Cardiovascular disease and type 2 diabetes mellitus: Investigation of underlying mechanisms

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A thesis by

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Abstract

Cardiovascular disease (CVD) remains the leading cause of death in the United Kingdom and is associated with a huge burden of morbidity. Within the group of cardiovascular diseases coronary artery disease (CAD) is the single largest cause of death. Death rates from CAD have been falling since the 1970s predominantly due to a reduction in the prevalence in major risk factors such as cigarette smoking. Type 2 diabetes mellitus (DM) is an important risk factor for CVD. Type 2 DM is increasing in prevalence and there is concern that this will contribute to an increase in the burden of CVD. Reducing cardiovascular risk in patients with type 2 DM has to date focussed on tight blood pressure and glycaemic control together with statin therapy to achieve tight low density lipoprotein cholesterol (LDL) targets. Recent studies such as ADVANCE and ACCORD have highlighted some of the limitations with this approach. Important vascular abnormalities underlying the development of CAD include endothelial dysfunction and increased arterial stiffness. Some of the mechanisms underlying these abnormalities are thought to include increased oxidative stress, inflammation, insulin resistance and dyslipidaemia. These processes in patients with type 2 DM are currently not fully understood. It is hoped that through increased understanding of these processes new strategies for reducing cardiovascular risk in patients with type 2 DM can be identified.

This study aimed to investigate some of the processes thought to underlie CVD in patients with type 2 DM namely endothelial dysfunction, arterial stiffness, oxidative stress and dyslipidaemia. Finally this study aimed to assess the impact of two cardiovascular prevention strategies (statin therapy and increased physical activity) on these processes believed to underlie the development of CVD.

One hundred and twenty six patients with CAD (36 patients with type 2 DM, 90 patients without diabetes) and 80 controls (64 healthy controls and 16 varicose vein controls) were recruited as

part of the VASCAB study. In these patients *in vivo* and *ex vivo* endothelial function studies were performed. Indicators of arterial stiffness were measured using pulse wave velocity and pulse wave analysis techniques. Superoxide levels were assessed in vascular tissue, mononuclear cells and whole blood. LDL and high density lipoprotein cholesterol (HDL) subfractions were analysed in patients with CAD. To assess the impact of intensive statin therapy and tight LDL targets, endothelial function and vascular superoxide levels were compared in patients recruited as part of the VASCAB study (2007 cohort) to a group of patients recruited in 2003. Finally patients attending the cardiac rehabilitation programme following surgical revascularisation were recruited to assess the impact of increased physical activity on endothelial function and oxidative stress.

Endothelial function was impaired in patients with CAD compared to controls. In patients with CAD, type 2 DM was associated with greater impairment of endothelial function compared to patients with CAD alone. Superoxide levels in the vasculature, mononuclear cells and whole blood were similar in patients with and without type 2 DM. Type 2 DM was associated with significantly lower HDL levels and a preponderance to small dense LDL compared to patients without diabetes. Arterial stiffness was increased in patients with CAD compared to controls. There was however no significant difference in arterial stiffness in patients with type 2 DM and CAD compared to patients with CAD alone. Intensive statin therapy was associated with lower LDL levels and improved endothelial function but no change in vascular superoxide levels. Following the cardiac rehabilitation programme endothelial function was improved and HDL levels increased. There were no changes in levels of oxidative stress.

Endothelial dysfunction in patients with type 2 DM may partly account for the increased cardiovascular risk and worse cardiovascular outcomes seen in this group of patients. Increased oxidative stress did not explain the endothelial dysfunction associated with type 2 DM. The dyslipidaemia that was associated with type 2 DM (low HLD and small dense LDL levels) may

partly explain the increased endothelial dysfunction observed. Targeting endothelial dysfunction may therefore be a strategy for reducing cardiovascular risk in patients with type 2 DM. Intensive statin therapy and increased physical activity were both associated with improvements in endothelial function. The lack of evidence for increased arterial stiffness in patients with type 2 DM may reflect deficiencies in the methods used for assessing arterial stiffness. However this study highlights the difficulties of assessing arterial stiffness clinically and raises questions regarding the impact of type 2 DM on commonly used measures of arterial stiffness.

Future prospective studies assessing the impact of improving endothelial function in patients with type 2 DM on cardiovascular outcomes are required.

Declaration

The work described in this thesis was performed during my period as a clinical research fellow at the Division of Cardiovascular and Medical Sciences (now Institute of Cardiovascular and Medical Sciences), University of Glasgow.

In the 2003 cohort patient recruitment and clinical examinations were performed by Dr Sammy Al Benna. Dr Carlene Hamilton performed the vascular superoxide and organ bath studies for both the 2003 and 2007 cohorts.

Dr Maria Moreno performed the mononuclear cell superoxide studies. Mr Jim McCulloch performed the whole blood superoxide studies. Routine lipid analysis was performed by Professor Naved Sattar, Dr Lynne Cherry and Miss Christine Gourlay of the routine lipids section of the biochemistry department of Glasgow Royal Infirmary. Professor Muriel Caslake performed the LDL and HDL subfraction analysis.

The study was designed by me. All participant recruitment, clinical examinations, pulse wave studies and in vivo assessment of endothelial function was carried out by me. All statistical analyses and interpretation of results was performed by me. The writing of the thesis was entirely my own work.

Dr Ruth MacKenzie cultured primary endothelial cells from some of the patients recruited as part of this study. Results from these cultured cells together with some of the clinical and laboratory data forms part of her thesis titled “Oxidative stress in endothelial cells in patients with coronary artery disease” which was submitted to the University of Glasgow in September 2009.

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Contents

List of Abbreviations

ACC	American college of Cardiology
ACCORD	Action to Control Cardiovascular Risk in Diabetes study
ACEi	Angiotensin converting enzyme inhibitor
ACR	Albumin to creatinine ratio
ADVANCE	Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation study
AGE	Advanced glycation end products
AHA	American heart association
AIx	Augmentation index
ANBP2	Australian Blood pressure trial
AP	Augmented pressure
ARBs	Angiotensin receptor blockers
ASCOT	Anglo-Scandinavian Cardiac Outcomes Trial
AU	Arbitrary units
BHF	British heart foundation
BMI	Body mass index
BP	Blood pressure

CABG	Coronary artery bypass grafting
CAD	Coronary artery disease
CAFE	Conduit artery function evaluation study
CCB	Calcium channel blocker
CETP	Cholesterol ester transfer protein
CPH	1-Hydroxy-3-carboxy- 2,2,5,5-tetramethylpyrrolidine
CRP	C-reactive protein
CVA	Cerebrovascular accident
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DM	Diabetes mellitus
DPP	Diabetes prevention project
DPS	Diabetes prevention study
ED	Ejection duration
EDTA	Ethylenediaminetetraacetic acid
eNOS	Endothelial nitric oxide synthase
EPR	Electron paramagnetic resonance
FIELD	Fenofibrate intervention and event lowering in diabetes

FMD	Flow mediated dilatation
GCRC	Glasgow cardiovascular research centre
GTN	Glyceryl trinitrate
H_2O_2	Hydrogen peroxide
HbA_{1c}	Glycated haemoglobin
HDL	High density lipoprotein
HEPES	(4-(2-hydroxyethyl)-1-piperzineethanesulfonic acid
HO^-	Hydroxyl radical
HR	Heart rate
IGT	Impaired glucose tolerance
ILDL	Intermediate low density lipoprotein
JBS	Joint British societies
KCL	Potassium chloride
LDL	Low density lipoprotein
MAP	Mean arterial pressure
METS	Metabolic equivalent of task
mm	Milimetre
MPD	Mean particle diameter

NAD(P)H	Nicotinamide adenine dinucleotide phosphate
NO	Nitric oxide
NS	Not significant
O_2^-	Superoxide
$ONOO^-$	Peroxynitrite
PBS	Phosphate buffered saline
PMA	Phorbol 12-myristate 13-acetate
PP	Pulse pressure
PPD	Peak particle diameter
PWA	Pulse wave analysis
PWV	Pulse wave velocity
ROS	Reactive oxygen species
SBP	Systolic blood pressure
SEM	Standard error of the mean
SEVR	Subendocardial viability ratio
SNP	Sodium nitroprusside
SPAQ	Scottish physical activity questionnaire
SPSS	Statistical package for the social sciences

TIA	Transient ischaemic attack
TR	Time to reflected wave
UKPDS	United Kingdom prospective diabetes study
VADT	Vertans Affairs Diabetes trial
VA-HIT	Veterans affairs high-density lipoprotein intervention study trial
VASCAB	Vascular function in coronary artery bypass patients study
VLDL	Very low density lipoprotein
VSMC	Vascular smooth muscle cells
VV	Varicose vein

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Publications

Delles C, Dymott JA, Neisius U, Rocchiccioli JP, Bryce GJ, Moreno MU, Carty DM, Berg GA, Hamilton CA, Dominiczak AF. Reduced LDL-cholesterol levels in patients with coronary artery disease are paralleled by improved endothelial function: An observational study in patients from 2003 and 2007. Atherosclerosis. 2010 Jul; 211(1):271-7. Epub 2010 Jan 21.

Taurino C, Miller WH, McBride MW, McClure JD, Khanin R, Moreno MU, Dymott JA, Delles C, Dominiczak AF. Gene expression profiling in whole blood of patients with coronary artery disease. Clin Sci (Lond). 2010 Jul 9;119(8):335-43.

Presentations

Scottish Cardiovascular Forum, Belfast February 2007. JA Dymott, C Chow, JP Rocchiccioli, GA Berg, L Cherry, C Delles, CA Hamilton, AF Dominiczak. Current Cholesterol lowering therapy improves endothelial function (oral communication).

RMCSG research prize night 01/03/2007. JA Dymott, C Chow, JP Rocchiccioli, GA Berg, A Kirk, L Cherry, C Delles, CA Hamilton, AF Dominiczak. Endothelial function in patients undergoing coronary artery bypass grafting is improved in 2006 compared to 2003 (oral communication).

European Society of Hypertension Annual Meeting, Milan 2007. JA Dymott, MAJ Hutton, G Bryce, GA Berg, CA Hamilton, AF Dominiczak and C Delles. Validation of a non-invasive method to assess endothelial function PWA and organ bath experiments in patients with advanced cardiovascular disease (poster communication).

Diabetes UK Annual professional conference 2008. JA Dymott, FO Owala, CA Hamilton, JP Rocchiccioli, KJ MacArthur, J MacDougall, C Delles and AF Dominiczak Reduced Vascular superoxide production in patients with coronary artery disease and type 2 diabetes (poster communication). Diabetes UK Annual Professional conference 2009. JA Dymott, JP Rocchiccioli, DM Carty , CA Hamilton, AF Dominiczak and C Delles HDL is an important unaddressed cardiovascular risk factor (poster communication).

Caledonian Society for Endocrinology/Scottish Society of Physicians, Edinburgh, September 2010. JA Dymott, MU Moreno, JP Rocchiccioli, AM Miller, WH Miller, KJ Macarthur, MJ Caslake, AF Dominiczak, CA Hamilton, C Delles. Mechanisms underlying cardiovascular disease in patients with type 2 DM (CalSoc prize for best oral presentation).

1 Introduction

1.1 The clinical context

1.1.1 Cardiovascular disease

Cardiovascular disease (CVD) remains the leading cause of death in the United Kingdom and is associated with a huge burden of morbidity. The term CVD covers stroke, peripheral vascular disease and coronary artery disease (CAD) all of which have the same underlying pathophysiology; atherosclerosis. Within the group of CVD, CAD is the single largest cause of death. CAD causes over 90,000 deaths a year in the UK; approximately 20% of deaths in men and 14% death in women [1]. CAD is also the most common cause of premature death (death in the under 75s) in both men and women [1].

The most important modifiable risk factors for CVD are cigarette smoking and cholesterol levels followed by hypertension, diabetes mellitus, abdominal obesity and psychosocial factors. The INTERHEART study showed that these risk factors together with dietary factors, levels of physical activity and alcohol consumption accounted for 90% of the risk for acute myocardial infarction[2].

Death rates from CVD have been falling since the 1970s. Over half of this reduction (58%) is the consequence of a reduction in cardiovascular risk factors, most notably cigarette smoking[1]. The remainder of the reduction can be attributed to improvements in medical management of CVD [1]. The role of diabetes mellitus (DM) in the development of CVD and CAD is of particular interest as rates of this condition are dramatically increasing throughout the world. There is concern that the increase in type 2 DM may contribute to an increase in prevalence of CVD.

1.1.2 Type 2 DM

DM is diagnosed on the basis of elevated blood glucose levels. The current WHO guidelines are a fasting glucose level of greater than 7mmol/L or random glucose greater than 11.1 mmol/L[3].

These levels were set based on the levels of hyperglycaemia above which microvascular complications specific to diabetes occur (e.g. retinopathy).

Type 2 DM is the commonest type of diabetes accounting for between 80 and 95% of cases of diabetes. Classifying an individual as having type 2 DM remains a predominantly clinical diagnosis. Type 2 DM is diagnosed in a patient with hyperglycaemia, no evidence of significant insulin deficiency and no evidence of secondary causes of diabetes (e.g. pancreatic insufficiency).

The pathogenesis of type 2 DM is complex but involves both insulin resistance and inadequate insulin secretion. Obesity, in particular abdominal obesity is central to the development of type 2 DM; over 80% of patients with type 2 DM are obese. Blood glucose levels are normally kept within tight control. Oral intake is balanced against insulin dependent glucose uptake by muscle, adipose tissue and the liver, and glucose production by the liver. Obesity and inactivity reduce insulin sensitivity. Increased insulin production by the β-cells of the pancreas are able to compensate for this initially, however eventually this is insufficient and blood glucose levels start to rise [4].

Type 2 DM therefore develops over many years. It is preceded by a pre-diabetic state that starts with abdominal obesity and insulin resistance, followed by marginally elevated blood glucose levels, termed impaired glucose tolerance and impaired fasting glucose. Over time if nothing is done to halt the process a significant proportion of these patients will go on to develop type 2 DM [4].

The prevalence of type 2 DM is increasing dramatically, fuelled both by the obesity epidemic and the aging population. In 2000 the prevalence of diabetes was estimated to be 171 million worldwide (2.8%). Based purely on demographic data and in particular the aging population it is projected that by 2030, 366 million people worldwide will have diabetes (4.4%) [5;6]. This is

likely to be an underestimate as the calculations are based on the assumption that current levels of obesity remains constant which is unlikely [6]. The 2009 Scottish diabetes survey reported the prevalence of diabetes at 4.3% with 87.4% of people having type 2 DM. Once again this has been increasing over the recent years [7].

Type 2 DM is associated with a number of long term complications in the vasculature which are divided into microvascular (retinopathy, neuropathy and nephropathy) and macrovascular (CAD, stroke and peripheral vascular disease) complications.

1.1.3 Type 2 DM and CVD

CVD is the leading cause of death in patients with Type 2 DM accounting for 50-70% of all deaths [8]. Indeed patients with Type 2 DM have the same risk of myocardial infarction as patients who have already suffered a heart attack, leading many clinicians to consider type 2 DM to be a cardiovascular equivalent [9]. Similar to the situation in the general population there is evidence death rates from CAD in patients with type 2 DM are also falling [10]. However the prevalence of Type 2 DM is increasing rapidly and the concern is that the prevalence of CAD may also start increasing in unison with this. Tackling CAD aggressively in patients with Type 2 DM is therefore essential. CAD is not only more prevalent in patients with Type 2 DM but when it does occur the atherosclerotic lesions are more severe and diffuse [11;12].

Hyperglycaemia partly explains the increased cardiovascular risk associated with type 2 DM. however the risk associated with hyperglycaemia is modest compared to the overall cardiovascular risk associated with type 2 DM. A 1% increase in HbA_{1c} is associated with 18% increase in cardiovascular events [13].Other cardiovascular risk factors associated with type 2 DM such as hypertension and dyslipidaemia are as, if not more important in the development of CVD in type 2 DM. For example blood pressure shows a stronger association with CVD in patients with type 2 DM compared to blood glucose levels [14].

Insulin resistance is also an important cardiovascular risk factor independent of diabetes status [15-17]. Insulin resistance is associated with a characteristic dyslipidaemia (high triglycerides, low HDL and preponderance of small dense LDL particles), hypertension and obesity [18]. A number of interventions can improve insulin sensitivity including pharmacological agents such as metformin, increased physical activity and weight loss [4]. Measures of insulin sensitivity are not currently assessed in routine clinical practice, and targeting insulin resistance in the prevention of CVD is not currently a clinical priority.

Current strategies for reducing cardiovascular risk in patients with type 2 DM predominantly focus on blood pressure and lipid management together with glycaemic control. The evidence behind these strategies will be discussed below.

1.1.3.1 Hyperglycaemia

Hyperglycaemia can be assessed by blood glucose levels or glycated haemoglobin (HbA_{1c}). HbA_{1c} gives an indication of blood glucose levels over the preceding 6-8 weeks. Epidemiological studies have shown that hyperglycaemia, assessed by either HbA_{1c} or blood glucose levels, are an important risk factor for CVD [19;20]. Previous studies had found a linear relationship between levels of glycaemia and cardiovascular risk [20]. A recent large meta-analysis however found a non-linear relationship between fasting blood glucose levels and cardiovascular risk [21]. For fasting blood glucose levels between 3.9 and 5.59 there was no longer a significant association with cardiovascular risk [21]. Optimal blood glucose targets for the prevention of CVD are not clear. Based on the earlier epidemiological studies it was assumed that achieving tight glycaemic control with the aim of near normal blood glucose levels would lead to a reduction in CVD in patients with type 2 DM. Many cardiovascular prevention guidelines therefore recommend target HbA_{1c} levels of 7% or less [22-24]. Evidence from a number of randomised controlled trials has however questioned this strategy.

Managing hyperglycaemia

Managing hyperglycaemia usually begins with lifestyle interventions such as dietary modification and the recommendation to increase physical activity. This is then followed by oral agents; three main drug classes of which are currently in widespread clinical use (biguanides, thiazolidinediones and sulphonylureas). These can be divided into insulin sensitizers, biguanides (metformin) and thiazolidinediones, and insulin secretogogues such as sulphonylureas. Insulin is usually added once oral agents fail.

Based on the epidemiological evidence it was hypothesised that improving glycaemic control in patients with type 2 DM would reduce cardiovascular events. A number of randomised controlled trials have aimed to test this hypothesis; 4 of the key studies will be discussed below.

The UKPDS (UK Prospective diabetes study) study began in 1977 and reported in 1998 compared intensive glucose control compared to conventional treatment in 3867 patients with newly diagnosed type 2 DM [25]. Intensive blood glucose control was achieved with increased use of sulphonylureas and insulin compared to conventional treatment. The difference in HbA_{1c} was 7.0% in the intensive group compared to 7.9% in conventional group. There was no reduction in macrovascular disease in the intensive group compared to conventional group. Weight gain was higher in the intensive group and episodes of hypoglycaemia more common.

The ADVANCE study (Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation study) looked at intensive blood glucose control and vascular outcomes in approximately 11,000 patients with type 2 DM. Patients were randomised to standard glucose control or intensive glucose control [26]. All patients in the intensive group were prescribed the sulphonylurea, gliclazide together with other drugs as necessary to achieve an HbA_{1c} of 6.5% or less. The primary endpoint was the combined outcome of major macrovascular and microvascular events. After 5 years of follow-up the average HbA_{1c} level in

the intensive group was 6.5% compared to 7.3% in the standard group. There was a 10% reduction in the combined primary endpoint but this was achieved through a 21% relative reduction in nephropathy. There was no reduction in macrovascular events in the intensive group. All cause mortality was similar between the two groups. Severe hypoglycaemia occurred more frequently in the intensive group. At the end of follow-up mean weight was 0.7kg greater in the intensive group compared to standard.

The ACCORD study (Action to Control Cardiovascular Risk in Diabetes study) also aimed to test whether intensive glucose control would reduce macrovascular disease in patients with type 2 DM [27]. The study also randomised patients to aggressive blood pressure and lipid control in a double two-by-two factorial design. All ten thousand patients were randomised to either intensive or standard blood glucose control with the primary outcome non-fatal myocardial infarction or stroke or death from any cardiovascular cause. Blood glucose control was achieved in both groups through the combination of oral agents together with insulin. Within the intensive group there was higher prescription of all blood glucose lowering therapies but in particular the use of 3 or more agents, insulin and thiazolidinediones. The study was terminated by the safety monitoring committee 17 months early due to the finding of increased mortality in the intensive group. At the time the study ended HbA_{1c} levels were 6.4% in the intensive group compared to 7.5% in the standard group. All cause mortality was significantly increased in the intensive group with no reduction in major cardiovascular events. Hypogylcaemia and weight gain was more common in the intensive group.

Finally VADT (Veterans Affairs Diabetes Trial) assessed intensive versus standard control in 1791 military veterans and once again found no significant effect on cardiovascular death or all cause mortality in the intensive group [28]. The HbA_{1c} was 8.4% in the standard group compared to 6.9% in the intensive group.

In these studies improved glycaemic control did not result in a reduction in cardiovascular mortality and morbidity. Given the epidemiological evidence linking hyperglycaemia and CVD these findings are somewhat unexpected and the reasons for this discrepancy are not clear. The studies may have been underpowered since in all studies cardiovascular event rates were lower than expected. A recent meta-analysis published looking at 33 000 patients in 5 randomised controlled trials suggested that improved glycaemic control, as assessed by HbA_{1c}, is associated with a reduction in cardiovascular events without an increase in all cause mortality [29].

The increased weight gain and episodes of hypoglycaemia found in the intensive groups may also be important. The ACCORD study which found increased mortality in the intensive group achieved tight glycaemic control rapidly in patients with long standing diabetes. The risk of severe hypoglycaemia was therefore greatest in this study with rates of 24.1% in the intensive group compared to 17.6% in the standard group [27]. Hypoglycaemia is postulated as a cause of sudden death in patients with type 1 DM and may be a precipitant for cardiac arrhythmias through QT prolongation [30].

