55 | 8.94 | 11.65 | 10.9 |
| 30 | 14.32 | 16.01 | 7.96 | 10.15 | 9.9 |
| 35 | 20.38 | 18.05 | 10.18 | 9.54 | 10.18 |
| 40 | 24.22 | 20.96 | 14.77 | 11.58 | 12.24 |
| 45 | 28.8 | 23.61 | 19.29 | 14.32 | 15.1 |
| 50 | 32.6 | 25.64 | 22.67 | 16.92 | 17.06 |
| 55 | 34.57 | 27.46 | 24.8 | 19.14 | 19.57 |
| 60 | 33.53 | 28.6 | 26.62 | 21.23 | 21.89 |
| 65 | | | 28.13 | 23.14 | 24.14 |
| 70 | | | 29.32 | 24.4 | 15.31 |
| 75 | | | 30.4 | 25.68 | 26.48 |
| 80 | | | 31.33 | 26.76 | 27.16 |
| 85 | | | 32.15 | 27.68 | 28.34 |
| 90 | | | 32.8 | 28.62 | 29.91 |

**Pressure Flow Index (PFI) after Four Hours Machine Perfusion according to Duration of Warm or Cooled Ischaemia**

| Control | 30 minutes warm | 30 minutes cooled | 60 minutes warm | 60 minutes cooled | 90 minutes warm | 90 minutes cooled |
|---|---|---|---|---|---|---|
| 1.85 | 0.8 | 1.41 | 0.68 | 1.35 | 0.64 | 1.01 |
| 1.9 | 2.02 | 2.46 | 1.3 | 1.03 | 0.35 | 1.34 |
| | 1.14 | 1.39 | 0.72 | 2.25 | 0.43 | 1.06 |

**Laparoscopic Renal Cooling demonstrates a trend suggesting limitation of perfusate GST/100g concentration**

| 30 minutes warm | 30 minutes cooled | 60 minutes warm | 60 minutes cooled | 90 minutes warm | 90 minutes cooled |
|---|---|---|---|---|---|
| 42 | 44 | 24 | 34 | 59 | 29 |
| 39 | 10 | 53 | 37 | 49 | 37 |
| 14 | 20 | 38 | 37 | 72 | 36 |

# Appendix 3

# Appendix 3

## Chapter 5

## Peritoneal Cooling in NHBDs

### Core Renal Temperature

### In-Situ Perfusion only group (Temperatures $^{o}$C)

| Time minutes | PIG A | PIG B | PIG C | PIG D |
|---|---|---|---|---|
| 0 | 38.62 | 39.06 | 38.54 | 38.11 |
| 5 | 38.6 | 39.22 | 38.52 | 38.07 |
| 10 | 38.58 | 39.33 | 38.28 | 38.02 |
| 15 | 38.52 | 39.41 | 38.09 | 37.82 |
| 20 | 38.49 | 39.43 | 37.96 | 37.71 |
| 25 | 38.4 | 39.47 | 37.87 | 37.68 |
| 30 | 38.29 | 36.98 | 37.82 | 37.67 |
| 35 | 38.23 | 32.55 | 37.75 | 37.46 |
| 40 | 38.13 | 29.49 | 37.33 | 35.8 |
| 50 | 37.97 | 27.18 | 35.23 | 33.66 |
| 55 | 37.68 | 26.43 | 33.98 | 32.56 |
| 60 | 37.05 | 25.81 | 32.81 | 31.45 |
| 65 | 36.2 | 25.36 | 31.69 | 30.41 |
| 68 | 35.58 | 25.48 | 31.06 | 29.83 |
| 70 | 35.04 | 25.21 | 30.55 | 29.36 |
| 75 | 34.09 | 26.39 | 29.66 | 28.56 |
| 80 | 33.09 | 27.15 | 28.73 | 28 |
| 85 | 32.15 | 27.71 | 28.09 | 28.43 |
| 90 | 31.29 | 27.36 | 27.65 | 28.66 |
| 95 | 30.5 | 26.33 | 27.3 | 28.71 |
| 100 | 29.72 | 23.92 | 27 | 28.7 |
| 105 | 29.08 | 23.13 | 26.71 | 28.46 |
| 110 | 28.54 | 24 | 26.39 | 28.12 |
| 115 | 28.06 | 24.38 | 26.07 | 27.43 |
| 120 | 27.88 | 24.66 | 25.63 | 27.16 |