The method by which glycaemic control is achieved may be important. Clearly in the epidemiological studies low blood glucose levels occur via very different processes to those induced during the management of patients with type 2 DM. All the discussed studies used a combination of various oral agents with the addition of insulin as necessary. Within the UKPDS study a subgroup of overweight patients with intensive blood glucose control using metformin showed a reduction in all cause mortality compared to obese patients receiving conventional treatment[31]. The metformin group had significantly fewer myocardial infarctions compared to obese group receiving conventional management however rates of myocardial infarct did not differ from the other intensive therapy groups.[31]. In the more recent studies thiazolidinediones were commonly prescribed, particularly in the ACCORD trial [26]. Recently there has been increasing concern with possible increased risk of CVD associated with thiazolidinediones [32].

At present there is insufficient evidence from randomised control trials to advocate attempting to achieve near normal blood glucose levels specifically for the prevention of CV disease in patients with type 2 DM. Furthermore in some circumstances tight glycaemic control may actually be harmful.

1.1.3.2 Hypertension

Hypertension is an important risk factor for CVD and is more prevalent in patients with type 2 DM. In the general population reducing blood pressure by an average of 12/6mmHg reduces the incidence of CAD by 20% with comparable reductions in patients with and without type 2 DM [33]. The relationship between blood pressure and incidence of macrovascular disease in patients with type 2 DM is stronger than the relationship with hyperglycaemia [34] Controlling blood pressure is therefore one of the cornerstones in preventing CVD in patients with type 2 DM. The majority of studies have shown blood pressure to be a continuous risk factor for CVD. With the lower the blood pressure the lower the cardiovascular risk and with no level of blood pressure which is not associated with further risk reduction[33;34]. When to introduce antihypertensive therapy is therefore a balance between benefit and adverse effects of treatment and dependant on overall cardiovascular risk. In high risk patients such as those with type 2 DM and established CVD lower blood pressure targets are therefore recommended [22-24]. There was until very recently little evidence from randomised controlled trials to support this strategy of intensive blood pressure lowering in patients with type 2 DM. The blood pressure arm of the ACCORD study aimed to address whether very tight blood pressure targets would reduce CVD in patients with type 2 DM[35]. The results of the ACCORD trial showed no benefit with targeting systolic blood pressure to less than 120mmHg compared to 140mmHg[35]. In addition, patients in the intensive group experienced more adverse events attributed to antihypertensive therapy [35]. The optimal blood pressure target for the reduction of cardiovascular events in patients with type

2 DM is therefore not clear however based on the evidence there appears to be little addition benefit from tighter blood pressure control.

Achieving blood pressure control appears to be the more important factor than choice of antihypertensive therapy. Meta analysis has failed to show any class of anti-hypertensive to be superior in preventing CVD [33]. There is however a disparity between the expected reduction in CVD with blood pressure lowering based on epidemiology evidence and the actual reduction achieved in randomised controlled trials [36]. Much of this evidence comes from early trials that used beta-blockers and diuretics raising the question whether newer agents would be superior. The Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT) suggested increased benefit from the combination of calcium channel blocker (CCB) with angiotensin converting enzyme inhibitor (ACEi) compared to β-blocker and diuretic in a population of patients with hypertension and at least 3 other cardiovascular risk factors including type 2 DM [37]. This difference may simply reflect slightly (although not significant) lower blood pressure in the CCB/ACEi group. Other possible explanations are improved metabolic profile or differences in central pressures with ACEi and CCB combination [37;38]. What is known is that ACEi and ARBs significantly reduce progression of kidney disease in patients with DM [39]. For this reason ACEi/ARBs are commonly used as first line antihypertensives in patients with type 2 DM.

1.1.3.3 Dyslipidaemia

Type 2 DM and other insulin resistant states are associated with a typical pattern of lipid abnormalities that is characterised by low HDL levels and elevated triglyceride levels LDL levels are comparable to those in the general population [40].

LDL cholesterol

Epidemiological studies have shown increasing prevalence of CVD with increasing levels of LDL [41]. Lowering cholesterol by any means, diet, drugs or even ileal bypass, significantly

reduces rates of cardiovascular disease [42;43]. The majority of the evidence for lowering LDL however comes from the numerous "statin" trials. HMG-COA reductase inhibitors or "statins" are a safe and well tolerated class of medication that lower cholesterol levels by inhibiting HMG-CoA reductase which is the rate limiting step in the production of cholesterol. The meta-analysis by the Cholesterol Trialists Collaboration, looked at 90 056 participants in 14 statin trials[44]. This analysis showed that the 5 year incidence of coronary events, revascularization and stroke was reduced by approximately 20% for every 1 mmol reduction in LDL. Importantly this reduction in cardiovascular disease was seen irrespective of starting levels of LDL with benefit continued to be seen in patients with normal and even low LDL levels. These results were seen both in patients with and without diabetes. Increasingly guidelines are therefore recommending ever lower targets for LDL levels in patients at high risk of CVD. This includes patients with established CVD and patients with type 2 DM (table 1.1).

Table 1.1. LDL targets for patients at high risk of CVD

Organisation	**Most recent LDL target**	**Previous LDL target**
JBS*	<2mmol/L (2005)	<3mmol/L (2000)
Joint Task Force**	<2mmol/L (2007)	<3mmol/L (2003)
AHA/ACC***	<1.8 (2006)	<2.6 (2001)

*JBS; Joint British Societies' guidelines on prevention of cardiovascular disease in clinical practice 2005 [22] 2000 [45] **Joint task force; Joint Task Force of European and other Societies European task force on cardiovascular disease prevention in clinical practice 2007 [24] 2003 [46]. ***AHA/ACC, American Heart Association, American College of Cardiology Guidelines for Secondary Prevention for Patients with coronary and Other Atherosclerotic Vascular Disease 2006 [23] 2001[47].

Levels of LDL are not higher in patients with type 2 DM compared to the general population. In the Framingham Heart Study the prevalence of high LDL levels in men and women with DM was 9% and 15% respectively compared to 11% and 16% in men and women without DM [40]. However as LDL is such a strong risk factor for CVD and statins are so efficient and well tolerated lowering LDL in patients with diabetes has been a major target for reducing CVD.

HDL cholesterol

Low HDL levels are a feature of the dyslipidemia associated with type 2 DM and other insulin resistant states. Low HDL levels are an important risk factor for CVD. This relationship was first identified in the 1970s through the Framingham heart study and a number of subsequent studies since [48-50]. HDL can be raised by lifestyle factors including weight loss, dietary manipulation and increased physical activity [51-53]. There is currently no pharmacological agent available that raises HDL alone. The cholesterol ester transfer protein (CETP) inhibitor torcetrapib which specifically raised HDL has been withdrawn due to increased cardiovascular events [54]. Statins have very minor effect on HDL levels[44]. Fibrates, thiazolidinediones and niacin can increase HDL to varying degrees but all also have a number of other effects [55-57]. In summary although low HDL is an important risk factor for CVD it is probably one that is not at present being adequately addressed in clinical practice.

1.1.3.4 Other strategies: lifestyle factors: obesity and physical activity

Lifestyle modification is an important part of the management of type 2 DM. Modifying oral intake and levels of physical activity are usually the initial steps recommended for improving glycaemic control. Obesity and levels of physical activity are also risk factors for CVD so important targets in the quest to reduce CVD [2;58].

Obesity

Obesity is defined by a body mass index (BMI) of 30kg/m^2 or greater[59]. Waist circumference is clinically the best method for detecting increased visceral fat[59]. The prevalence of obesity is increasing rapidly throughout the world [60]. Obesity, particularly central obesity is pivotal in the development of type 2 DM and is also an important risk factor for CVD [2;61]. Hypertension, dyslipidaemia and type 2 DM are all more prevalent in people who are obese [60].

The driving force behind the obesity epidemic is a combination of reduced physical activity and the greater availability of energy rich foods. This can be illustrated by the dramatic increases in prevalence of type 2 DM seen when rural communities migrate to cities and adopt western style diets [62;63]. It therefore follows that the management of obesity should focus on increasing physical activity and reducing energy consumption. Studies show that weight loss of only 5%-10% result in improvements in blood glucose levels, lipids and blood pressure[64]. Even modest weight reduction can be extremely difficult to achieve[65]. There is therefore interest in the development of pharmacological agents to augment the weight loss that can be achieved with lifestyle modification. To date pharmacological agents had little success with rimonabant and sibutramine both being withdrawn due to safety concerns[66;67]. The only pharmacological agent currently available specifically aimed at tackling obesity is orlistat. Orlistat impairs the absorption of fat and only has modest effects on weight loss[68]. Obesity is a cardiovascular risk factor that is probably not adequately addressed in the management of patients with type 2 DM.

Sedentary lifestyles

For over 50 years it has been recognised that sedentary lifestyles are an important risk factor for CVD. In 1953 the now classic epidemiological study by Morris et al.[58] showed that the conductors compared to bus drivers had lower rates of CAD. Furthermore when conductors were diagnosed with CAD it tended to be later in life with a lower mortality. This difference was

partly attributed to the conductors being more physically active at work compared to bus drivers. Since then numerous studies have confirmed a reduction in levels of CVD with increased levels of physical activity [69-71]. Physical activity reduces blood pressure increases HDL and reduces risk of type 2 DM, however there also appears to be an independent effect of physical activity [69].

For the prevention of CVD it is recommended that individuals take 30 minutes of regular physical activity most days of the week [22]. The 2008 Scottish health survey estimates only 39% of adults met these recommendations[72].

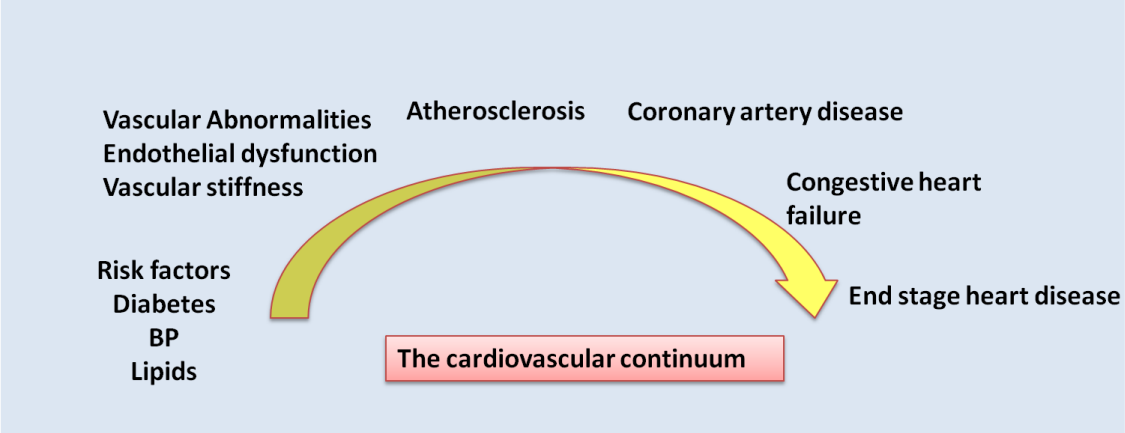
1.1.4 Summary

Type 2 DM is associated with increased risk of CVD. Tackling the traditional risk factors such as LDL and hypertension can partly reduce this risk. Hyperglycaemia is an important risk factor for CVD but current evidence does not support strict blood glucose lowering for the prevention of CVD. Lifestyle factors such as obesity and sedentary lifestyles are important risk factors for both type 2 DM and CVD and are therefore attractive targets although ones that are often difficult to modify. In view of these difficulties novel strategies for reducing CVD in patients with DM are required.

1.2 Mechanisms underlying CVD: the cardiovascular continuum

A greater understanding of the mechanisms underlying the development of CVD in patients with type 2 DM may help identify novel cardiovascular management strategies. CVD can be viewed as a continuum that spans from patients with cardiovascular risk factors, through vascular abnormalities such as endothelial dysfunction and arterial stiffness, to the development of atherosclerotic lesions and eventually established end stage disease (figure 1.1) [73]..

Figure 1.1 The cardiovascular continuum. (Adapted from Dzau et al. [73])

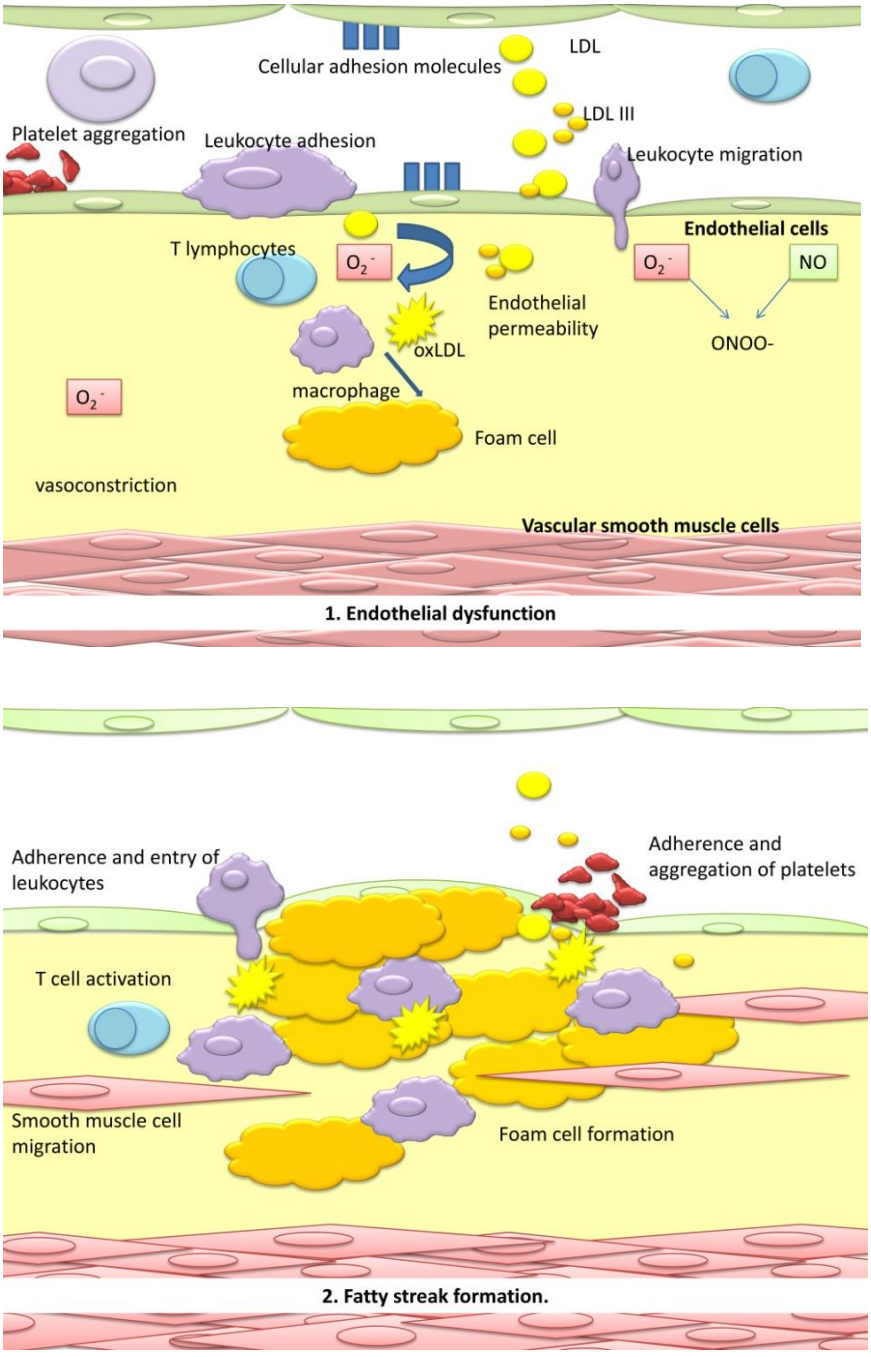


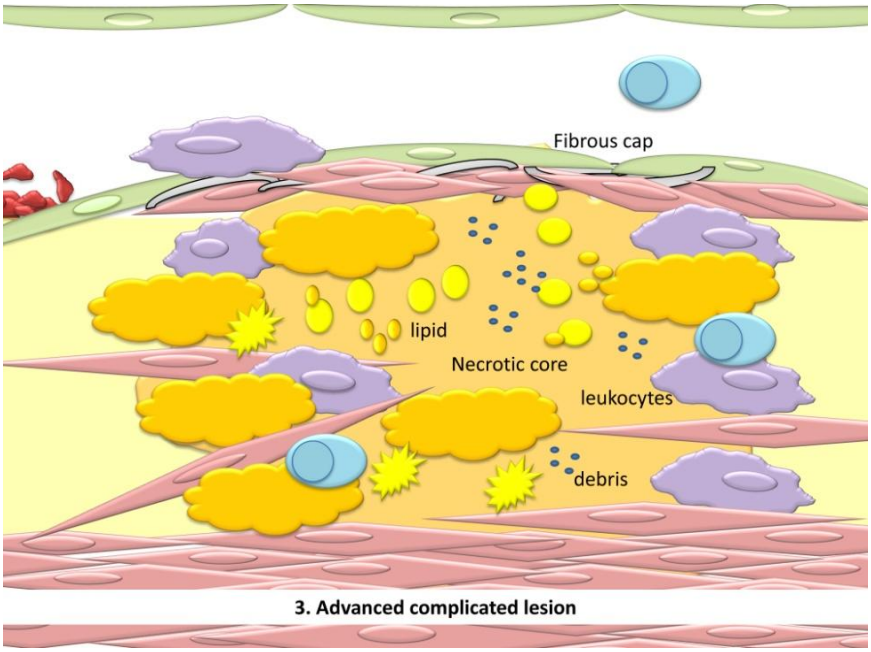
1.2.1 The atherosclerotic lesion

The underlying pathological abnormality in CVD is the atherosclerotic lesion. Atherosclerotic lesions occur in the arterial system predominantly at the sites of bifurcations. Atherosclerotic lesions develop slowly over many years, with the initial abnormality; fatty streaks developing in adolescence [74] . Extracellular deposition of lipids is followed by an inflammatory infiltrate of monocytes and T lymphocytes to form fatty streaks [74]. Monocytes develop in to macrophages and scavenge modified lipids to form foam cells [75]. The inflammatory infiltrate and foam cells secrete inflammatory mediators and produce reactive oxidative species resulting in the migration and proliferation of vascular smooth muscle cells[76]. This process continues with further lipid deposition, inflammatory infiltration and vascular smooth muscle cell proliferation. As the lesion develops focal necrosis occurs at the centre; attracting further inflammatory cells and formation of fibrous tissue[74]. The lesion is now termed a complex lesion consisting of a fibrous cap overlying a core of lipid and necrotic tissue. The lesion may continue to expand producing clinical symptoms secondary to gradual reduction in arterial flow. The plaque may also rupture with thrombus forming lending to acute occlusion of the vessel[74-77].

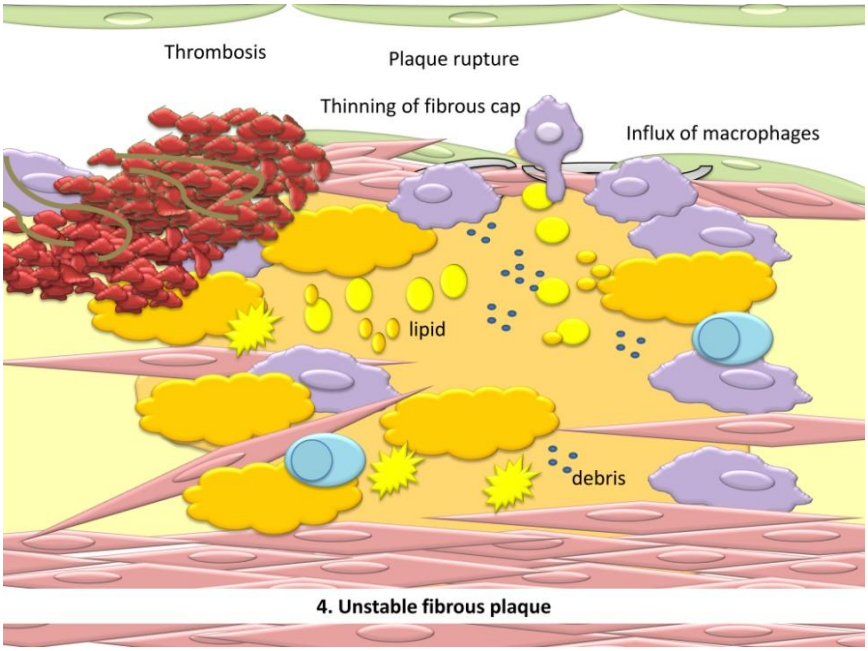
Multiple processes are involved in the initial development and progression of atherosclerotic plaques. Important processes in the development of atherosclerosis are endothelial dysfunction, oxidative stress, inflammation and increased arterial stiffness[74;76;78-80]. Cardiovascular risk factors such as dyslipidemia, hyperglycaemia and hypertension are understood to act through these processes to contribute to the development of atherosclerosis and CVD[78;79;81].

Figure 1.2 The atherosclerotic process (Adapted from Ross[74]).





3. Advanced complicated lesion



4. Unstable fibrous plaque

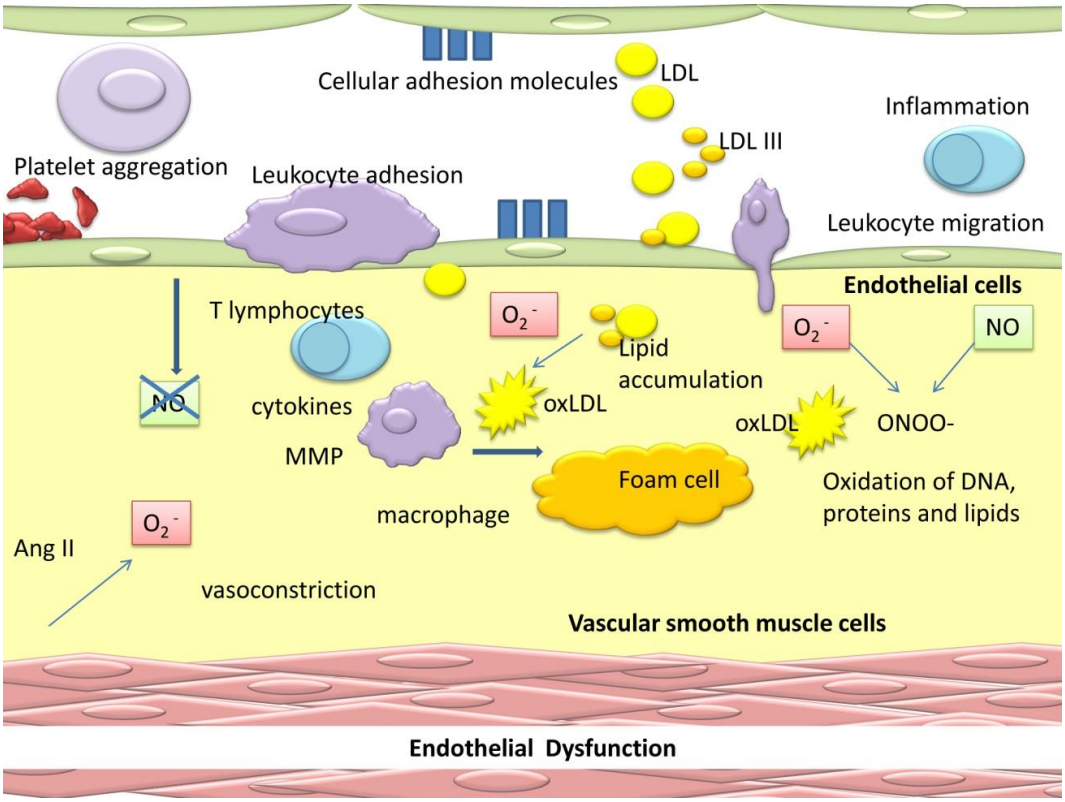
1.2.2 Endothelial dysfunction

Disruption of the normal endothelial function or endothelial dysfunction is one of the earliest steps in the development of atherosclerosis[82;83]. The basic structure of the human vasculature consists of adventitial tissue, vascular smooth muscle cells and finally the endothelium. The

endothelium is composed of a single layer of endothelial cells and is pivotal in maintaining normal vascular function. In health the endothelium maintains the balance between vasodilation and vasoconstriction, pro-thrombotic and anti-thrombotic, inflammatory and anti-inflammatory processes[82;83]. Production of nitric oxide (NO) is one of the key mediators of these processes. NO is produced by endothelial nitric oxide synthase (eNOS) through the conversion of L-arginine to L-citrulline[78]. NO is a potent vasodilator and the balance between production of NO and vasoconstrictors such as endothelin maintains vascular tone. In the vascular smooth muscle cells (VSMC) NO activates guanylate cyclase resulting in cGMP mediated vasodilation[78]. In addition NO reduces platelet adhesion, leukocyte migration and proliferation of VSMC[78;84]. In health these processes are balanced and produce a vascular phenotype that prevents the development of atherosclerosis.

Endothelial dysfunction describes a pathological state in which many of the normal functions of the endothelium are disrupted. Impairment of normal endothelial function therefore results in a complex array of abnormalities including disruption of normal vaso-regulation, increased adhesion and migration of leukocytes, platelet aggregation and vascular smooth muscle cell proliferation[83]. All of which as discussed are key steps in the development of atherosclerotic plaques. Reduced NO bioavailability is fundamental to the development of endothelial dysfunction[84]. The hallmark of endothelial dysfunction is therefore attenuated NO dependent vasodilation[85].

Figure 1.3 Endothelial dysfunction (Adapted from Hamilton et al.[79])



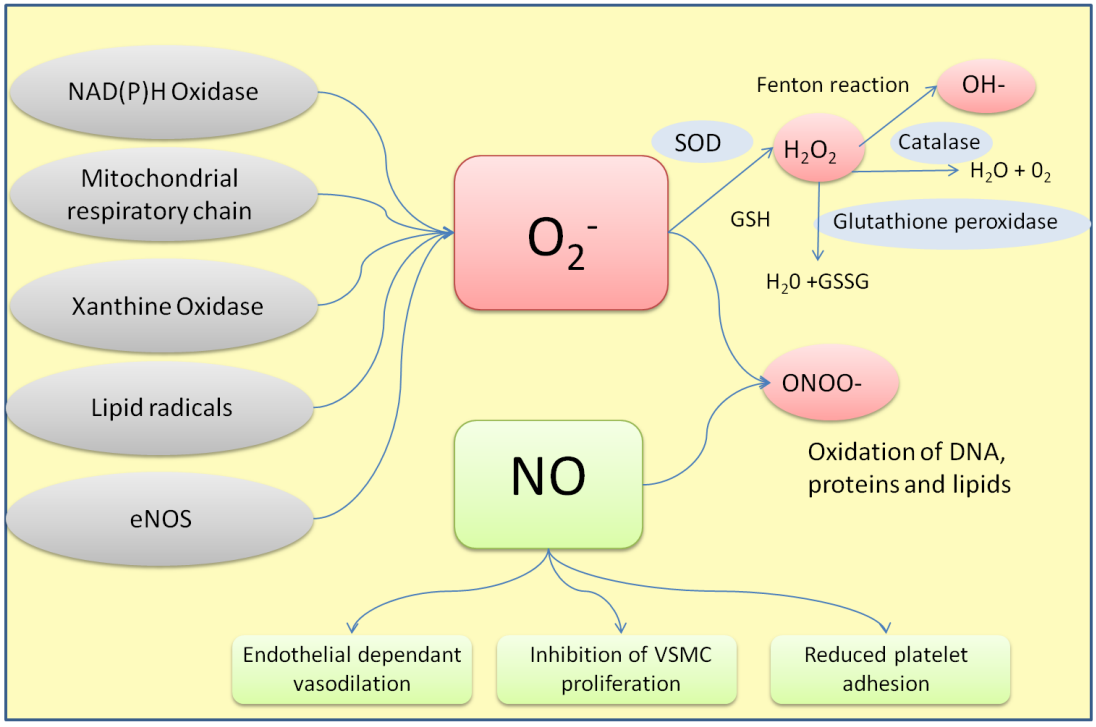
1.2.3 Oxidative stress

Reactive oxygen species (ROS) are a group of highly oxidative molecules and are produced in all cells. They include superoxide (O_2^-), hydroxyl radical ($HO^·$) hydrogen peroxide (H_2O_2), peroxynitrite ($ONOO^-$) and lipid radicals among others[84;86]. Of these O_2^- is one of the most important ROS. O_2^- is the principle ROS produced by the vasculature and inflammatory cells[86;87]. In addition O_2^- produces further ROS through chain reactions. For example, O_2^- rapidly scavenges NO to produce peroxynitrite[84] Not only does this reduce the bioavailability of NO but peroxynitrite itself is highly oxidative[84]. ROS are produced during normal cellular function and play a physiological role in intracellular signalling[88]. However due to the highly reactive nature of ROS complex antioxidant scavenging systems are in place to keep levels in balance. For example O_2^- is converted by superoxide dismutase to H_2O_2[88]. H_2O_2 is converted to water by

catalase and glutathione peroxidase[88]. Increased oxidative stress occurs when there is an imbalance between the production of ROS and the antioxidant systems that scavenge these molecules. Increased oxidative stress can therefore result from either increased production of ROS or impaired scavenging systems or a combination of both.

Increased oxidative stress and ROS exacerbate many of the proatherogenic processes underlying the development of CVD. Oxidative stress contributes to the development of endothelial dysfunction. ROS such as O_2^- react rapidly with NO[84;89]. Reduced bioavailability of NO results in impaired endothelial function manifested by loss of vasodilation, platelet aggregation and inflammation[90]. Increased levels of ROS also potentiate the formation of oxidized LDL a key substance in the formation and maintenance of atherosclerotic lesions[91]. Furthermore oxidized LDL can itself increase levels of oxidative stress [91]. ROS exacerbate inflammation in the vessel wall through the increased adhesion and migration of monocytes [92]. Increased oxidative stress can contribute to proliferation and migration of vascular smooth muscle cells[90].

Figure 1.4 Oxidative stress (Adapted from Hamilton et al.[79]).



1.2.4 Arterial stiffness

As part of the aging process vessels become increasingly stiff and this process can be accelerated by the presence of cardiovascular risk factors such as hypertension, hyperlipidaemia and diabetes[80;93]. The mechanical properties of arteries are complex however in simple terms normal arterial physiology protects the microcirculation from pressure-induced damage and ensures adequate perfusion of the coronary vessels during diastole[94]. Increased arterial stiffness can alter these normal processes and contribute to the development of cardiovascular disease.

Under normal conditions the aorta transforms the pulsatile on-off blood flow of the left ventricle in to less pulsatile flow in distal vessels and smooth non pulsatile flow in capillaries[94]. This system protects the microcirculation from damage due to high pressures and ensures the coronary arteries have sufficient blood flow in diastole to meet metabolic requirements of the heart. The ability of the aorta and proximal arteries to buffer the pulsatile flow during systole

depends in part on the compliance of these vessels[94]. The phenomenon of wave reflection also plays a role in normal arterial function. Changes in vessel diameter and stiffness occur as the arterial tree branches and tapers. This results in reflection of a proportion of the propagating pressure wave[95]. In ideal circumstances the reflected wave returns to the central aorta in diastole enhancing coronary perfusion. Furthermore wave reflection reduces the transmission of pulsatile energy to the microcirculation[96]. Increased arterial stiffness reduces compliance of aorta and proximal vessels. This results in a reduction in the buffering properties of these vessels and early return of the reflected wave[97]. Reflected waves arriving in late systole increase or augment central systolic pressure increasing left ventricular afterload and altering coronary artery perfusion[95]. These changes contribute to left ventricular hypertrophy, worsening of coronary ischaemia, and increased vessel wall stress with risk of atherosclerotic plaque rupture[96].

1.3 CAD and the role of surgical revascularisation

As discussed CVD encompasses stroke, peripheral vascular disease and CAD all of which have the same underlying pathogenesis. The focus of this study will be patients with CAD undergoing elective surgical revascularisation or coronary artery bypass grafting (CABG). Patients undergoing CABG are an attractive study group for a number of reasons. Firstly they represent a group of patients with severe atherosclerotic disease. Secondly they are well phenotyped with all patients having undergone thorough investigation including diagnostic angiography to confirm the diagnosis of severe CAD prior to surgery. Thirdly there is the opportunity to obtain samples of the by-pass grafts for *ex vivo* studies that are surplus to clinical requirements.

Revascularisation techniques, either percutaneous or surgical, for patients with established CAD have become an important and effective management option. For most patients with left main stem coronary artery lesions or triple vessel CAD surgical revascularisation is the treatment of choice[98]. Approximately 1600 patients undergo CABG in Scotland annually. In 2009, 45.7% of

patients undergoing CABG had triple vessel disease and 42.1% had left main stem lesions. Approximately 24% of these patients had diabetes[99]. Overall short and long term outcomes following CABG are good. In patients undergoing CABG in Scotland in patient mortality is 2.3%, with 10 year survival of 26.3%[99]. These rates are similar to those reported in other studies[100;101]. Both short and long term outcomes are worse in patients with diabetes compared to patients without diabetes[101-103]. CABG in patients with diabetes is however associated with improved long term survival compared to percutaneous intervention[101;103]. CABG is therefore the management of choice for patients with diabetes and multivessel CAD[104].

1.4 Study aims and objectives

Type 2 DM is associated with increased cardiovascular risk. Recent studies such as ACCORD and ADVANCE have highlighted some of the limitations with current cardiovascular prevention strategies for patients with type 2 DM. This study has three broad aims. By studying patients with severe CAD undergoing CABG this study firstly aims to investigate some of the mechanisms underlying cardiovascular disease and secondly to assess the impact of type 2 DM. Finally the effect of two CV prevention strategies on these mechanisms will be evaluated.

Chapter 3 focuses on the assessment of arterial stiffness. Arterial stiffness is understood to play an important role in the development of CVD. Clinical assessment of arterial stiffness may assist in the management and prevention of CVD. The hypothesis was that patients with CAD would have increased arterial stiffness compared to healthy controls. Secondly patients with CAD and type 2 DM would have increased arterial stiffness compared patients with CAD alone.

The aims of chapter 3 were therefore:

1. To use pulse wave velocity (PWV) and pulse wave analysis (PWA) techniques to assess indicators of arterial stiffness in patients with CAD compared to healthy controls.

2. To use PWV and PWA techniques to assess indicators of arterial stiffness in patients with CAD and type 2 DM compared to patients with CAD alone.
3. To investigate the use of simpler indicators of arterial stiffness such as augmentation index compared to aortic PWV (current gold standard for assessing arterial stiffness).

Since endothelial dysfunction is understood to be a pivotal process underlying the development of cardiovascular disease this is the focus of chapter 4. The hypothesis was to confirm that patients with CVD would have impaired endothelial function compared to healthy controls and secondly to confirm that patients with type 2 DM and CAD would have greater impairment of endothelial function compared to patients with CAD alone.

The aims of chapter 4 were therefore to:

1) Investigate endothelial function using *ex vivo* and *in vivo* techniques in patients with CAD compared to healthy controls
2) In patients with CAD assess the impact of type 2 DM on endothelial function.
3) To evaluate the use of a non-invasive PWA based technique for assessing endothelial function *in vivo*.

Chapter 5 focuses on oxidative stress as this is understood to be one of the major mechanisms underlying both endothelial function and CV disease. The hypothesis was that patients with CAD would have increased oxidative stress compared to healthy controls. Furthermore that patients with CAD and type 2 DM would have increased levels of oxidative stress compared to patients with CAD alone.

The aims of chapter 5 were therefore to:

1) Measure superoxide levels in vascular tissue, mononuclear cells and whole blood as an indicator of levels of oxidative stress.

2) Compare levels of oxidative stress in patients with CAD compared to healthy controls.
3) Measure levels of oxidative stress in patients with CAD and type 2 DM compared to patients with CAD alone
4) Evaluate the use of whole blood superoxide as a simple direct measure of reactive oxygen species and an indicator of levels of oxidative stress.

Chapter 6 aims to investigate whether dyslipidaemia in patients with type 2 DM and CAD might partially account for the impaired endothelial function found in patients with type 2 DM. The hypothesis was that patients with type 2 DM would have lower HDL levels and increased triglyceride levels and small dense LDL particles compared to patients without diabetes. Furthermore that these abnormalities may partially explain the endothelial dysfunction associated with type 2 DM described in chapter 4.

The aims of chapter 6 were therefore to assess triglyceride, HDL and LDL levels in patients with type 2 DM and CAD compared to patients with CAD alone. Furthermore to investigate LDL and HDL particle size in patients with CAD and type 2 DM compared to patients with CAD alone.

LDL reduction by statin therapy is a major strategy for management of CVD in patients with and without DM; this is the focus of chapter 7. LDL levels are an important risk factor for CVD and determinant of endothelial function. Over the years guidelines have recommended increasingly tight LDL targets. The hypothesis was that patients managed under these newer guidelines would have lower LDL levels, reduced oxidative stress and improved endothelial function compared to patients managed during older guidelines. Furthermore that this effect would be greater in patients with type 2 DM and CAD who are understood to have greater impairment of endothelial function and increased oxidative stress compared to patients without DM.

The aims of chapter 7 were to assess LDL levels, oxidative stress and endothelial function in patients with CAD undergoing CABG in 2007 compared to a group of patients investigated in 2003.

Levels of physical activity are low in the general population. Sedentary lifestyles are an important risk factor for CVD and type 2 DM. The focus of chapter 8 is therefore the effect of increased physical activity on processes underlying CVD. The hypothesis was that in patients who attended exercise classes as part of cardiac rehabilitation would have improved endothelial function and reduced oxidative stress compared to patients who elected not to attend exercise classes.

The aims of chapter 8 were firstly to assess endothelial function and oxidative stress in patients before and after 12 weeks of exercise classes that form part of the cardiac rehabilitation programme. Secondly to assess the impact of number of classes attended on these variables.

2 Methods

2.1 Participants

2.1.1 VASCAB study

The CAD group consisted of patients with stable coronary artery disease attending for elective CABG. Volunteers were recruited at preoperative assessment clinic at the Western Infirmary, Glasgow between October 2006 and February 2008. This clinic took place on a Wednesday afternoon and included patients from across the West of Scotland. Patients were recruited the week preceding by-pass surgery. Recruits were examined in the clinical research facility at the BHF Glasgow Cardiovascular Research Centre (BHF GCRC) the afternoon prior to surgery (the day of hospital admission). Patients were asked to avoid large meals for 3 hours prior to assessment and to avoid smoking. Patients were asked to take their medication as usual. Exclusion criteria were atrial fibrillation, co-existing valvular heart disease, type 1 DM and previous CABG. Classification of diabetes was made following case note review and history using the WHO guidelines for classification of diabetes[3]. This was done to ensure that patients treated with insulin only were not misclassified.

Two hundred and sixteen patients undergoing CABG were eligible for recruitment into the study. Thirty (14%) patients declined to be part of the study upon initial approach in the pre-operative clinic. Sixty (28%) further patients did not attend the initial appointment at the BHF GCRC. Cancellation of appointments was usually the consequence of either a change in the date or location for the patients operation.

The control group consisted of both patients attending for elective VV removal and participants recruited from local health clubs.

Control (or healthy) saphenous veins were obtained from patients attending for elective VV removal who were recruited from the vascular surgery unit at Gartnavel hospital during pre-

operative assessment by the surgical team. Exclusion criteria were previous history of CAD (including angina, myocardial infarction and CABG), type 1 or type 2 DM, hypertension requiring two or more antihypertensive agents and untreated hypercholesterolaemia. Patients were examined 8 weeks after surgery in clinical research facility at the BHF GCRC.

Patients undergoing surgery for VV tend to be younger with a higher proportion of females compared to CABG patients. In view of this a further group of healthy controls were recruited. This group were age and sex matched to the CABG patients and recruited from local health clubs in response to advertisements. Exclusion criteria were previous history of CAD (including angina, myocardial infarction and CABG), type 1 or type 2 DM, hypertension requiring two or more antihypertensive agents and untreated hypercholesterolaemia.

2.1.2 2003 cohort

The 2003 group consists of patients with CAD and controls attending for VV surgery that were studied between February 2003 and February 2004. Detailed history and clinical examination was taken between 1 and 7 days prior to surgery. The control groups were used to confirm consistency of vascular function and O_2^- studies between 2003 and 2007.

2.1.3 Exercise study

Patients attending cardiac rehabilitation following coronary artery bypass grafting were recruited. The rehabilitation assessment clinic was held in the Western Infirmary, Glasgow on Friday mornings and run by the cardiac rehabilitation nurses and physiotherapists. Patients were assessed in the clinic approximately 6 weeks after discharge following CABG. Detailed assessment of the patient's recovery from surgery was evaluated by the cardiac rehabilitation team. Exercise tolerance was then assessed by the physiotherapists using a validated step test or shuttle walk test [105;106]. Cardiac rehabilitation consists of a 10 week programme which includes twice weekly exercise classes. The exercise classes are adapted to an individual's exercise

capacity and consist of 15 minutes warning up and down and 30 minutes cardiovascular training. Patients are asked to work at 65-80% of age predicted maximal heart rate based on self assessment of pulse rate and use of the Borg scale (Borg's perceived exertion scale).

Seventy seven patients were eligible for recruitment in to the exercise study. Thirty four patients agreed to participate in the study.

Patients were assessed at BHF GCRC centre prior to commencing cardiac rehabilitation and then 12 weeks later. Patients attended the research centre in the morning following an overnight fast.

Assessment of physical activity and functional capacity

Levels of physical activity were assessed before and after cardiac rehabilitation programme using Scottish physical activity questionnaire (SPAQ see figure 10.2 in supplementary data). This is based on the more widely used Stanford 7 day recall questionnaire. In the SPAQ the language has been modified for use with a Scottish population and to enable the questionnaire to be self reported rather than interviewer based. SPAQ has been validated and shown to be a good estimate of levels of physical activity compared to objective measures using accelerometers [107]. Functional capacity before and after cardiac rehabilitation was assessed by physiotherapists using the shuttle walk test or chester step test[105;106].

2.2 Ethics

All studies were performed to comply with the declaration of Helsinki. All studies were approved by the local ethics committee and approval letters are enclosed in section 10. All participants gave informed written consent.

2.3 Clinical examination

All clinical studies were performed in the clinical research facility at the BHF GCRC except for the 2003 cohort. Details for the clinical examination in the 2003 cohort are provided separately below.

All participants completed questionnaires (see section 10.1 in supplementary data) providing detailed information on lifestyle and medical history. Blood pressure was measured according to current WHO guidelines. Participants were seated for 5 minutes prior to blood pressure measurement. The arm was supported at the level of the heart. Any tight clothing was removed. Blood pressure was measured three times using a calibrated oscillometric device (Omron HEM-750CP). The appropriate cuff size was used to ensure that the bladder encircled at least 80% of the arm. The first recording was discarded. The average of the second and third recordings was used in subsequent analysis.

Height was measured with the patient barefoot and to the nearest millimetre. Tanita Body Composition Analyzer BC-418MA (Tanita UK limited) was used to measure weight. Participants were weighed fully clothed with an appropriate clothing allowance subtracted (0.5kg for light summer clothes, 1kg for normal indoor wear). Waist and hip measurements were measured using an anthropometric tape measure. Waist circumference was taken as the smallest circumference around the abdomen with the participant relaxed. Hip circumference was measured as the largest measured circumference at the level of the buttocks.

In the 2003 cohort detailed history and clinical examination was taken between 1 and 7 days prior to surgery.

2.4 Routine biochemistry

Venous blood samples were taken using Vacutainer® system. Plasma samples were centrifuged and stored at -70^{o} C. Cholesterol, triglyceride, HDL and high-sensitivity C-reactive protein

(CRP) assays were performed by the staff (Professor Naved Sattar, Dr Lynne Cherry and Miss Christine Gourlay) of the routine lipids section of Glasgow Royal Infirmary. Cholesterol and triglycerides were measured using enzymatic colorimetric methods in Roche/Hitachi modular P analyser. Very low density lipoprotein (VLDL) and LDL cholesterol results were calculated using Friedewald equation (LDL= [total cholesterol]-[HDL]-[Triglyceride]/2.19). VLDL= [total cholesterol-LDL]. CRP was measured using particle-enhanced immunoturbidimetric assay (Roche diagnostics).

Glycated haemoglobin (HbA_{1c}) and urinary albumin:creatinine ratio (ACR) were assessed by a latex immunoagglutination inhibition method using the Bayer DCA 2000® (Bayer Diagnostics) Venous blood glucose was obtained using Accu-Chek® Advantage (Roche Diagnostics).