**Peritoneal Cooling group**
**Temperatures $^{o}C$**

| Time minutes | PIG D | PIG E | PIG F | PIG G |
|---|---|---|---|---|
| 0 | 39.59 | 44.7 | 39.55 | 40.97 |
| 5 | 39.59 | 44.65 | 39.51 | 40.66 |
| 10 | 39.58 | 44.64 | 39.47 | 40.39 |
| 15 | 39.51 | 44.33 | 39.34 | 40.17 |
| 20 | 39.42 | 44.14 | 39.41 | 40.36 |
| 25 | 39.31 | 43.99 | 39.44 | 40.49 |
| 30 | 39.21 | 44.23 | 39.62 | 40.19 |
| 35 | 38.83 | 30.99 | 30.69 | 39.91 |
| 40 | 26.22 | 23.16 | 25.07 | 32.73 |
| 45 | 23.98 | 22.86 | 23.09 | 28.2 |
| 50 | 22.25 | 22.34 | 20.24 | 23.95 |
| 55 | 20.63 | 22.06 | 19.96 | 20.86 |
| 60 | 19.39 | 20.58 | 19.1 | 18.23 |
| 65 | 20.12 | 19.95 | 18.34 | 17.57 |
| 70 | 20.21 | 18.59 | 17.5 | 17.85 |
| 75 | 20.7 | 16.99 | 17.05 | 17.9 |
| 80 | 20.77 | 16.39 | 16.27 | 18.08 |
| 85 | 19.91 | 16.06 | 16.18 | 18.78 |
| 90 | 19.93 | 15.7 | 16.86 | 17.85 |
| 95 | 19 | 15.25 | 17.18 | 16.98 |
| 100 | 18.9 | 15.91 | 16.88 | 17.6 |
| 105 | 18.76 | 15.2 | 16.37 | 17.72 |
| 110 | 17.9 | 14.99 | 16.88 | 18.26 |
| 115 | 17.29 | 15.11 | 16.84 | 18.31 |
| 120 | 17.06 | 15.5 | 16.7 | 18.34 |

**Microdialysis – Markers of Ischaemia**

**Lactate**
**(mmol/l)**

**Control organs – normal perfusion**

| Time minutes | Control 1 | Control 2 |
|---|---|---|
| 0 | 1.09 | 0.48 |
| 20 | 1.05 | 1.82 |
| 40 | 5.13 | 6.82 |
| 60 | 7.59 | 7.68 |
| 80 | 8.79 | 8.13 |
| 100 | 8.66 | 8.49 |
| 120 | 8.91 | 8.55 |

**Lactate**
**In-situ perfusion group**

| Time minutes | ISP A | ISP B | ISP C | ISP D |
|---|---|---|---|---|
| 0 | 0.88 | 0.67 | 0.99 | 0.78 |
| 20 | 1.34 | 1.17 | 1.44 | 1.29 |
| 40 | 3.4 | 3.33 | 3.68 | 3.49 |
| 60 | 6.44 | 6.34 | 6 | 5.94 |
| 80 | 6.29 | 6.01 | 6.19 | 6.38 |
| 100 | 6.64 | 6.11 | 6.24 | 6.67 |
| 120 | 6.1 | 5.99 | 6.21 | 6.6 |

**Lactate**
**Peritoneal Cooling group**

| Time minutes | PC A | PC B | PC C | PC D |
|---|---|---|---|---|
| 0 | 0.41 | 0.68 | 0.8 | 0.61 |
| 20 | 1.04 | 1.23 | 1.51 | 1.12 |
| 40 | 4.31 | 3.91 | 3.04 | 3.74 |
| 60 | 4.82 | 3.88 | 3.14 | 3.81 |
| 80 | 4.64 | 3.84 | 2.99 | 3.67 |
| 100 | 5.2 | 3.85 | 3.21 | 3.9 |
| 120 | 4.6 | 3.8 | 3.15 | 3.58 |

**Pyruvate**
**µmol/l**

**Control group**

| Time minutes | Control 1 | Control 2 |
|---|---|---|
| 0 | 74 | 56 |
| 20 | 78 | 107 |
| 40 | 24 | 27 |
| 60 | 0 | 12 |
| 80 | 0 | 0 |
| 100 | 0 | 0 |
| 120 | 0 | 0 |