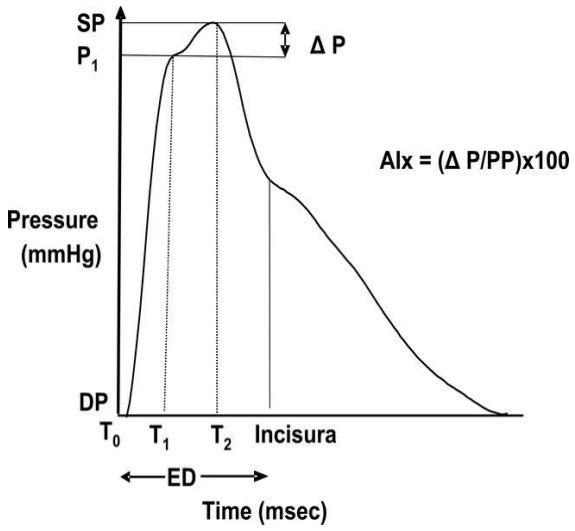
2.5 Pulse wave analysis

Pulse wave analysis (PWA) was performed using the SphygmoCor® device (AtCor medical). This system uses a tonometer connected to an electronics module to non-invasively record a peripheral pressure waveform. All PWA measurements were performed at the right radial artery. The wrist was slightly dorsiflexed and supported, the maximal pulsation of the radial artery was located. The tonometer was then placed over the site of maximal pulsation and gentle pressure applied. The position of the tonometer was adjusted until good quality pressure pulse tracings were obtained. Ten seconds of good quality pulse pressure tracings were then captured using the SphygmoCor® software.

The shape of the tracing was inspected. The shape will change according to the age of the patient and cardiovascular risk factors; however the basic features should remain constant (figure 2.1). The following were checked in all tracings; an initial sharp upstroke rising to an initial peak (T1) followed by a second shoulder (T2) and finally a notch marking closure of the

aortic valve. Waveforms lacking these features usually reflect incorrect placement of the tonometer and were therefore repeated following repositioning.

Figure 2.1 Typical Pulse wave tracing (from Williams *et al.*[38]).



T0, time at the start of the waveform. T1, time from start of waveform to first peak. T2, time from start of waveform to second peak. ED ejection duration i.e. time form start of waveform to closure of aortic valve (incisura) SP, systolic pressure. DP, diastolic pressure, PP pulse pressure (SP-DP). P1, difference between the minimum pressure and the pressure at T1. Δ P, Augmented pressure, difference between SP and P1. AIx ,Augmentation index, Augmented pressure divided by PP expressed as a percentage.

As well as the shape the recording was checked for quality. Quality control indices check pulse wave variables are within limits set using the configuration settings and include average pulse height, pulse height variation, diastolic variation. The quality index is an indicator of the overall quality of the captured data and was calculated by the software by assigning a weighting to each of the quality control indices. Recordings with a quality index of 80% or greater were accepted. Overlaid recorded data was also checked. This area displays a visual guide to how well individual pulses can be overlaid to form an average pulse. This is visually checked to ensure little variability. Baseline PWA recordings were made in duplicate.

For each patient where the PWA tracings were of an acceptable quality the following variables from the aortic pressure waveforms were recorded; Time to the peak/shoulder of the first and second pressure wave components (T1, T2). The pressure at T1 (P1 height). The pressure difference between P1 and the maximal pressure during systole (ΔP or augmentation). AIx; augmented pressure as a percentage of pulse pressure (ΔP/PP) x100. Time to reflected wave (TR) and ejection duration time from start of waveform to closure of aortic valve.

2.6 Pulse wave velocity

Pulse wave velocity (PWV) was measured non-invasively using SphygmoCor® device (AtCor medical, model SCOR_Vx, NSW, Australia). The system uses a high-fidelity tonometer (SPC-301, Millar Instruments) and ECG leads connected to an electronics module to non-invasively record a peripheral artery pressure and ECG waveforms. PWV is determined from the distance between artery sites and the delay between the R wave in the ECG and the sharp systolic up-stroke of the pulse wave.

Measurements were recording in a quiet room at 20^{o}C. Participants were asked not to smoke or take caffeinated drinks for 3 hours prior to study. Participants were rested for 10minutes supine prior to recording commencing. Participants were asked to lie still and refrain from talking during the study. The ECG leads were connected and ECG tracing inspected to confirm sinus rhythm. Supine blood pressure was measured using a calibrated oscillometric device (Omron HEM-750CP).

2.6.1 Brachial PWV

The participant's wrist was slightly dorsiflexed and the radial pulse located. The distance from the maximal pulsation of the radial artery to the suprasternal notch was measured. The patient was asked to slightly extend their neck and the carotid pulse was located. The distance from the

most prominent pulsation of the carotid artery to the suprasternal notch was recorded. All measurements were in millimetres (mm) to the nearest mm.

Pulse pressure wave forms were initially recorded at the radial artery in the right arm using the tonometer. Once again the participant's wrist was slightly dorsiflexed and the maximum pulsation of the radial artery located. The tonometer was placed over the maximal pulsation of the radial artery and the position adjusted until a good quality waveform was obtained. Pulse pressure recordings were then obtained for the carotid artery. With the patients neck slightly extended maximal pulsation of the carotid artery was located and pulse pressure tracings recorded. Capture time was set at 10 seconds. The Intersecting tangent method was used to identify the onset of the pressure pulse. This algorithm uses the point formed by the intersection of a line tangent to the initial systolic upstroke of the pressure waveform and a horizontal line through the minimum point. Brachial PWV were recorded in duplicate.

2.6.2 Aortic PWV

The above method was then employed to obtain aortic PWV readings. The distance from the maximal pulsation of the femoral artery to the umbilicus and then to the suprasternal notch was recorded. Maximum pulsation of the femoral artery was located and pressure tracings recorded. Pressure tracings were then captured from the carotid artery. Aortic PWV readings were also recorded in duplicate.

2.7 Assessment of endothelial function

2.7.1 Ex vivo assessment of endothelial function; organ bath studies

Residual segments of saphenous veins were obtained during CABG surgery of patients with CAD. Control saphenous veins were obtained from patients undergoing elective VV surgery. In the operating theatre vessels were immediately stored in sterile saline solution before being transferred to the research laboratories at BHF GCRC. On arrival the vessels were then

transferred into Krebs HEPES solution (10mmol) and stored under refrigeration until the following day. Directly prior to the experiment, vessels were cleaned of adherent fat and connective tissue, and then sliced into rings of 2-3mm length. The rings were transferred onto metal hooks and suspended in organ chambers filled with 10ml oxygenated Krebs buffer solution (37°; 7.4 pH), containing indomethacin (0.02 mmol/L) to inhibit prostanoid-mediated vasoactive effects. The isometric tension studies were performed using a Grass FT03 force transducer and displayed using a MacLab dedicated computer.

After stabilisation at a resting tension for approximately 1 hour, the vessels were activated with potassium chloride (KCl) 0.1M. Vessels were then washed out for 30 minutes and the KCl repeated. After activation, the vessels were washed out and allowed to rest for 30 minutes before any vasoactive agents were added. Resting tension was adjusted to 1g.

To assess endothelium dependent vasodilation the vessels were pre-contracted with phenylephrine $3x10^{-6}$M before measurement of relaxation to calcium ionophore A23187. The dose-response curve to calcium ionophore was then carried out from concentrations 10^{-8} to 10^{-5}M. Calcium ionophore stimulates the synthesis and release of nitric oxide by increasing intracellular calcium, and is therefore an endothelium dependent vasodilator.

To assess endothelium independent vasodilation a dose-response curve to sodium nitroprusside (10^{-8} to $3x10^{-6}$M; David Bull Laboratories, UK) was carried out. Vessels were preconstricted with phenylephrine $3x10^{-6}$M before measurement of relaxation to sodium nitroprusside (SNP). SNP acts as a NO donor therefore causing endothelium independent vasodilation.

Endothelial function studies in both the 2003 cohort and the VASCAB study (2007 cohort) were performed under the supervision of the same experienced researcher Dr Carlene Hamilton.

2.7.2 *In vivo* assessment of endothelial function

In vivo assessment of endothelial function was performed using the methods described by Wilkinson et al.[108] and Hayward et al.[109]. This method uses PWA to assess change in AIx following administration of salbutamol (endothelial dependent vasodilation) and GTN (endothelial independent vasodilation). Within observer variability of AIx measurement was assessed in 10 healthy volunteers. AIx was measured twice on day 1 and then measurements repeated 24-48 hours later, to check for validity of repeated AIx measurements.

Measurements were recording in a quiet room at 20^{o}C. Participants were rested for 10 minutes supine prior to the recording commencing. Participants were asked to remain still and to refrain from talking throughout the test. Supine blood pressure was measured using a calibrated oscillometric device (Omron HEM-750CP).

Basal PWA recordings were made in triplicate according to the methods described in section 2.1.4. Following the capture of basal PWA recordings 400µg of inhaled salbutamol (Salamol®; IVAX, UK) was administered via volumatic spacer device (Allen and Hanburys, Middlesex, UK). Salbutamol was administered via the use of a spacer device with assistance from the researcher to ensure reliable drug delivery as inhaler devices can be difficult to use without practice.

Salbutamol causes reduced pulse wave reflection[110]. These effects appear to be due to endothelial dependent NO release as they are ameliorated with the infusion of the eNOS inhibitor L-NMMA[111]. It is likely that salbutamol simulates NO release through the L-arginine NO pathway[111].

PWA recordings were obtained in triplicate at 5, 10, 15 and 20 minutes following salbutamol. These timings are based on the findings by Wilkinson et al.[108] that the maximal change in AIx to

salbutamol occurred at 12±3 and 11±3 minutes in patients with hypercholesterolaemia and healthy controls respectively.

The average peripheral AIx was calculated at each time point. Peripheral AIx was used since it was felt to be preferable to use the raw readings rather than central recordings calculated using a generalised transfer function. Change in peripheral AIx from baseline was calculated for each time point. The maximum change in peripheral AIx from baseline was used as a measure of endothelium dependent vasodilation.

Following salbutamol administration participants were then rested for 5 minutes. A further 3 PWA recordings were performed. 400µl sublingual GTN (Nitrolingual®, Lipha, UK) was administered. GTN is an NO donor resulting in endothelium independent vasodilation [112]. PWA was recorded in triplicate 5 minutes later. The change in peripheral AIx from baseline was then calculated. This was used as a measure of endothelial independent vasodilatation.

2.8 Oxidative stress studies

2.8.1 Vascular O_2^- measurement

Vascular O_2^- studies in both 2003 and VASCAB study (2007) group were performed under the supervision of the same experienced researcher Dr Carlene Hamilton.

Residual segments of saphenous veins were obtained during CABG surgery of patients with CAD. Control saphenous veins were obtained from patients undergoing elective VV surgery. In the operating theatre vessels were immediately stored in sterile saline solution before being transferred to the research laboratories at BHF GCRC. On arrival the vessels were then transferred into Krebs HEPES solution (10mmol) and stored under refrigeration until the following day.

The following morning blood vessels were carefully dissected free of loose connective tissue, divided into 3-4 mm segments, and weighed. The vessels were then incubated in Krebs buffer at pH 7.4±0.02.

Vascular·O_2^- was measured by lucigenin-enhanced chemiluminescence. Lucigenin-enhanced chemiluminescence is the most commonly used chemiluminescence technique for the detection of O_2^-[113]. When O_2^- reacts with lucigenin a series of reactions occur culminating in the release of a photon. The photon release can then be detected using a scintillation counter. Lucigenin based techniques are specific for O_2^- and have been widely used. One of the major concerns is that lucigenin can itself react with oxygen to produce O_2^- and therefore levels may be overestimated. This phenomenon can be partly overcome by using low doses of lucigenin [114]. Vascular O_2^- was measured in a liquid scintillation counter (Hewlett Packard model Tricarb 2100TR) in out of coincidence mode with a single active photomultiplier tube. Low dose lucigenin (5µmol/L) was used as described by Berry et al.[114]. Counts were obtained at 2 minute intervals and quantified with a xanthine/xanthine oxidase calibration curve for·O_2^- generation. Results were reported as pmol min^{-1} mg $tissue^{-1}$. In all experiments, O_2^- production was measured in paired samples.

2.8.2 Electron paramagnetic resonance spectroscopy studies

Electron paramagnetic resonance (EPR) spectroscopy measures the absorption of microwave radiation by molecules such as free radicals following stimulation by an electromagnetic field. The amplitude of the EPR signal is proportional to the numbers of free radicals in the sample. Interactions between the nuclei and unpaired electrons contribute to the magnetic field applied by the spectrometer. This results in several peaks being detected by the spectrometer. Since most ROS are short lived, compounds that form stable adducts with free radicals have been developed. These compounds are specific to one ROS species and produce a characteristic EPR spectrum[113;115].

2.8.2.1 Mononuclear cell O_2^- measurement

Venous blood was collected using the vacutainer® system in to ethylenediaminetetraacetic acid (EDTA) tubes. Samples were kept at room temperature and processing begun within 30 minutes of venesection. Total white cell count and differential white cell count were obtained using coulter®A^c·T diff 2^{TM} Analyser (Beckman coulter).

Mononuclear cells were isolated from venous blood using a Ficoll gradient as described by Fortuno et al.[116]. Ten ml of venous blood was diluted with 10ml of 0.9% saline. This diluted blood was carefully layered on top of 10ml of lymphoprep^{TM} The sample was centrifuged for 25minutes at 20^{o}C and 800g so as to produce a buffy coat of mononuclear cells between the plasma and erythrocytes. The buffy coat was carefully removed and 50ml of phosphate buffered saline (PBS) added. The isolated mononuclear cells were washed twice in PBS, through centrifugion for 10min at room temperature and 300g. Finally the pellet containing the isolated mononuclear cells was re-suspended in 1ml of PBS. For the duration of the study the isolated mononuclear cells were kept under soft agitation at 37^{o}C using a thermomixer to prevent cell aggregation. Total white cell count and differential white cell count were again obtained using coulter®A^c·T diff 2^{TM} Analyser (Beckman coulter). The isolated white cells were then diluted to give a final concentration of $5x10^6$ cells/ml.

Mononuclear O_2^- levels were detected by electron paramagnetic resonance (Bruker BioSpin e-scan R, Bruker Corporation) with the spin probe 1-Hydroxy-3-carboxy- 2,2,5,5-tetramethylpyrrolidine (CPH, Noxygen). CPH reacts with O_2^- to produce a stable nitroxide radical with half life of several hours[115].

Basal mononuclear cell O_2^- levels were recorded initially. 3µl of CPH (final concentration 500uM) and 2µl of PBS were added to 55µl of the isolated mononuclear cells. The sample was mixed and immediately transferred to a thin glass capillary and placed in EPR machine.

Instrument settings used were: centre field of 3375G, modulation amplitude of 2.27G, sweep time of 5.24s, sweep width of 60G and 10 scans. Counts were recorded once a minute for 10 minutes and the rate of O_2^- anion production (counts/minute) was calculated by means of a standard curve. Basal O_2^- levels were recorded in duplicate and the mean of recordings taken.

Stimulated O_2^- release was measured using the protein kinase C activator Phorbol 12-myristate 13-acetate (PMA Sigma-Aldrich Company Ltd). PMA causes NAD(P)H oxidase-dependant O_2^- release from mononuclear cells [116]. 3µl of CPH and 2µl of PMA (Final concentration 3.2uM) was added to 55µl of isolated mononuclear cells. The sample was mixed and immediately transferred to glass capillary for EPR O_2^- measurement. Stimulated O_2^- release was recorded in duplicate and the mean of recordings taken. To adjust for slight variations in capillary diameter, a note was made of capillary batch number used and result adjusted against standard. Intra assay variability, assessed in 3 independent experiments, performing 5 measurements within 90 minutes after blood collection, was 10%. Inter assay variability, as analyzed in 7 independent experiments performing 3 measurements, was 10%.

2.8.2.2 Whole blood O_2^- measurement

Venous blood was collected via the Vacutainer® system in to lithium-heparin tubes and placed immediately on ice. Samples were analysed within 30 minutes of venesection. 475µl of whole blood was added to 25µl of CPH (final concentration 500uM). Sample was transferred to a glass capillary and placed in EPR machine for O_2^- measurement. Machine settings were: centre field of 3375G, modulation amplitude of 2.27G; sweep time of 5.24s; sweep width of 60G and 10 scans. Whole blood O_2^- measurements were recorded in duplicate and the mean of readings taken. To adjust for slight variations in capillary diameter, a note was made of capillary batch number used and result adjusted against standard.

2.9 Detailed lipid profiles

LDL and HDL size and relative proportions in plasma were determined using non-denaturing gradient gel electrophoresis using 2-16% polyacrilamide gels (Alamo Gels, San Antonio, TX) as previously described [117]. Using in house standards prepared by density gradient ultracentrifugation LDL was divided in to three fractions; LDL I (28.92-31.38nm), LDL II (25.88-27.06nm) and LDL III (24.5-25.5nm). The gels were stained with comassie blue and scanned using an imaging densitometer (Model GS-700, Biorad, Hemel Hempstead). LDL subfraction is reported as a percentage of total LDL. LDL peak particle diameter (PPD) was reported as the size of the major LDL subfraction. HDL mean particle diameter (MPD) and LDL-MPD were calculated to give the mean diameter across the entire HDL or LDL profile.

2.10 Statistical analysis

Statistical analyses were performed using SPSS (version 15; SPSS Inc.,Chicago,IL,USA) software. In text and tables, data are expressed as mean ± standard deviation or median [interquartile range] as appropriate. Normal distribution of data was examined by the Kolmogorov-Smirnov test and by visual inspection of Q-Q plots. Unpaired Student's *t*-tests were performed for comparison of normally distributed data. Wilcoxon test was used for comparison of data that were not normally distributed. Fisher's exact test was used for comparison of categorical data. 95% confidence intervals were calculated for the difference between means where appropriate. Paired *t*-tests were used for repeated measurements in the exercise study. Pearson's correlation coefficients were been calculated where indicated. Multiple linear regression analysis was used to investigate determinants of endothelial function in chapters 4 and 6. In the full model all variables were forced into the model. In the stepwise model variables with a significance of ≥0.1 were removed. A *P*-value of less than 0.05 (two-sided) was considered significant.

3 Arterial Stiffness

3.1 Introduction

Arterial stiffness refers to important changes in the mechanical properties of arteries that contribute to the development of cardiovascular disease (CVD). The ability to assess these changes is of interest to help stratify cardiovascular risk and as a therapeutic target.

3.1.1 Mechanisms underlying increased arterial stiffness

As the structure of the vasculature changes the mechanisms underlying increased arterial stiffness alter. In the walls of the aorta and large arteries varying amounts of elastin and collagen are the main determinants of compliance[94]. Alterations in the proportion and composition of elastin and collagen that occur with aging and disease contribute to increased arterial stiffness in these vessels [93;118]. In medium size conduit arteries and the microcirculation vascular smooth muscle quantity and function is important in determining the compliance and calibre of vessels [94]. Endothelial function in these vessels therefore may contribute to arterial stiffness [119].

3.1.2 Assessing arterial stiffness

Arterial stiffness is a concept, a descriptive term, which cannot be absolutely quantified by one single measure. There are a number of different variables that can be measured as indicators of degree of arterial stiffening. As discussed the causes and underlying mechanisms of arterial stiffness vary according to the vessel being studied and this needs to be taken into account when interpreting studies. Incorporating simple non-invasive methods of assessing arterial stiffness into clinical practice is of interest for quantifying cardiac risk and making arterial stiffness a therapeutic target. Indeed the 2007 European Society of Hypertension guidelines recommend the use of arterial stiffness measures, aortic pulse wave velocity (PWV), in the assessment of patients with hypertension [120]. The guidelines do however acknowledge that methods for assessing arterial stiffness are currently not widely available outside the research setting. For assessment of arterial stiffness to become a routine part of clinical practice tests need to be non-

invasive and easy to perform. Furthermore the tests need to provide valuable prognostic information independent of currently available risk factors. The use of PWV, augmentation index (AIx) and central pulse pressure (PP) will be discussed below as methods for assessing aspects of arterial stiffness in clinical practice.

3.1.3 PWV

3.1.3.1 Basic principles and measurement of PWV

The speed at which a pulse wave travels in a vessel is related to distensibility of the vessel, the stiffer the vessel the faster the pulse wave will travel. The velocity or speed off a pulse wave is therefore an indicator of arterial stiffness. PWV is obtained by measuring the time for a pulse wave to travel a specified distance. In the SphygmoCor® system the pulse wave is captured noninvasively using applantation tonometry at a proximal site and a distal site. Time to the foot of the wave is measured and gated against the R wave in an ECG recorded simultaneously. The distance is then measured and the PWV in m/s can be calculated. Aortic PWV is measured from carotid to femoral artery. Peripheral PWV can be measured from carotid to radial or brachial arteries or femoral to dorsalis pedis or posterior tibial arteries. Aortic PWV has recently been recognised as the gold standard measurement of arterial stiffness [121]. The utility of peripheral measurement of PWV is currently uncertain [121].

3.1.3.2 Aortic PWV and CVD

Age and hypertension are the most important determinants of aortic PWV [122]. Aortic PWV is increased in patients with cardiovascular risk factors and established CVD [123]. Other factors associated with increased aortic PWV include hypercholesterolaemia, diabetes mellitus (DM), endothelial dysfunction and sedentary lifestyles [124-128]. However the relative contribution of these other factors in determining aortic PWV is unclear. Indeed a recent meta-analysis has

suggested that other cardiovascular risk factors in addition to age and hypertension only make a small contribution to aortic PWV [122].

As discussed the compliance of large vessels such as aorta is principally determined by elastin and collagen. Aging is associated with a reduction in both function and quantities of elastin in the walls of the aorta [93]. It is thought that cardiovascular risk factors such as hypertension and DM accelerate this process; however the underlying mechanisms are not fully understood. Mechanisms thought to be involved include Angiotensin II stimulated collagen formation, advanced glycation end products (AGE) and inflammation [80]. Acute changes in blood pressure can also affect aortic stiffness. As blood pressure increases the aorta becomes stiffer due to recruitment of collagen fibres [118]. Short term changes in blood pressure therefore need to be taken into consideration when interpreting studies assessing PWV.

Aortic PWV is a predictor of future cardiovascular events in a number of different patient groups including patients with end stage renal failure, essential hypertension and type 2 DM [129-132]. Strategies to reduce aortic PWV are therefore a possible therapeutic target in the management and prevention of CVD.

3.1.3.3 Strategies to reduce PWV

There are limited studies assessing the effect of interventions on aortic PWV and no long term outcome studies. Endurance trained male athletes have lower aortic PWV compared to less active aged matched men [126] The effects of exercise training programmes on aortic PWV are however not known. The effect of antihypertensive agents on aortic PWV is also unclear. McKenzie et al.[133] showed no effect on aortic PWV with either angiotenisn-converting enzyme inhibitors (ACEi), diuretics, beta blockers or calcium channel blockers (CCB), however this was a short term study. In patients with type 2 DM Angiotensin-receptor blockers (ARBs) decreased aortic PWV more than CCB and this effect was independent of blood pressure lowering [134].

Novel agents such as inhibitors of AGE formation and agents that break down AGE cross links are of interest although development of these agents has been slow [135].

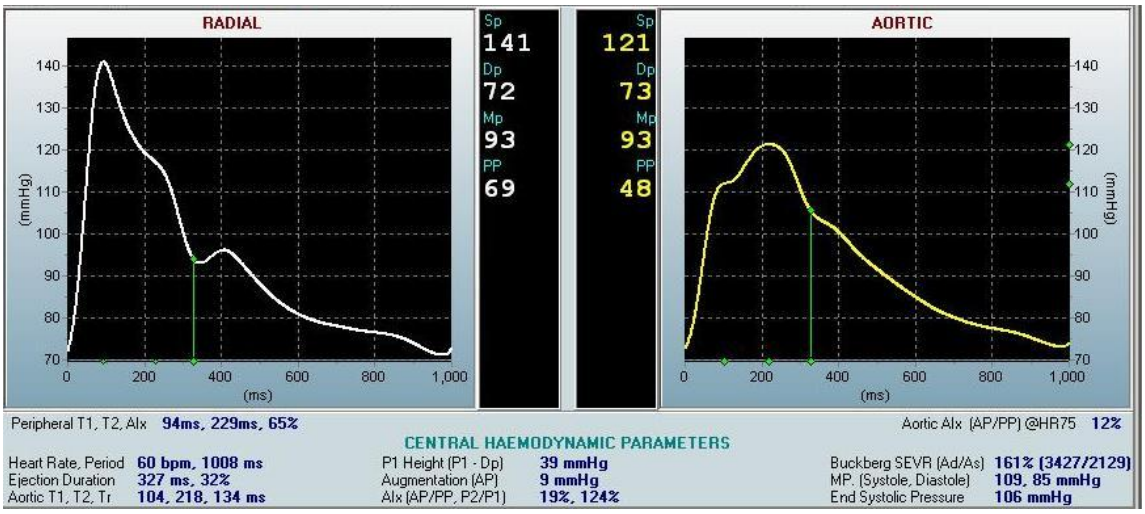
3.1.3.4 Limitations of aortic PWV

Aortic PWV is considered the gold standard for assessment of arterial stiffness and is recommended in 2007 ESH guidelines for the assessment of patients with hypertension [121]. Aortic PWV is in theory easy to perform but there can be a number of difficulties in certain patient groups [121]. The accuracy of the PWV result is dependent on the accuracy of the distances measured [136]. In the calculation of aortic PWV this can be difficult for a number of reasons. Measuring the distances between the femoral pulsation and the sternum may be inaccurate particularly in obese patients. In addition the surface measurement that aim to estimate the length of the aorta may be inaccurate in more elderly individuals with tortuous aortas. Obtaining pulse wave recordings at the femoral artery may also be difficult particularly in overweight patients. They are also more intrusive due to the intimate nature of recording femoral waveforms. Simpler methods for assessing arterial stiffness are therefore attractive. It is possible to calculate brachial PWV by recording the pulse wave at the radial and carotid artery. Certainly this would resolve a number of problems seen with aortic PWV, as the distance measurements are simpler and recording pulse wave at the radial less technically challenging compared to recording femoral waveforms. However in patients with end stage renal failure PWV in peripheral arteries had no prognostic value [137]. In addition it is not clear whether brachial PWV correlates well with aortic PWV. Cameron found that only Aortic PWV and not brachial PWV correlated with age in patients with type 2 DM [138]. It is possible that since the brachial artery is not affected by CVD to the same degree as the aorta, PWV measurements in this artery do not reflect the degree of CVD. Finally PWV gives information only on the speed of pulse wave travel and no information on the consequences of this, i.e. no indication of changes in wave reflection/central pressures [118].

3.1.4 Pulse wave analysis

In addition to measuring the speed of flow in vessels the shape of the pulse wave can give information on vascular function and degree of arterial stiffness [97]. Pulse wave analysis (PWA) can be performed through applantation tonometry at the radial artery. A transfer factor can then be applied to generate the aortic or central pulse wave [139]. Alternately waveforms can be captured directly at the carotid artery without the need for a transfer function. A number of parameters can then be calculated including central blood pressure measurements and AIx [97].A typical pulse wave tracing using the SphygmoCor® system is shown in figure 3.1.

Figure 3.1 Typical pulse wave tracing produced by the SphymoCor® system



Sp, systolic pressure, Dp diastolic pressure, Mp, mean arterial pressure, PP, pulse pressure.ms, milliseconds. T1 time from start of waveform to first peak. T2 time from start of waveform to second peak. TR time to start of reflected wave. P1, difference between the minimum pressure and the pressure at T1 AP, Augmentation difference between central Sp and P1. AIx augmentation index (AP pressure divided by PP expressed as a percentage).SEVR, subendocardial viability ratio.

3.1.4.1 AIx

A number of variables can be obtained from analysis of the waveform however the AIx has been proposed as a single reading that provides an assessment of wave reflection and a surrogate marker of arterial stiffness [97].

3.1.4.2 Basic principles and assessment of AIx

AIx is defined as the difference between the second and the first systolic peaks expressed as a percentage of the pulse pressure [97]. In other words AIx is the percentage of the systolic pressure wave attributable to wave reflection. AIx depends on the shape of the forward wave and the timing of the reflected wave [118]. The shape of the forward wave is determined by left ventricular outflow and elasticity of aorta [118]. The timing of the reflected wave is influenced by a number of factors including gender, height, reflected wave amplitude and vessel stiffness [96]. So although AIx is determined in part by arterial stiffness other factors are also important. AIx has been advocated by some to be suitable as a surrogate marker for arterial stiffness [140]. Whereas others advise that AIx has a role in complementing PWV recordings to provide information on the consequences of increased PWV on wave reflections and central blood pressures[121]. The use of AIx has been much debated and its usefulness in the clinical assessment of cardiovascular risk is far from certain [141].

Central blood pressure readings and AIx can be obtained directly from the carotid artery or from reading taken at the radial artery with central recordings calculated using a transfer function [139;142]. Recording good quality waveforms at the carotid artery is difficult and it has been shown that the correlation between central AIx obtained from carotid and radial arteries is relatively poor [143]. Most studies therefore use radial measurements and a generalised transfer function to produce central readings. The use of a generalised transfer factor to generate central pressure readings from peripheral pulse waves was investigated by Chen et al.[139] in 24 patients undergoing cardiac catherization using non-invasive measures at the radial artery and invasive

measures in the aorta. These recordings were used to generate a generalised transfer function that was able to calculate central aortic pressures from the radial waveform. This generalised transfer function was subsequently validated in 400 subjects by Gallagher etc al.[142]. There are concerns that this generalised transfer function may not be applicable to all patient groups. Hope et al.[144] showed there was significant error when the transfer function was used to generate central pressures compared to invasive measures and this was particularly marked in patients with type 2 DM. It was advocated that a specific diabetes transfer function should be used. This may also be important in other groups of patients.

3.1.4.3 Determinants of AIx

Age, gender, height and heart rate are all important determinants of AIx and need to be taken into consideration when comparing AIx between groups [143;145-147]. Age is a key determinant of AIx however this relationship is not linear. AIx increases more in younger individuals [145]. Some studies have shown a plateau in older patients whereas others have reported a decline [145;148]. This is different to the relationship seen with PWV and age in which the increase is more prominent in older individuals [145]. Gender is also an important determinant of AIx with women having higher AIx at all ages. [147]. Part of this difference can be explained by height. Body height is important factor determining AIx as with taller statue, wave reflections occur later due to increased aortic length [149]. However height does not fully explain the differences in AIx between men and women and the underlying mechanisms are not fully understood [146].Heart rate is also an important determinant of AIx. There is a significant inverse relationship with AIx with an increase of 10 beats per minute being associated with approximately a 4% decrease in AIx [140].

3.1.4.4 AIx and CVD

The data surrounding the significance of AIx in the assessment of CVD is mixed. AIx has been found to be correlated with cardiovascular risk factors [150]. It is also increased in patients with

hypercholesterolaemia [151] and inversely related to endothelial function [128]. Weber et al.[152] found that in patients undergoing diagnostic angiography AIx is correlated with coronary artery plaque load. This relationship however was only seen in younger individuals; in patients over 60 years this relationship was no longer observed [152].The prognostic significance of AIx for the prediction of future cardiovascular events is unclear. Increased AIx has been shown to be an independent predictor of all cause mortality and cardiovascular mortality in patients with end stage renal failure [153]. In a further study by Weber et al.[154] AIx adjusted for heart rate was also an independent predictor of cardiovascular events in patients undergoing percutaneous coronary interventions. However in a substudy of the Australian Blood Pressure trial (ANBP2) Dart et al.[155] found carotid arterial waveforms were not predictive of cardiovascular events in elderly women with hypertension whereas brachial pressures were. Chirinos et al.[156] showed that in patients with established coronary artery disease (CAD) augmented pressure was predicted of all cause mortality but not AIx. Most recently the Conduit Artery Function Evaluation (CAFE) study also found augmented pressure but not AIx was significantly associated with the composite clinical endpoint of all cardiovascular events and renal failure [38].

3.1.4.5 AIx and type 2 DM

There is also a great deal of debate surrounding the use of AIx in patients with DM. The presence of increased arterial stiffness as assessed by a number of methods in patients with type 2 DM is supported by a wealth of data [157-160] . In view of the difficulties discussed early with measuring aortic PWV particularly in obese individuals it is clear why a simpler method such as AIx would be attractive in patients with type 2 DM. The utility of AIx in patients with type 2 DM is far from clear however. Some groups have found increased AIx in patients with type 2 DM while others have not. Brooks et al.[161] found elevated AIx in patients with type 2 DM. Whereas Lacy et al.[127] found no increase in AIx in a group of patients with type 2 DM despite an increase in aortic PWV. These findings have been reported by other groups although the reasons

underlying this discrepancy are uncertain. Maple-Brown et al.[162] found no increase in AIx in patients with type 2 DM compared to obese individuals without type 2 DM. In this study AIx was found to be inversely related to obesity. One mechanism may be dampening of the reflected wave through fat, similar to the observation that wave reflection can be reduced by applying external pressure to the arm [163]. Whether this explanation is correct is unclear and whether it is part of the explanation for the findings in patients with type 2 DM (the majority of who are obese) is uncertain.

3.1.4.6 AIx and PWV

Although AIx is partly determined by PWV the relationship between AIx and PWV is not straightforward. As already discussed AIx is determined by a number of factors in addition to PWV. Yasmin et al.[164] showed that brachial PWV was weakly correlated with AIx (R=0.29) although this relationship was stronger when men and women were analysed separately. A number of studies have however shown no correlation between AIx and aortic PWV. Kelly et al.[165] showed that aortic PWV was correlated with AIx but this relationship was no longer seen after adjusting for age. Lemogoun et al.[166] also found no correlation between aortic PWV and AIx in healthy men. These findings call in to question the use of AIx as a surrogate for arterial stiffness.

3.1.4.7 Strategies to reduce AIx

Vasodilator drugs such as ACEi, ARBs and CCB can reduce AIx index to a greater degree than other antihypertensive agents[38;167;168]. ACEi and β blocker have similar effects on brachial blood pressure but ACEi decrease AIx to a greater extent [167]. β blockers have little effect on wave reflection but can increase augmented outgoing pressure wave during systole as a result of heart rate and timing of systolic ejection [168]. A sub study of the ASCOT trial, the CAFE study showed combination of amlodipine and perindopril was associated with a significantly greater reduction

in AIx compared to atenolol and thiazide regime despite similar effects on brachial cuff blood pressure [38]. The combination of ACEi and CCB in the ascot study was associated with improved cardiovascular outcomes compared to a β blocker/thiazide based regime [37]. In the CAFE study although central pulse pressure was significantly associated with cardiovascular events and procedures augmentation index was not [38].

3.1.4.8 Limitations of AIx

Radial artery tonometry is less technically demanding than pulse wave capture at femoral or carotid arteries. This is the major advantage of AIx over aortic PWV. In addition AIx provides information on wave reflection, an important consequence of increased arterial stiffness. However as discussed there are a number of concerns regarding the use of AIx for the assessment of arterial stiffness, including the effect of age, heart rate, height and gender, the applicability of a generalised transfer function, and the use of AIx in certain groups of patients. AIx needs further assessment to determine whether it provides useful information in the assessment of CVD.

3.1.5 Central blood pressure readings; Pulse Pressure

3.1.5.1 The theory and assessment

PP is the difference between systolic blood pressure and diastolic blood pressure. PP measures the impairment of the buffering function of larger arteries. In addition early return of the reflected wave will contribute to increased PP [169]. PP calculated from cuff measurements taken at the brachial artery is the simplest available marker for assessing arterial stiffness [169]. There is however increasing interest in the use of central pressure measurements calculated from non-invasive pulse wave recordings taken at the radial artery using applantation tonometry as discussed above.

3.1.5.2 PP and CVD

Brachial PP is predictive of CVD in healthy individuals , untreated and treated hypertension and in patients with type 2 DM [170-172]. Central PP may be a better assessment of cardiovascular risk as brachial PP tends to overestimate central PP in the young and underestimate in the elderly [169]. This is supported by the finding that central PP was a better predictor of carotid intima media thickness than brachial PP [173]. Carotid PP has also been shown to predicted CAD severity more accurately than brachial pressure [174]. Central PP was also a predictor of cardiovascular mortality in patients with end stage renal failure [175]. However Dart et al.[155] found that central pressures were not predictive of cardiovascular events in hypertensive women whereas brachial readings were. There is little evidence to support that reducing PP reduces cardiovascular events independently of systolic blood pressure[169].

3.1.6 Summary

Arterial stiffness is an important step in the cardiovascular continuum. PWV is the gold standard measurement of arterial stiffness and is an important prognostic indicator in patients with CVD and is increased in patients with type 2 DM. There a number of technical difficulties with measuring aortic-PWV and simpler methods are therefore attractive clinical tools. Two such methods are brachial PWV and AIx. The value of these two simpler techniques is as yet not clear and further evaluation of these techniques is required.

3.2 Aims and objectives

The hypotheses were that patients with CAD would have increased arterial stiffness (as assessed by aortic PWV and AIx) compared to controls. Furthermore in a subgroup of patients with type 2 DM and CAD these markers of arterial stiffness would be increased compared to patients with CAD alone.

The aims of this study were:

1. To investigate aortic PWV in healthy controls and patients with CAD with and without type 2 DM.
2. To investigate the correlation between brachial and aortic PWV.
3. To investigate the use of AIx in patients with CAD with and without type 2 DM.

3.3 Methods

PWA and PWV measurement were performed in patients with CAD and healthy controls recruited as part of the VASCB study. Detailed descriptions of the methods used are provided in chapter 2.

3.4 Results

3.4.1 PWA

Seventy four patients with CAD and 70 healthy controls attended for pulse wave recordings. In 13 patients with CAD and 7 healthy controls the quality of recordings was inadequate for analysis. Demographics and clinical characteristics are shown in table 3.1. Patients with CAD were older and the group contained a higher proportion of males compared to controls. Prevalence of hypertension was significantly higher in the CAD group although brachial systolic and diastolic blood pressure recordings were not significantly different. Patients with CAD were more overweight compared to controls as illustrated by higher body mass index. There was significant usage of statins, ACEi/ARB, CCB and nitrates in the CAD group compared to controls. Biochemistry results are shown in table 3.2. Participants that attended for PWA studies were not significantly different from those who did not attend. Demographics, clinical characteristics and basic biochemistry for all patients recruited are available in the supplementary tables (chapter 10).

PWA results are shown in table 3.3. Both brachial and central pulse pressure was higher in patients with CAD compared to controls. Heart was slower in patients with CAD compared to controls. Time to reflected wave was shorter in CAD patients compared to controls. Augmented pressure was higher in CAD group compared to controls. There was no significant difference in peripheral AIx, central AIx or central AIx corrected for heart rate between controls and CAD (figure 3.2).

Since the control group contained more women than CAD and sex is an important determinant of AIx males were analysed separately (table 3.4). When males were analysed alone there was a trend to higher peripheral, central and central AIx corrected for heart rate but this was not significant. The male CAD patients had a significantly lower heart rate compared to healthy controls and shorter time to first reflected wave.

PWA results for patients with and without type 2 DM are shown in table 3.5. Once again there was no significant difference in peripheral AIx, central AIx or central AIx corrected for heart rate. There was no difference in time to start of reflected wave between the two groups.

3.4.2 PWV

Of the 74 CAD patients attending for pulse wave recordings brachial PWV results were available for 60 patients and aortic PWV for 16 patients. Fourteen brachial PWV recordings and 18 aortic PWV recordings were rejected due to poor quality. In a further 26 CAD patients there was insufficient time for aortic PWV measurements. Fourteen patients with CAD declined to have recordings performed at groin. Of 70 controls attending for pulse wave studies, 57 brachial PWV were available for analysis (13 rejected due to poor quality) Forty two aortic PWV recordings from controls were available for analysis (18 rejected due to poor quality, 10 participants declined to have recordings performed at groin). Demographics and clinical characteristics are shown in table 3.6. Biochemistry results are shown in table 3.7.

Aortic PWV was significantly faster in CAD compared to controls (10.16±2.17 vs. 8.42±1.75, *P*=0.003) (figure 3.5). Brachial PWV was significantly slower in patients with CAD compared to controls (8.22±1.43 m/s vs. 9.05±1.41m/s P=0.002, figure 3.6).

In patient with CAD with and without type 2 DM there was no difference in brachial PWV 8.14±1.67 (n=16), vs.8.25±1.67 (n=44), *P*=NS. Due to the small number of aortic PWV results in patients with CAD it was not possible to compare these groups.

There was a significant correlation between brachial PWV and aortic PWV (R=0.399, P=0.005) When the two groups were analysed separately this relationship only remained in the control group (r=0.527, P=0.001). In patients with CAD there was no correlation between aortic and brachial PWV (figure 3.7).

Table 3.1 Demographics and clinical characteristics for patients with CAD and controls undergoing PWA studies

	CAD (n=61)	Control (n=63)	*P*-value
Age (years)	66.8±9.3	60.0±10.2	<0.0001
Male (%)	48(78.7)	38 (60.3)	<0.0001
Systolic BP, mm Hg	142.1±20.2	137.8±19.4	NS
Diastolic BP, mm Hg	78.3±10.4	81.7±10.5	NS
Heart rate (beats/min)	63.1±9.3	68.3±12.4	0.01
BMI, kg/m^2	29.0±4.1	26.1±3.5	<0.0001
Current smokers (%)	4 (6.6)	3(4.8)	NS
Type 2 DM (%)	17(27.9)	0	n/a
Hypertension (%)	35(57.4)	16(25.4)	0.002
Myocardial Infarction (%)	33 (54.1)	0	n/a
TIA/CVA (%)	3(4.9)	1 (1.6)	NS
Chronic renal failure (%)	3 (4.9)	0	NS
Heart failure (%)	7 (11.5)	0	n/a
Aspirin (%)	56 (91.8)	6(9.5)	<0.0001
Other antiplatelet agent (%)	18 (29.5)	1 (1.6)	<0.0001
Statin (%)	56 (91.8)	9 (14.3)	<0.0001
ACEi/ARB (%)	35 (57.4)	6(9.5)	<0.0001
Beta-blocker (%)	47 (77)	4 (6.3)	<0.0001
Calcium channel blocker (%)	25 (41)	3 (4.8)	<0.0001
Nitrate (%)	41 (67.2)	0	<0.0001
Diuretic (%)	11(18)	5 (7.9)	NS
Oral hypoglycaemic agent (%)	10 (16.4)	0	n/a
Insulin (%)	4 (6.6)	0	n/a

Continuous variables are mean± standard deviation. Discrete variables are absolute numbers and percentage (%) BP, blood pressure, TIA; transient ischaemic attack, CVA, cerebrovascular accident. ACEi, Angiotensin-converting enzyme inhibitor, ARB, Angiotensin receptor blocker.

Table 3.2 Biochemistry results for patients with CAD and controls undergoing PWA studies

	CAD (n=61)	Control (n=63)	*P*-value
Cholesterol (mmol/L)	4.01±0.91	5.71±1.17	<0.0001
Triglycerides (mmol/L)	1.88±0.91	1.54±0.79	0.026
LDL(mmol/L)	1.97±0.70	3.50±1.07	<0.0001
HDL(mmol/L)	1.17±0.27	1.50±0.35	<0.0001
CRP (mg/L)	4.76±10.74	2.12±2.46	0.036
HbA_{1c} (%)	6.13±1.12	5.50±0.29	<0.0001
Urinary ACR (mg/mmol)	3.05±6.79	1.33±0.97	0.045

All variables mean ± Standard deviation. LDL, low density lipoprotein; HDL, high density lipoprotein; CRP, C-reactive protein; HbA_{1c}, glycated haemoglobin; urinary ACR, urinary albumin:creatinine ratio.