**Pyruvate**
**In-situ perfusion group**

| Time minutes | ISP A | ISP B | ISP C | ISP D |
|---|---|---|---|---|
| 0 | 46 | 68 | 134 | 197 |
| 20 | 34 | 29 | 68 | 179 |
| 40 | 15 | 0 | 12 | 63 |
| 60 | 0 | 0 | 0 | 0 |
| 80 | 0 | 0 | 0 | 0 |
| 100 | 0 | 0 | 0 | 0 |
| 120 | 0 | 0 | 0 | 0 |

**Pyruvate**
**Peritoneal cooling group**

| Time minutes | PC a | PC B | PC C | PC D |
|---|---|---|---|---|
| 0 | 36 | 23 | 61 | 57 |
| 20 | 63 | 29 | 31 | 124 |
| 40 | 20 | 16 | 14 | 25 |
| 60 | 0 | 0 | 0 | 0 |
| 80 | 0 | 0 | 0 | 0 |
| 100 | 0 | 0 | 0 | 0 |
| 120 | 0 | 0 | 0 | 0 |

**Glucose**
**(mmol/l)**

**Control group**

| Time minutes | Control 1 | Control 2 |
|---|---|---|
| 0 | 2.43 | 0.91 |
| 20 | 2.92 | 1.66 |
| 40 | 1.91 | 0.13 |
| 60 | 1.19 | 0 |
| 80 | 0.6 | 0 |
| 100 | 0.24 | 0 |
| 120 | 0 | 0 |

**Glucose**
**In-situ perfusion group**

| Time minutes | ISP A | ISP B | ISP C | ISP D |
|---|---|---|---|---|
| 0 | 2.61 | 3.47 | 2.16 | 2.67 |
| 20 | 3.07 | 3.53 | 3.01 | 2.99 |
| 40 | 1.15 | 3.15 | 2.04 | 1.29 |
| 60 | 0.89 | 1.64 | 0 | 0.35 |
| 80 | 0.14 | 0.99 | 0 | 0 |
| 100 | 0 | 0.76 | 0 | 0 |
| 120 | 0 | 0.45 | 0 | 0 |

**Glucose**
**Peritoneal Cooling group**

| Time minutes | PC A | PC B | PC C | PC D |
|---|---|---|---|---|
| 0 | 1.23 | 1.29 | 3.31 | 2.5 |
| 20 | 0.76 | 2.13 | 2.11 | 2.05 |
| 40 | 0.13 | 2.51 | 1.03 | 0.77 |
| 60 | 0 | 0.51 | 0.71 | 0.34 |
| 80 | 0.14 | 0.5 | 0.1 | 0.33 |
| 100 | 0.13 | 0.4 | 0 | 0.99 |
| 120 | 0 | 0.54 | 0 | 0.31 |

**Glycerol**
**(µmol/l)**

**Control group**

| Time minutes | Control 1 | Control 2 |
|---|---|---|
| 0 | 79 | 65 |
| 20 | 106 | 171 |
| 40 | 446 | 350 |
| 60 | 729 | 712 |
| 80 | 922 | 870 |
| 100 | 1070 | 978 |
| 120 | 1027 | 1021 |

**Glycerol**
**In-situ perfusion group**

| Time minutes | ISP A | ISP B | ISP C | ISP D |
|---|---|---|---|---|
| 0 | 43 | 57 | 61 | 51 |
| 20 | 120 | 115 | 138 | 128 |
| 40 | 393 | 364 | 401 | 351 |
| 60 | 410 | 380 | 489 | 399 |
| 80 | 492 | 459 | 543 | 487 |
| 100 | 536 | 463 | 628 | 594 |
| 120 | 540 | 459 | 633 | 587 |

**Glycerol**
**Peritoneal cooling group**

| Time minutes | PC A | PC B | PC C | PC D |
|---|---|---|---|---|
| 0 | 41 | 38 | 51 | 42 |
| 20 | 104 | 111 | 112 | 99 |
| 40 | 179 | 187 | 132 | 168 |
| 60 | 279 | 264 | 198 | 256 |
| 80 | 270 | 298 | 202 | 264 |
| 100 | 319 | 297 | 213 | 279 |
| 120 | 333 | 301 | 224 | 280 |

**Machine Perfusion**

**Perfusion Flow Index**
**(ml/min/100g/1.73m$^2$)**

| Controls | ISP | PC |
|---|---|---|
| 0.58 | 1.02 | 1.88 |
| 0.36 | 0.76 | 1.58 |
| | 1.26 | 1.64 |
| | 1.31 | 1.41 |

# Publications