Table 3.3 Pulse wave analysis results in patients with CAD and Controls

	CAD (n=61)	Control (n=63)	*P*-value
Brachial Systolic BP, mm Hg	135.5±17.6	131.7±18.0	NS
Brachial Diastolic BP, mmHg	74.5±9.5	77.5±10.4	NS
Brachial Pulse Pressure, mm Hg	61.1+14.2	54.2+12.6	0.011
Brachial Mean Arterial Pressure, mmHg	94.8±11.7	96.8+12.5	NS
Peripheral AIx	88.5±12.5	84.6±16.5	NS
Heart rate, bpm	57.3±7.3	60.6±9.6	0.031
Central Systolic BP, mm Hg	126.4±17.4	122.8±18.1	NS
Central Diastolic BP, mm Hg	75.1±9.6	78.5±10.4	NS
Central Pulse pressure	51.3±13.5	44.3±12.5	0.003
Central AIx (%)	31.5±7.7	29.0±11.0	NS
Central AIx corrected for HR (%)	23.1±7.0	22.1±10.8	NS
Augmentation, mm Hg	16.7±7.6	13.7±7.6	0.036
P1 height, mm Hg	34.6±7.8	30.6±7.0	0.003
T1, ms	108.2 ±9.8	112.2±9.9	0.025
T2, ms	236.6±18.1	240.7±18.9	NS
TR, ms	140.1±10.7	147.7±15.6	0.004
ED, ms	338.8±23.8	337.6±21.4	NS

HR heart rate. T1 time from start of waveform to first peak. T2 time from start of waveform to second peak. TR time to start of reflected wave. ED ejection duration ie time from start of waveform to closure of aortic valve. Augmentation difference between central SBP and P1. AIx augmentation index (Augmented pressure divided by pulse pressure expressed as a percentage).

Table 3.4 PWA results for males only

	CAD males only (n=47)	Healthy controls males only (n=27)
Brachial SBP, mm Hg	135.1 ±16.9	136.4±19.0
Brachial DBP, mm Hg	74.7 ±9.1	78.9± 9.1
Brachial PP, mm Hg	60.4 ±13.1	57.6± 14.7
Brachial MAP, mmHg	94.9 ±11.4	99± 11.9
Peripheral AIx	89.1±13.5	83.7± 15.3
Heart rate, bpm	56.2 ±6.5*	60.2±6.5
Central SBP, mm Hg	126.2 ±17.4	126.9 ±19.1
Central DBP	75.4 ±9.1	79.7 ±9.3
Central PP	50.8 ±13.3	47.2 ±10.7
Central AIx (%)	31.6 ±8.3	27.9± 10.7
Central AIx corrected for HR (%)	22.8 ±7.5	20.9 ±10.0
Augmentation, mm Hg	16.7 ±8.2	14.0 ±8.2
P1height, mm Hg	109.5±12.3	113.0 ±14.0
T1, ms	108.5 ±10.2	112.7 ±10.5
T2, ms	237.5± 17.9	237.6± 17.4
TR, ms	140.5 ±11.0**	146.7 ±12.7
ED, ms	340.1± 24.3	333.2 ±18.7

*P=0.027 **P=0.031. SBP, systolic blood pressure, DBP diastolic blood pressure, PP, pulse pressure, MAP, mean arterial pressure, HR heart rate. T1 time from start of waveform to first peak. T2 time from start of waveform to second peak. TR time to start of reflected wave. ED ejection duration ie time from start of waveform to closure of aortic valve. Augmentation difference between central SBP and P1. AIx augmentation index (Augmented pressure divided by pulse pressure expressed as a percentage).

Table 3.5 PWA results for patients with CAD with and without type 2 DM

	CAD and T2DM (n=17)	CAD alone (n=44)
Brachial SBP, mm Hg	135.7±10.8	135.4±19.7
Brachial DBP, mm Hg	71.5±9.6	75.5±9.3
Brachial PP, mm Hg	64.1±13.8	59.9±14.4
Brachial MAP, mmHg	91.8±8.5	95.9±12.6
Peripheral AIx (%)	85.8±11.8	89.5±12.8
Heart rate, bpm	59.9±6.5	56.3±7.4
Central SBP, mm Hg	124.6±9.1	127.1±19.8
Central DBP, mm Hg	72.1±9.6	76.3±9.5
Central PP, mm Hg	52.4±11.1	50.9±14.4
Central AIx (%)	29.7±7.2	32.2±7.8
Central AIx corrected for HR (%)	22.6±6.8	23.3±7.2
Augmentation, mm Hg	15.6±5.1	17.1±8.4
P1 height, mm Hg	36.8±8.7	33.8±7.3
T1, ms	108.7±9.6	108.0±10.0
T2, ms	228.5±14.7	239.8±18.5*
TR, ms	139.9±10.0	140.2±11.1
ED, ms	332.4±22.4	341.3±24.2

**P*=0.028. T1 time from start of waveform to first peak. T2, duration from start of waveform to second peak. TR time to start of reflected wave. ED ejection duration. P1, difference between the minimum pressure and the pressure at T1. Augmentation difference between Systolic pressure and P1. AIx Augmentation index augmented pressure divided by pulse pressure expressed as a percentage.

Figure 3.2 Peripheral AIx in patients with CAD and healthy controls

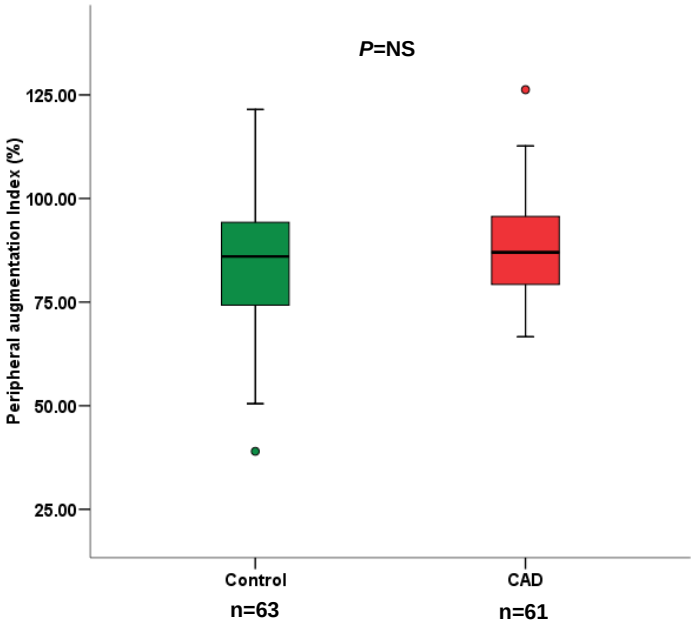


Figure 3.3 Central AIx in patients with CAD and healthy controls

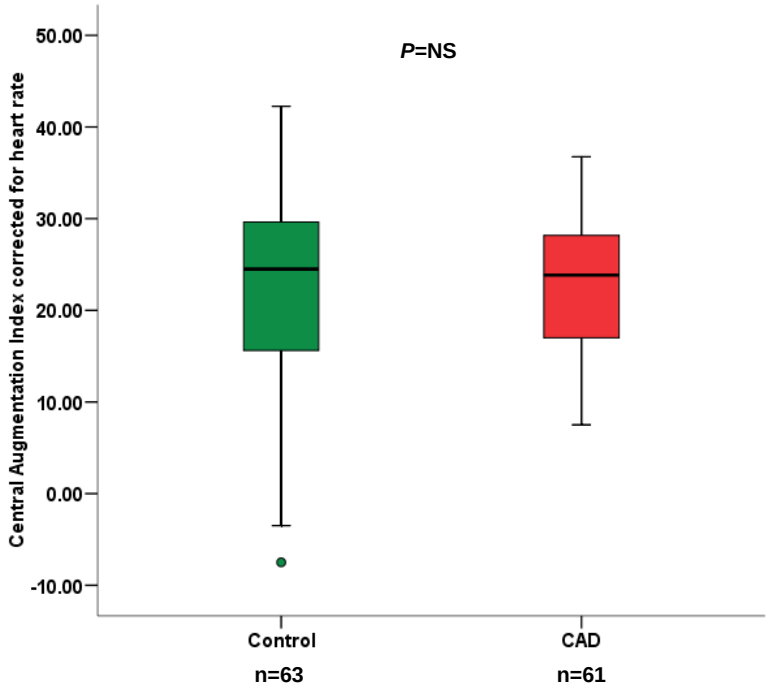


Figure 3.4. Central AIx corrected for heart rate in patients with CAD and healthy controls

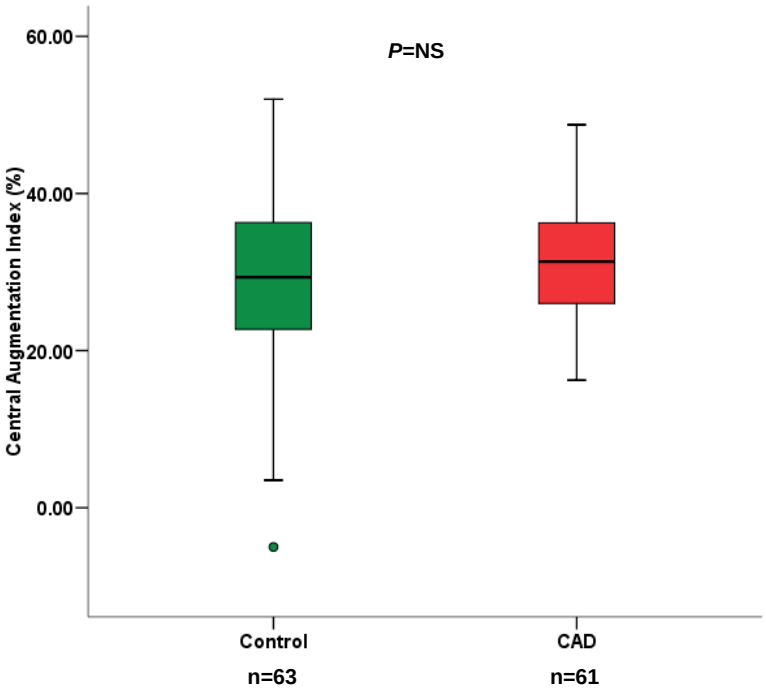


Table 3.6 Demographics and clinical characteristics for participants PWV studies

	CAD (n=60)	**Control (n=57)**	***P*-value**
Age (years)	65.2±9.9	60.1±10.3	0.007
Male (%)	49(81.7)	30(52.6)	0.001
Systolic BP, mm Hg	139.4±21.3	138.3±19.4	NS
Diastolic BP, mm Hg	77.4±11.0	81.7±10.5	0.031
Pulse Pressure (mmHg)	62.0±15.8	56.5±13.4	0.046
Heart rate (beats/min)	63.8±11.1	69.2±12.9	0.016
BMI, kg/m^2	29.3±4.5	25.8±3.6	<0.0001
Current smokers (%)	6(10)	4(7)	NS
Type 2 DM (%)	16(26.7)	0	n/a
Hypertension (%)	32(53.3)	18(31.6)	NS
Myocardial Infarction (%)	34(56.7)	0	n/a
TIA/CVA (%)	4 (6.7)	1(1.8)	NS
Chronic renal failure (%)	3(5)	0	n/a
Heart failure (%)	8 (13.3)	0	n/a
Aspirin (%)	53(88.3)	6(10.5)	<0.0001
Other antiplatelet agent (%)	19 (31.7)	1(1.8)	<0.0001
Statin (%)	56(93.3)	6(10.5)	<0.0001
ACEi/ARB (%)	36(60)	6(10.5)	<0.0001
Beta-blocker (%)	49 (81.7)	5(8.8)	<0.0001
Calcium channel blocker (%)	21(35)	3(5.3)	<0.0001
Nitrate (%)	40 (66.7)	0	<0.0001
Diuretic (%)	11(18.3)	4(7)	NS
Oral hypoglycaemic agent (%)	12 (20)	0	n/a
Insulin (%)	4 (6.7)	0	n/a

Continuous variables are mean ± standard deviation. Discrete variables are absolute numbers and percentage (%) BP, blood pressure, TIA; transient ischaemic attack, CVA, cerebrovascular accident. ACEi, Angiotensin-converting enzyme inhibitor, ARB, Angiotensin receptor blocker.

Table 3.7 Biochemistry results for healthy controls and patients with CAD in PWV study

	CAD (n=60)	Control (n=57)	*P*-value
Cholesterol (mmol/L)	4.09±0.93	5.76±1.14	<0.0001
Triglycerides (mmol/L)	2.03±0.95	1.51±0.84	0.002
LDL(mmol/L)	2.00±0.73	3.53±1.02	<0.0001
HDL(mmol/L)	1.15±0.26	1.54±0.40	<0.0001
CRP (mg/L)	4.33±9.88	1.96±2.23	0.024
HbA_{1c} (%)	6.19±1.22	5.50±0.30	<0.0001
Urinary ACR (mg/mmol)	3.15±6.87	1.38±0.99	NS

All variables mean ± standard deviation. LDL, low density lipoprotein; HDL, high density lipoprotein; CRP, C-reactive protein; HbA_{1c}, glycated haemoglobin; urinary ACR, urinary albumin:creatinine ratio.

Figure 3.5. Aortic PWV in patients with CAD compared to controls

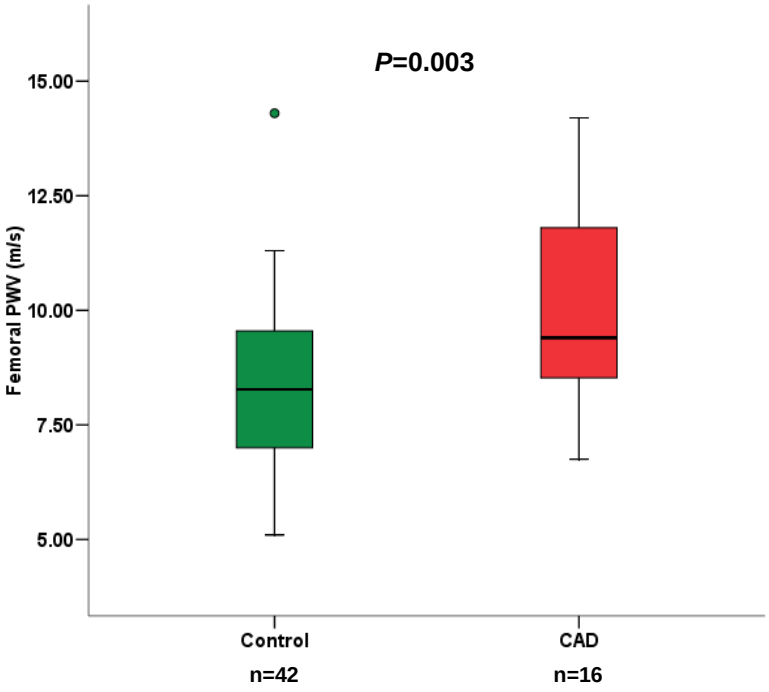


Figure 3.6 Brachial PWV in patients with CAD compared to controls

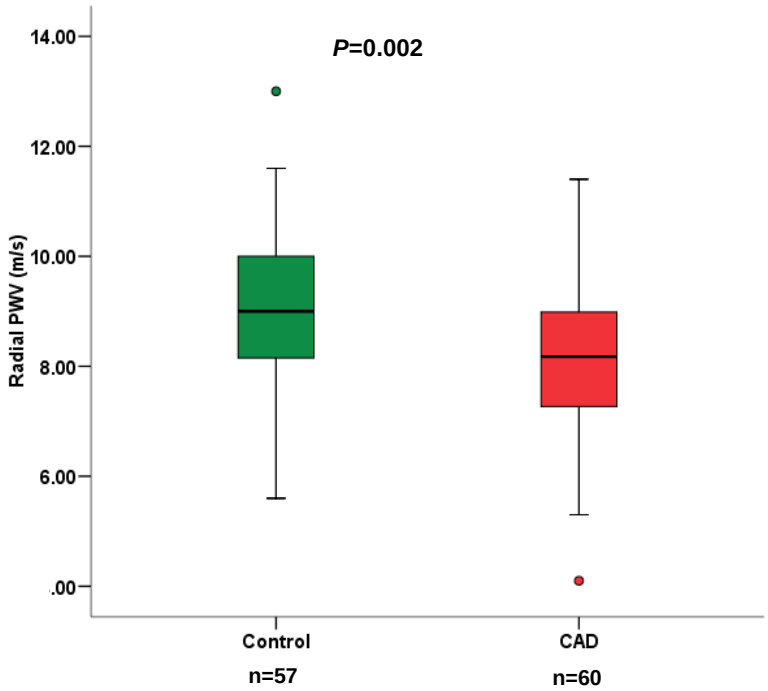
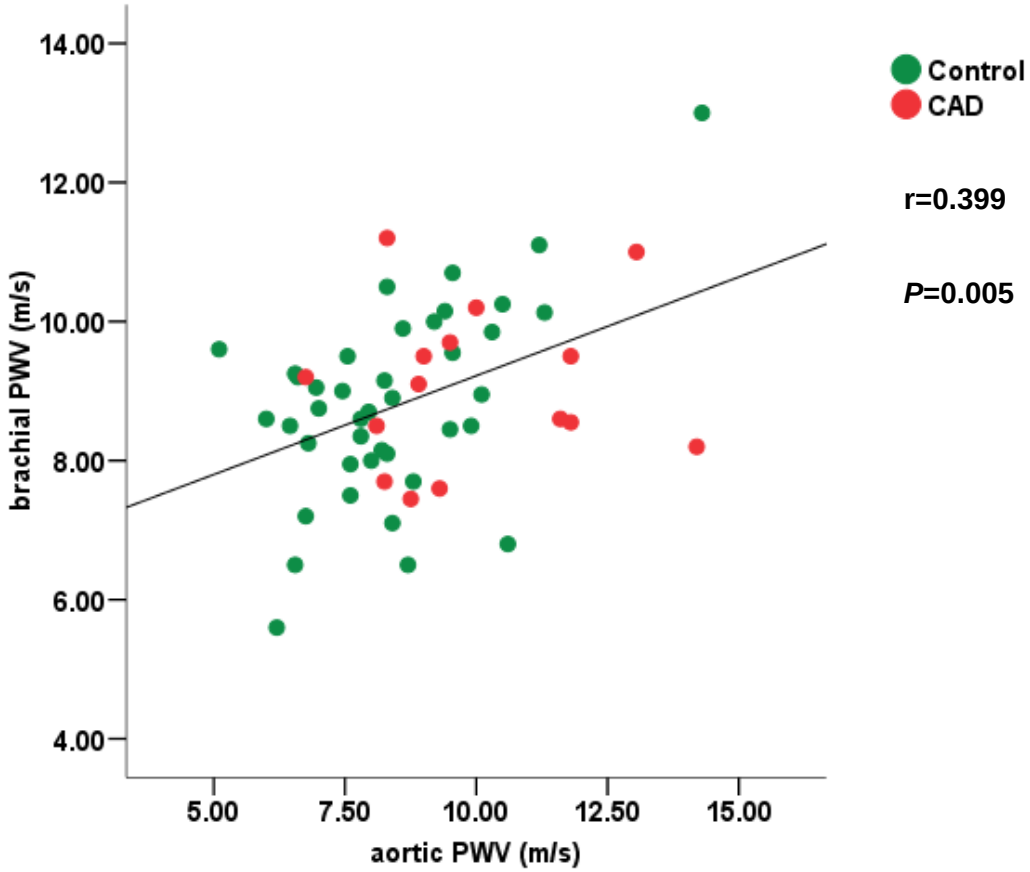


Figure 3.7 Brachial and aortic PWV



3.5 Discussion

Arterial stiffness was increased in patients with CAD compared to controls as shown by increased aortic PWV, increased brachial and central PP, and shorter time to reflected wave. Despite this evidence for increased arterial stiffness in patients with CAD there was no significant difference in brachial PWV or AIx between patients with CAD and healthy controls. The hypothesis of increased arterial stiffness in patients with type 2 DM and CAD compared to patients with CAD alone could not be supported by the available data. There were no significant differences in PP (brachial or central) or time to reflected wave in patients with type 2 DM and

CAD compared to those with CAD alone. AIx was similar in patients with and without type 2 DM.

Our finding of increased aortic PWV in patients with established CAD is in keeping with other studies. Increased aortic PWV has been found in patients with cardiovascular risk factors and established cardiovascular disease [123]. The difficulties of obtaining aortic PWV recordings have been previously discussed and were highlighted in our study by the significant number of patients in whom it was not possible to obtain aortic PWV recordings. Brachial PWV with radial recordings is simpler to perform and therefore an attractive alternative. The results of previous studies question the use of brachial PWV. Cameron et al.[138] found no correlation between brachial PWV and age. Pannier et al.[137] found that brachial PWV was not predictive of cardiovascular events in patients with end stage renal failure whereas aortic PWV was. Although there was a correlation between aortic PWV and brachial PWV in all patients studied this relationship was not found in patients with CAD when the groups were analysed separately. The lack of correlation between PWV measured in peripheral sites is perhaps not surprising as it is likely there are different mechanisms contributing to stiffness in different arterial segments. This study adds further evidence against the use of peripheral measurement of PWV for the assessment of arterial stiffness.

In addition to aortic PWV a number of other markers of arterial stiffness were significantly different in patients with CAD compared to controls. Brachial and central PP was elevated in patients with CAD compared to those without. Time to reflected wave was decreased in patients with CAD. PP is well established as a marker of arterial stiffness that has previously been shown to be elevated in patients with cardiovascular risk factors and predictive of future cardiovascular events [170-172]. Time to reflected wave has been less extensively studied. Time to reflected wave is inversely correlated to aortic pulse wave velocity and may be a substitute for PWV[176]. The prognostic significance of time to reflected wave compared to aortic PWV is not known.

AIx was not significantly different between patients with CAD and controls. There are a number of possible explanations for this finding. AIx is affected by a number of physiological variables that may account for this finding. There is strong relationship between heart rate and AIx[140]. Heart rate was significantly higher in controls compared to patients with CAD. However heart rate and AIx are inversely related so a fall in heart rate is associated with an increase in AIx [140]. The faster heart rate in controls therefore should have increased the difference in AIx between the two groups. Furthermore when central AIx corrected for heart rate (Central AIx corrected for a heart rate of 75 beats per minute by SphygmoCor® software) was analysed there remained no significant difference between the two groups. Gender is also an important determinant of AIx, with women having higher augmentation index compared to men at all ages [147]. This is partly a reflection of difference in height however there is also an independent effect of sex [146;149]. Our control group contained more females compared to the CAD group which may have resulted in increased AIx in this group. When men were analysed alone there was a tendency to increased AIx in the CAD group although this was not significant. Age is an important determinant of AIx [145]. Our control group was significantly younger than patients with CAD, which should again be associated with lower AIx. However the mean age in our control group was 60 and CAD group 65 years. This may account for the high AIx recordings seen in both groups. Weber et al.[152]found in patients undergoing diagnostic coronary angiography over 60 years old AIx readings were similar in patients with and without CAD and all AIx reading were high. In this study AIx recordings were higher in both CAD group and control compared to the study by Weber et al.

Medication use in patients with CAD may have had an impact on AIx. Vasodilators such as ACEi/ARBs, CCB and nitrates can reduce AIx and central pressure components to a greater extent than brachial blood pressure recordings[38;167]. Therefore despite the similar brachial blood

pressure indices between the patients and controls the use of medications such as ACEi and CCB in the CAD patients may have lowered AIx in this group.

There was a trend to increased brachial and central PP and reduced time to reflected wave in patients with type 2 DM compared to those without, although these differences were small and not statistically significant. It was not possible to assess aortic PWV in patients with type 2 DM due to small numbers. It is well established that arterial stiffness is increased in patients with type 2 DM [157-160]. There is less information regarding arterial stiffness in patients with type 2 DM and established CAD. Lacy et al.[127] found the time to reflected wave was reduced and aortic PWV increased in patients with type 2 DM compared to healthy controls. Time to reflected wave was shorter in their patients with type 2 DM 134±1.8 ms compared to our recordings 139±10ms. Brachial pulse pressure and central pulse pressure were higher in our study in both patients with and without diabetes compared to the patients in the study by Lacy et al.[127]. This is not surprising given the increased age of patients in this study. There are a number of explanations for the findings in this study. The numbers of patients in this study are small and this may have been underpowered to detect a difference between the two groups. The recent meta-analysis by Cecelja et al.[122] showed that diabetes status only accounted for a small amount of the variability in aortic PWV. The finding of similar AIx in patients with and without type 2 DM is in keeping with other studies [127]. The mechanisms underlying this are not fully understood.

Our results highlight the limitations of using simpler surrogates such as augmentation index for the assessment of arterial stiffness. Our finding that AIx was not increased in patients with CAD whereas other indicators of arterial stiffness were increased adds further evidence to previously published studies that question the use of AIx as a surrogate marker of arterial stiffness[127;166]. The difficulty of using aortic PWV in certain groups has been highlighted by this study. The use of aortic PWV may be more applicable to selected groups with early disease in whom

measurement may be more straightforward. TR may be useful parameter that can be easily recorded from pulse wave and warrants further investigation.

Further studies are required to investigate the effect of lowering aortic PWV on cardiovascular outcomes. The use of time to reflected wave may be a simpler measure of arterial stiffness compared to aortic PWV however the prognostic value of this recording needs further evaluation.

There are clearly a number of limitations with this study. One of the major limitations with this study is the composition of the control group. The control group was significantly younger and contained more women compared to the CAD group. Age is one of the most important determinants of arterial stiffness. Gender has important differences in augmentation index.

The very small numbers in this study particularly for PWV measurements limits the interpretation and analysis of these results. Although can detect differences not able to determine whether provide any useful prognostic information. The study design meant patients with CAD were seen on the day of admission for surgery. The time available for study tests was limited by clinical commitments and this impacted on the number of patients who had aortic PWV recordings performed.

The observational nature of this study clearly limits the conclusions that can be drawn. No conclusions can be drawn regarding causation. Vasodilator medications can impact on AIx and PWV. Medications were not withheld prior to examinations. Assessing patients on usual medications gives probably more clinically relevant information. However it is difficult to separate the impact of medications and mechanisms underlying pathological processes. There were no differences in markers of arterial stiffness in patients with type 2 DM compared to those without.

4 Endothelial function

4.1 Introduction

Endothelial dysfunction plays a fundamental role in the development of cardiovascular disease (CVD)[82;83]. It is therefore an attractive therapeutic target in the management and prevention of CVD.

4.1.1 Assessment of endothelial dysfunction

Clinical assessment of endothelial function is important not only to help understand the pathophysiology of CVD but also in the evaluation of strategies targeting coronary artery disease (CAD). An ideal tool for assessing endothelial function clinically would be non-invasive, sensitive, reproducible and simple. At present such a test does not exist.

Currently there are a number of different methods for assessing endothelial function in different vascular beds. Although the endothelium has numerous functions, assessment in the mainstay concentrates on measurement of endothelium dependent vasodilation. A stimulus known to cause nitric oxide (NO) release from endothelial cells such as shear stress or acetylcholine is applied. Dilatation of vessel or vessels is then measured as an indicator of endothelium dependent vasodilation. In order to show that observed changes are an endothelium dependent effect and not due impaired vascular smooth muscle function a stimulus that acts directly on vascular smooth muscle cells is tested in parallel.

4.1.1.1 Ex vivo assessment of endothelial function.

Segments of vessels can be assessed in organ bath experiments which enable detailed pharmacological studies. Clearly the major limitation is the availability of vessels. Furthermore the tests are not repeatable over time and results need to be interpreted cautiously as the vessels are no longer *in situ* with ongoing exposure to circulating factors. Patients undergoing coronary artery bypass grafting (CABG) for CAD are a good source of vessels[87;177-181]. Short segments of the grafts surplus to clinical requirements can be obtained at the time of surgery. Available

vessels include left internal mammary arteries, radial arteries and saphenous veins. It is saphenous veins that are used most commonly. Although veins are not affected by atherosclerotic lesions to the same extent of arteries endothelial dysfunction has been demonstrated in both veins and arteries taken from patients with CAD[178]. Given the limited availability of arterial samples the use of veins is therefore considered appropriate.

Control vessels from patients without CVD are even more difficult to obtain. It is possible to access saphenous veins from otherwise healthy patients attending for elective varicose vein (VV) removal. Endothelial function in these vessels is significantly better than that of patients with CAD[179]

4.1.1.2 In vivo assessment of endothelial function

There are currently a number of methods for assessing endothelial function *in vivo* in either conduit vessels or the microcirculation. Each method has different disadvantages and as yet no method is easily applicable for large scale clinical studies.

Endothelial function in the coronary arteries can be assessed during cardiac catheterization. Acetylcholine is infused and change in arterial diameter measured. With an intact endothelium there is vasodilation whereas endothelial dysfunction is associated with vasoconstriction[182]. Endothelium independent vasodilation is assessed following sub lingual administration of the NO donor glyceryl trinitrate (GTN). Cardiac catherization is attractive as the results provide direct assessment of endothelial function in coronary arteries, a major target of the atherosclerotic process. However this method of assessment is only applicable to patients with established CAD and is not easily repeatable due to the inherent risks associated with cardiac catherization.

Venous plethysmography is a technique that can be used to assess microvascular endothelial function[183]. The brachial artery is catheterized and pharmacological agents of interest infused.

Blood flow in the forearm is measured non-invasively using strain gauge plethysmography, with increases in blood flow being related to vascular reactivity. Although this technique is generally considered safe it does involve arterial catheterization and is therefore not completely without risk. It is not applicable for large studies or studies requiring repeated assessments of endothelial function.

The current gold standard for non-invasive assessment of endothelial function is flow-mediated dilatation (FMD)[112]. FMD measures change in diameter of a conduit vessel following a period of ischaemia. The brachial artery is the most commonly studied vessel. A sphygmomanometer cuff is placed on the forearm distal to the brachial artery and inflated to suprasystolic blood pressure for 4-5 minutes and the cuff is released. The resulting reactive hyperaemia increases shear stress leading to NO release and therefore endothelium dependent vasodilation[184]. Endothelium independent vasodilation is assessed by response of brachial artery to sublingual GTN. FMD is widely used and correlates well with coronary vascular endothelial function[112].However FMD is technically difficult and results are inconsistent when performed by inexperienced operators[185]. This together with the expense of equipment currently limits the use of FMD.

Alternative non-invasive techniques of endothelial function are therefore needed. One of these alternatives is the pulse wave analysis based method established by Hayward et al.[109] and validated by Wilkinson et al.[108]. This method assesses changes in pulse wave reflection resulting from vascular dilation. Endothelial function can therefore be assessed using agents know to cause endothelium dependent vasodilation. Inhaled salbutamol results in endothelial dependent vasodilation and a fall in pulse wave reflection[108-110]. Salbutamol directly infused in to the brachial artery has no effect on pulse wave reflection. The result is therefore an assessment of endothelial function in the microcirculation[110]. Sublingual GTN can be used to assess endothelial independent vasodilation. Endothelial function assessed by changes in pulse wave reflection following salbutamol correlates well with endothelial function assessed by venous

occlusion plethysmography and FMD[108;186]. However currently there are no long term studies looking at the prognostic value of this technique or interventional studies.

4.1.2 Endothelial dysfunction in cardiovascular disease

Endothelial dysfunction is found in patients with established CAD[87;109;179;182;187;188]. Furthermore endothelial dysfunction occurs early in the development of CVD and is present prior to the development of overt atherosclerotic plaques[112]. Endothelial dysfunction is therefore an attractive therapeutic target early in the cardiovascular continuum.

In patients with established cardiovascular disease the persisting endothelial dysfunction has important implications. Impaired endothelial function as assessed by FMD, during cardiac catheterisation or venous plethysmography is an important prognostic factor for future cardiovascular events in patients with established disease[187-189]. The increased risk associated with endothelial dysfunction appeared to be independent of established CV risk factors[188]. It is thought in patients with CAD that persisting endothelial dysfunction continues to drive progression of the atherosclerotic process and may contribute to episodes of myocardial ischemia, angina and plaque stability[187;190;191]. Currently however there are no prospective studies linking improved endothelial function with improved cardiovascular outcomes.

4.1.3 Endothelial dysfunction in type 2 diabetes mellitus

Endothelial dysfunction is a key feature of type 2 diabetes mellitus (DM)[110;192-195]. What is less clear is whether this is purely an endothelial abnormality. Some groups have reported normal endothelium independent vasodilation[110;195] whereas others have described both impaired endothelium dependent and independent vasodilation[193;194].

Endothelial dysfunction is also found in patients with insulin resistant states prior to the development of overt hyperglycaemia and type 2 DM. For example endothelial dysfunction has been described in women with gestational diabetes, obesity and individuals with the metabolic

syndrome[196-198]. Suggesting that insulin resistance is important and endothelial dysfunction is not simply a consequence of hyperglycaemia. Insulin has important actions on the vasculature causing vasodilation through endothelium derived NO[199]. In individuals with insulin resistance this effect may be attenuated[197]. Signalling pathways underling the vascular response to insulin are similar to those involved in insulin mediated glucose uptake[199]. Therefore there may be a common mechanism underlying both the development of endothelial dysfunction and insulin resistance.

The mechanisms underlying endothelial dysfunction in patients with type 2 DM and other insulin resistant states are not fully understood. There are a number of proposed mechanisms underlying diabetes related endothelial dysfunction these include increased oxidative stress, inflammation, dyslipidemia and increased arginase activity[200-202]. However most of the evidence points to increased oxidative stress as the predominant underlying cause[91].

Endothelial dysfunction is predictive of future cardiovascular events in patients with type 2 DM[203]. Endothelial dysfunction seen in type 2 DM may in part explain the increased burden of cardiovascular disease associated with type 2 DM and is therefore an attractive therapeutic target.

4.1.4 Endothelial dysfunction and dyslipidaemia

Hypercholesterolaemia has consistently been found to be associated with impaired endothelial function[108;204;205]. Elevated cholesterol levels are important across the cardiovascular continuum. Endothelial function correlated with low density lipoprotein (LDL) cholesterol levels in both children with familial hypercholesterolaemia and patients with severe CAD undergoing revascularisation[112;179].

Cholesterol lowering by a number of methods either using statins, dietary methods or apheresis improves endothelial function[81]. The improvement in endothelial function observed with statins

appears to be due to both lipid dependent and independent effects. This is demonstrated by the findings from three studies published by John et al.[206-208] investigating both the short and longer term effects of statin therapy on endothelial function. An initial study showed forearm blood flow improved after 24 weeks of treatment with fluvastatin compared to placebo[207]. Infusion of the NO synthase inhibitor L-NMMA reversed the improvement in endothelial function suggesting that increases in NO availability were partly responsible for this finding[207]. A further study John el al.[206] showed that endothelial function is also improved after short term statin therapy. Two weeks treatment with cerivastatin was associated with improvements in forearm blood flow and this once again was partly mediated by increased NO availability[206]. Furthermore these effects of statins on endothelial function appear partly to be independent of lipid lowering effects. A subsequent study by John et al.[208] showed just three days of statin therapy was associated with improvements in endothelial function assessed by plethysmography. Assessment of endothelial function was repeated after 14 days of statin therapy and there were no further improvements seen in endothelial function despite further reduction in LDL levels[208]. The improvements in endothelial function appeared to be in part mediated by decreased oxidative stress as vitamin C infusion improved endothelial function before but not after statin therapy[208].

The effect of high density lipoprotein (HDL) levels on endothelial function can be difficult to determine as low HDL levels are usually associated with other lipid abnormalities. However HDL levels also appear to be an important determinant of endothelial function. Lupatelli et al.[209] measured FMD in 107 patients attending a lipid clinic with either elevated LDL or triglyceride levels. When FMD was divided into tertiles HDL was significantly lower in patients with the lowest FMD. Triglyceride levels were also elevated in this group whereas LDL levels were similar. Strategies to increase HDL levels independently of altering metabolic abnormalities are limited. Therefore evidence from interventional studies linking increased HDL levels with changes in endothelial function is also limited. In a small study by Spieker et al.[210] infusion of

reconstituted HDL reduced endothelial dysfunction seen in patients with hypercholesterolaemia. Kuvin et al.[211] also showed that treatment with niacin improved FMD in patients with CAD compared to controls. The change in FMD was strongly correlated with level of HDL achieved.

The effect of hypertriglyceridaemia on endothelial function is less clear. Triglyceride levels have been shown to be an important determinant of endothelial function. Schnieder et al.[212] investigated endothelial function in patients with hypercholesterolaemia. In these patients triglyceride levels were an important determinant of endothelial function independent of other cardiovascular risk factors[212]. FMD has also been shown to be impaired in men with elevated triglyceride levels compared to men with triglyceride levels in the normal range[213]. However in the men with hypertriglyceridaemia HDL levels were significantly lower and fasting insulin levels higher compared to men with normal triglyceride levels[213]. It may therefore be other metabolic abnormalities such as low HDL driving endothelial dysfunction associated with hypertriglyceridaemia. In a study by Gudmundsson et al.[214] no acute impairment in endothelial function was observed following triglyceride infusion. Strategies to lower triglyceride levels (such as fibrate therapy) alter a number of other metabolic abnormalities therefore direct evidence from interventional studies linking elevated triglycerides and endothelial dysfunction is lacking. The direct effect of hypertriglyceridaemia on endothelial function is not clear.

4.1.5 Endothelial dysfunction and hypertension

The effect of hypertension alone on endothelial function is not clear. Some studies have reported endothelial dysfunction in patients with essential hypertension[215;216]. However a number of studies have found preserved endothelial function in essential hypertension[217;218].The conflicting results may be partly attributable to different assessment methods or a result of coexisting risk factors that tend to cluster in hypertension. Chan et al.[219] found that endothelial function as assessed by forearm blood flow was correlated with other cardiovascular risk factors but not blood pressure. However Preik et al.[220] showed that hypertension when present with other risk

factors has an additive detrimental effect on endothelial function. Furthermore endothelial function is predictive of future cardiovascular events in patients with hypertension[221].

Conflicting results have also been reported regarding the effects of blood pressure treatment on endothelial function. In some but not all studies both calcium channel blockers, Angiotensin converting enzyme inhibitors (ACEi) and Angiotensin receptor blockers (ARBs) have been associated with improvements in endothelial function[81;222].

4.1.6 Endothelial function and oxidative stress

Impaired endothelium dependent vasodilation arises secondary to reduced NO bioavailability[78]. This could result from decreased production of NO (reduced eNOS, reduced cofactors for eNOS, impaired eNOS signalling) or accelerated NO degradation. Increased NO degradation by reactive oxygen species (ROS) is thought to be one of the key mechanisms underlying endothelial dysfunction[84]. Hypercholesterolaemia, type 2 DM and hypertension have all been associated with increased levels of ROS such as superoxide[87;116;179]. Furthermore administration of antioxidants has been shown to improve endothelial function[223;224].

4.1.7 Summary

Endothelial dysfunction is an important step in the development of cardiovascular disease. It is a feature found throughout the cardiovascular continuum from patients with single risk factors to those with established CVD. Endothelial function is an important predictor of future cardiovascular events. Strategies aimed at tackling endothelial function are therefore attractive for the management of patients with both established CVD through to those with isolated risk factors. The current methods for assessing endothelial function that are available have various drawbacks limiting their clinical application. A better understanding of methods underlying endothelial dysfunction together with simpler assessment techniques are required.

4.2 Aims

The hypotheses were firstly to confirm that patients with CAD would have impaired endothelial function compared to healthy controls. Secondly to confirm that patients with CAD and type 2 DM would have greater impairment of endothelial function compared to patients with CAD alone. It was hypothesised that impairment in endothelial function would remain despite use of currently available secondary prevention therapies.

The aims of the study were:

- To assess endothelial function using *in vivo* and *ex vivo* methods in patients with severe CAD undergoing CABG (with and without type 2 DM) compared to healthy controls.
- To assess the impact of type 2 DM on endothelial function in patients with CAD.
- To evaluate whether a non invasive pulse wave analysis (PWA) based technique can be used to assess endothelial function in patients with established CAD.

4.3 Methods

In vivo and *Ex vivo* endothelial function studies were performed in participants recruited as part of the VASCAB study. A detailed description of participant recruitment, clinical examination, and methods for *in-vivo* and *ex vivo* assessment of endothelial function is given in chapter 2.

4.4 Results

4.4.1 *Ex vivo* vascular function studies in patients with CAD

Ex vivo vascular function studies were performed in 49 out of a total of 126 CAD patients recruited. The reasons for organ bath results not being available for a patient recruited for the study were insufficient samples of vein, the vessel not being vital before studies completed, and date or location of operation being changed. Sixteen patients attending for elective VV surgery were recruited to provide control saphenous vein samples; of these 16 patients, 10 vessels were

suitable for organ bath experiments. Reasons for unavailable organ bath studies include insufficient size of sample received and vessel not being vital before studies completed. In both patients with CAD and patients with VV clinical characteristics (age, sex, history of diabetes etc) did not differ from patients in whom vessels were not available.

Participant characteristics for CAD and control (VV) participants are shown in table 4.1. Table 10.1 in the supplementary data shows participant characteristics for all patients recruited within the study for comparison. Table 10.2 in the supplementary data shows participant characteristics for healthy controls compared to all patients with varicose veins. Patients with VV as expected are younger than the CAD patients with a lower proportion of males within the group. Although 49% of CAD patients had a history of hypertension there was no significant difference in blood pressure between the two groups. This is probably reflects the universal use of one or more antihypertensive agent in the CAD patients. There was high usage of secondary prevention therapies (aspirin, statins, ARBs/ACEi and beta-blockers) within the CAD patients.

The biochemistry results for participants in the *ex vivo* endothelial function study are shown in table 4.2. Total cholesterol and LDL levels were lower in the CAD group compared to VV controls, reflecting the high prevalence of statin prescription. HDL levels were lower in the CAD group. Table 10.3 in the supplementary data shows biochemistry results for all study participants for comparison. Table 10.4 in the supplementary data shows the biochemistry results for healthy controls compared to patients with VV. Again the participants in whom vessels were available did not differ from all patients recruited for the study.

Endothelium dependent vasodilation as assessed by maximum relaxation of saphenous veins to calcium inophore A23187 is shown in figure 4.2. Maximal relaxation following calcium inophore was significantly lower in saphenous veins from patients with CAD compared to controls in keeping with impaired endothelial function (43.1±15.7% vs. 61.9±16.3. %, P=0.001).

95% Confidence intervals for the difference between mean relaxation to calcium inophore in patients with CAD compared to controls was -7.8 to-29.7%. Endothelium independent vasodilation as assessed by maximum relaxation of saphenous veins to SNP is shown in figure 4.3. There was no significant difference in endothelium independent vasodilatation between the 2 groups, 111.2±15.7 vs 101.3±6.2 in CAD and controls respectively. The 95% confidence intervals for the difference in mean relaxation to SNP between patients with CAD compared to controls was -6.5 to 26.5%.

Table 4.1 Clinical characteristics for patients with CAD and patients with VV in *ex vivo* vascular function study.

	CAD (n=49)	**VV control (n=10)**	***P*-value**
Age (years)	65.5±9.3	45.9±18.9	0.014
Male (%)	39(79.6)	4(40)	0.018
Systolic BP (mm Hg)	138.1±25.9	134.4±24.9	NS
Diastolic BP (mm Hg)	77.9±12.7	86.2±13.4	NS
Heart rate (beats/min)	64.2±13.9	68.6±13.3	0.04
BMI ($kg/m^{2)}$	29.4±4.4	26.9±1.9	NS
Current smokers (%)	2(4.1)	1(10)	NS
Type 2 DM (%)	14(28.6)	0	n/a
Hypertension (%)	24(49)	2(20)	NS
Myocardial Infarction (%)	24(49)	0	n/a
TIA/CVA (%)	3(6.1)	0	n/a
Chronic renal failure (%)	3(6.1)	0	n/a
Heart failure (%)	8(16.3)	0	n/a
Aspirin (%)	43(87.8)	1(10)	<0.0001
Other antiplatelet agent (%)	15(30.6)	0	0.049
Statin (%)	43(87.8)	1(10)	<0.0001
ACEi/ARB (%)	33(67.3)	1(10)	0.001
Beta-blocker (%)	36(73.5)	1(10)	<0.0001
Calcium channel blocker (%)	14(28.6)	1(10)	NS
Nitrate (%)	26(53.1)	0	0.001
Diuretic (%)	14(28.6)	1(10)	NS
Oral hypoglycaemic agent (%)	7(14.3)	0	n/a
Insulin (%)	3(6.1)	0	n/a

Continuous variables are mean±standard deviation. Discrete variables are absolute numbers and percentage (%) TIA; transient ischaemic attack, CVA, cerebrovascular accident, BP, Blood pressure, ACEi, Angiotensin-converting enzyme inhibitor, ARB, Angiotensin receptor blocker.

Table 4.2. Biochemistry results in patients with CAD and patients with VV for *ex vivo* vascular function study

	CAD (n=49)	**VV Control (n=10)**	***P*-value**
Cholesterol (mmol/L)	3.99±1.01	5.20±0.82	0.007
Triglycerides (mmol/L)	2.27±1.48	1.24±0.47	0.016
LDL (mmol/L)	1.86±0.80	3.00±0.85	0.004
HDL (mmol/L)	1.15±0.28	1.64±0.20	<0.0001
CRP (mg/L)	4.36±10.59	3.70±4.54	NS
HbA_{1c} (%)	6.07±1.11	5.42±0.19	NS
Urinary ACR (mg/mmol)	2.11±2.16	1.28±0.43	NS

All variables mean ± Standard deviation. LDL, low density lipoprotein; HDL, high density lipoprotein; CRP C-reactive protein;HbA_{1c}, glycated haemoglobin; urinary ACR, urinary albumin:creatinine ratio.

Figure 4.1 Maximal relaxation of saphenous veins to calcium inophore A23187 in patients with CAD and control patients with VV.

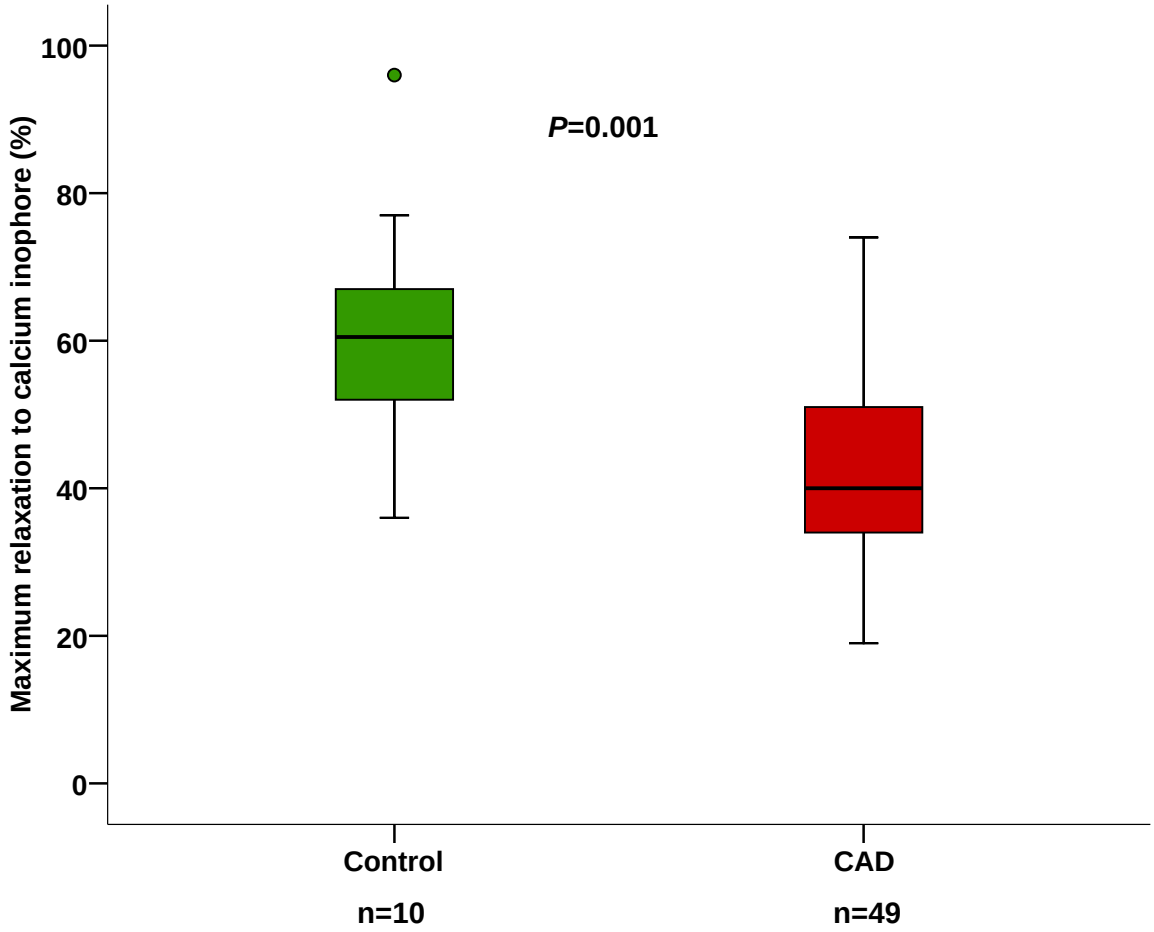
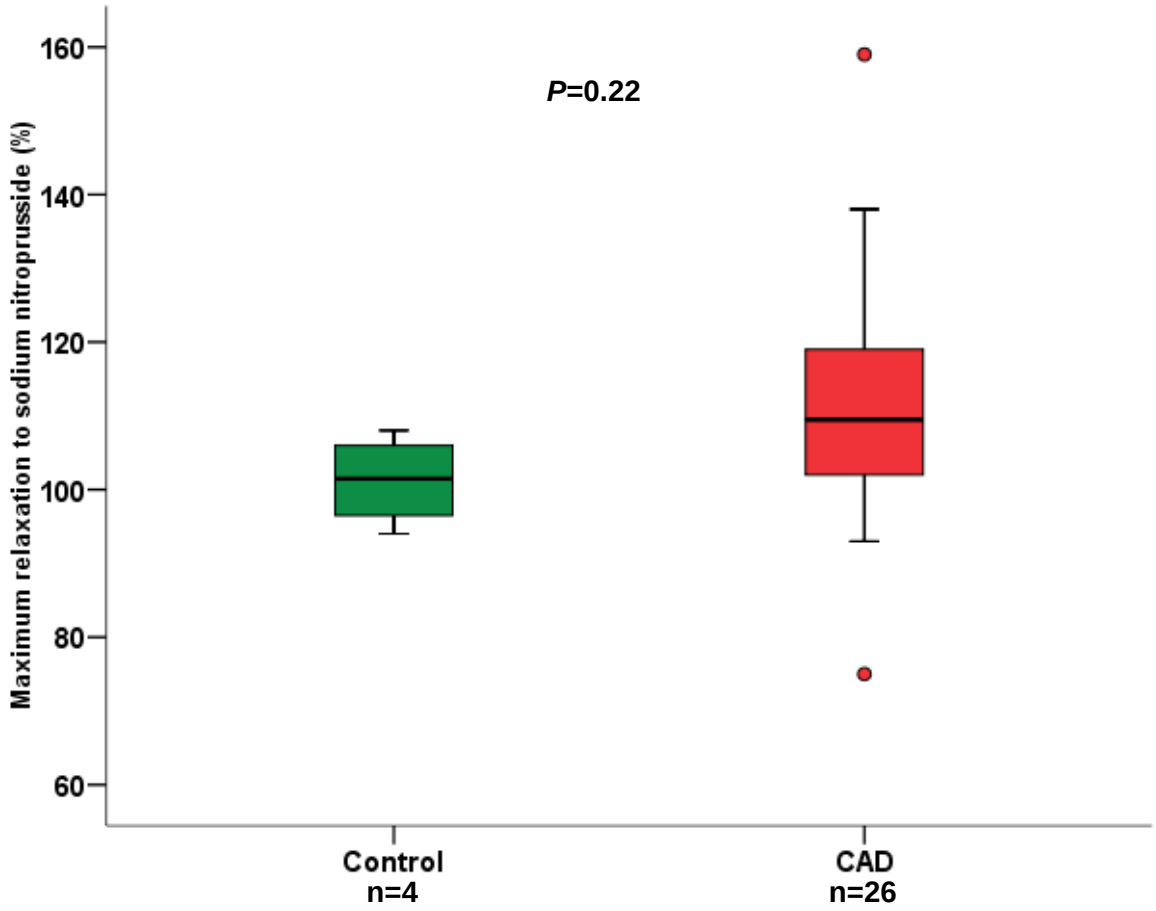


Figure 4.2 Maximal relaxation to sodium nitroprusside in saphenous veins from control patients with VV and patients with CAD



4.4.2 *Ex vivo* studies in patients with CAD and type 2 DM

Twenty eight per cent of the CAD patients had type 2 DM. Participant demographics and clinical characteristics are shown in table 4.3. Body mass index and prevalence of hypertension was higher in the patients with diabetes compared to those without. There were no other significant differences between the two groups. Biochemistry results are shown in table 4.4. HbA_{1c} was significantly higher in the patients with diabetes compared to those without. There

were no significant differences in cholesterol measures between the two groups. Endothelial function was worse in patients with CAD and diabetes compared to CAD alone (figure 4.4) Maximum relaxation to calcium ionophore was 33.9±10.8% in patients with type 2 DM compared to.46.8±15.9% in patients without diabetes (*P*=0.008).The 95% confidence intervals for difference between the means were -22.3 to-3.6%. There was no significant difference in endothelium independent vasodilation, 107.6±11.5 vs 119.5±21.3, 95% confidence intervals for the difference between the means were -6.17 to 30.1%. Results are shown in figure 4.5. Determinants of endothelial function were investigated using stepwise regression. Age, sex, CAD status, diabetes status, LDL and HDL levels were entered into a stepwise regression model. Of these variables diabetes status was the only significant predictor of endothelial function, R^2=0.157, *P*=0.003 (table 4.5).

Table 4.3 Demographics and clinical characteristics in patients with CAD with and without type 2 DM in *ex vivo* vascular function study.

	CAD alone (n=35)	**CAD and type 2 DM (n=14)**	***P*-value**
Age (years)	66±8.7	64±11.1	NS
Male (%)	27 (77%)	12(87.5)	NS
Systolic BP(mm Hg)	137.3±27.15	140.8±22.37	NS
Diastolic BP (mm Hg)	79.0±11.76	73.9±15.5	NS
Heart rate (beats/min)	64.3±12.79	63.8±17.79	NS
BMI, kg/m^2	28.7±4.11	31.9±4.67	0.047
Current Smokers (%)	1(2.9)	1(7.1)	NS
Hypertension (%)	12(34.3)	12(85.7)	0.004
Myocardial Infarction (%)	15(42.9)	9(64.3)	NS
TIA/CVA (%)	2 (5.7)	1(7.1)	NS
Chronic renal failure (%)	2(5.7)	1(7.1)	NS
Heart failure (%)	3(8.6)	5(35.7)	NS
Aspirin (%)	30(85.7)	13(92.9)	NS
Other antiplatelet agent (%)	12(34.3)	3(21.4)	NS
Statin (%)	30(85.7)	13(92.9)	NS
ACEi/ARB (%)	21(60)	12(85.7)	NS
Beta-blocker (%)	25(71.4)	11(78.6)	NS
Calcium channel blocker (%)	9(25.7)	5(35.7)	NS
Nitrate (%)	20(57.1)	6(42.9)	NS
Diuretic (%)	8(22.9)	6(42.9)	NS
Oral hypoglycaemic agent (%)	0	7(50)	n/a
Insulin (%)	0	3(21.4)	n/a

Continuous variables are mean±standard deviation. Discrete variables are absolute numbers and percentage (%) TIA; transient ischaemic attack, CVA, cerebrovascular accident, BP, Blood pressure, ACEi, Angiotensin-converting enzyme inhibitor, ARB, Angiotensin receptor blocker.

Table 4.4 Biochemical results for patients with CAD alone and patients with CAD and type 2 DM in ex vivo vascular function study

	CAD alone (n=35)	**CAD and type 2 DM (n=14)**	***P*- value**
Cholesterol (mmol/L)	4.02±1.07	3.91±0.91	NS
Triglycerides (mmol/L)	2.04±0.87	2.64±2.32	NS
LDL (mmol/L)	1.91±0.81	1.78±0.72	NS
HDL (mmol/L)	1.17±0.30	1.11±0.24	NS
CRP (mg/L)	5.13±12.58	2.72±3.34	NS
HbA_{1c} (%)	5.59±0.36	7.41±1.35	<0.0001
Urinary ACR (mg/mmol)	2.31±6.24	3.79±3.35	NS

All variables mean ± Standard deviation. LDL, low density lipoprotein; HDL, high density lipoprotein; CRP, C-reactive protein;HbA_{1c}, glycated haemoglobin; urinary ACR, urinary albumin:creatinine ratio.

Figure 4.3. Maximal relaxation of saphenous veins to calcium inophore A23187 in patients with CAD alone and patients with CAD and type 2 DM

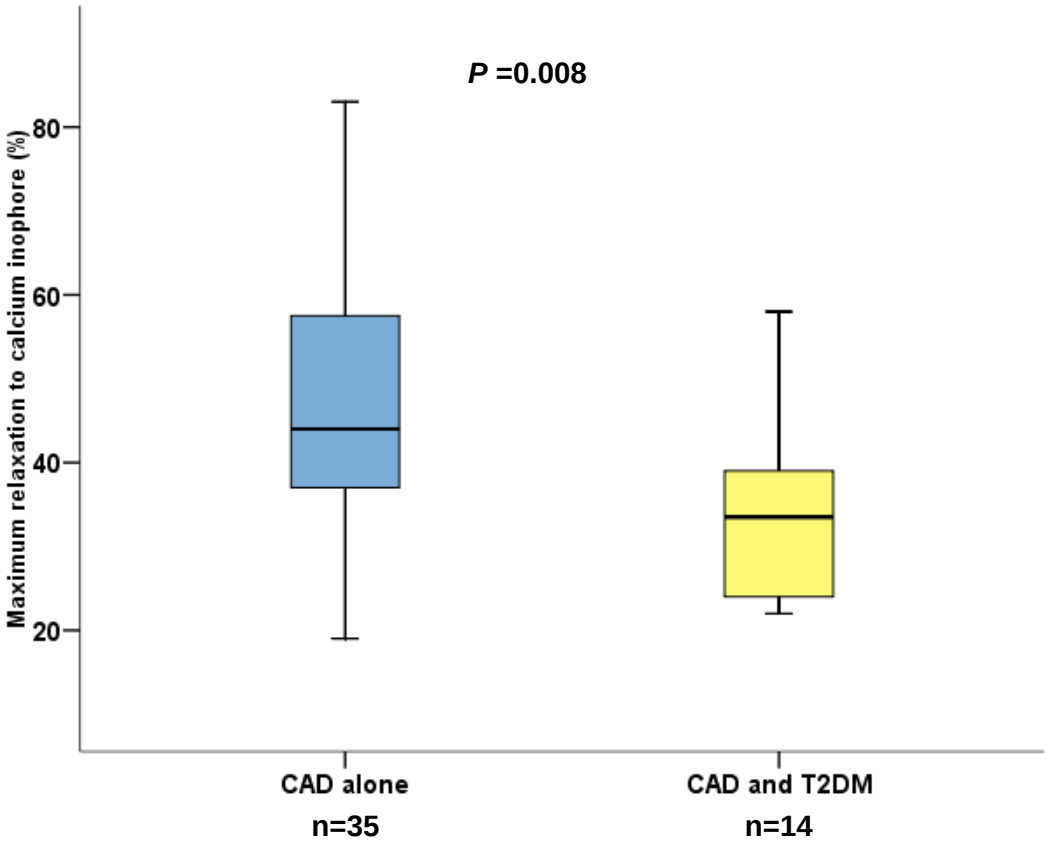


Figure 4.4 Maximal relaxation to sodium nitroprusside in saphenous veins from patients with CAD alone and patients with CAD and type 2 DM

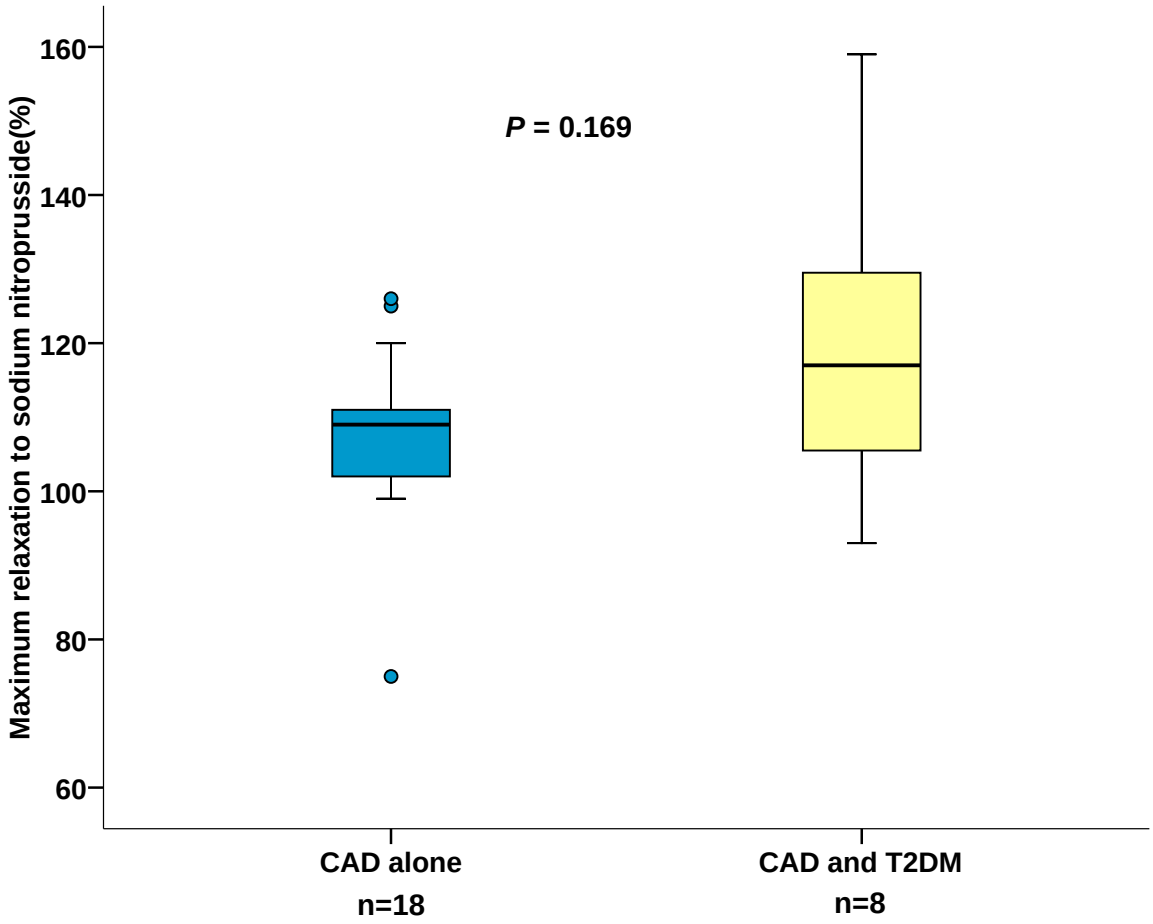


Table 4.5 Determinants of *ex vivo* endothelial function

	Full model (R^2=0.242)		Stepwise model (R^2=0.157)	
	β	*P*-value	β	*P*-value
Age	0.72	0.642	_	_
Sex (0=female,1=male)	0.113	0.415	_	_
CAD (0=no,1=yes)	-0.323	0.073	_	_
Type 2 DM (0=no,1=yes)	-0.383	0.006	-0.397	0.003
LDL-cholesterol	-0.018	0.907	_	_
HDL-cholesterol	-0.217	0.196	_	_

In the full model all variables were forced into the model. In the stepwise model variables with a significance of ≥0.1were removed. *B* indicates the partial correlation coefficients.

4.4.3 *In vivo* endothelial function studies

Reproducibility of AIx was assessed in 10 healthy controls. Intra-observer results for AIx measurements taken on the same day were -0.034±1.92% and -0.67±3.86% for measurements taken 24-48 hours later. *In vivo* endothelial function studies were performed in 22 patients with CAD and 30 healthy controls. Reasons for not performing *in vivo* vascular function studies included insufficient time for studies (74 CAD patients,18 controls) , participant unwilling to take either salbutamol or GTN (19 CAD patients and 18 controls) or PWA recording being of insufficient quality (33 CAD patients and 19 controls). Table 4.6 shows demographics and clinical characteristics for participants in in-vivo vascular function study. Table 4.7 shows biochemical analysis. Change in peripheral AIx following inhaled salbutamol was used to assess endothelial dependent vasodilation. Table 4.8 shows heart rate, systolic blood pressure, diastolic blood pressure and peripheral AIx at 5, 10, 15 and 20 minutes following salbutamol in patients with CAD and controls. In view of the variability to peak salbutamol effect between individuals the maximum change for each variable was also calculated. There was a significant increase in heart rate in both controls and patients with CAD following salbutamol compared to baseline.

However there was no difference between the maximum change in heart rate between the two groups. Overall there was no trend for change in systolic or diastolic blood pressure following salbutamol in either group with no difference in maximum change in either systolic or diastolic blood pressure. Peripheral AIx fell at all time points following salbutamol in controls, in CAD patients overall there was an increase in peripheral AIx 5 minutes post salbutamol but no other significant change. The maximum change in peripheral AIx post salbutamol was significantly greater in the control group indicating improved endothelial function compared to CAD,-7.32±8.1 vs -0.6±9.4 in controls and patients with CAD respectively. The 95% confidence intervals for the difference between the mean response of peripheral AIx to salbutamol in controls compared to patients with CAD were 1.80 to 11.65% (figure 4.6).

Heart rate, systolic blood pressure, diastolic blood pressure and peripheral augmentation index following GTN administration are shown in table 4.9. There was a significant increase in heart rate following GTN in both groups but the maximum change in heart rate was not different between the 2 groups. There were no significant changes in blood pressure following GTN. Peripheral augmentation index fell significantly following GTN administration. Healthy controls showed a greater response to GTN compared to patients with CAD; this was of borderline significance -16.8±8.65 *vs* -11.9±7.1, P=0.05. 95% confidence intervals for the difference between the mean change in AIx following GTN between healthy controls and patients with CAD was 2.4 to 4.85% (Figure 4.7).

In vivo endothelial function was assessed in 5 patients with type 2 DM and CAD compared to 17 patients with CAD alone. Peripheral AIx fell to a lesser degree in the patients with type 2 DM compared to those without diabetes however in view of the small numbers detailed analysis is not appropriate.

Twelve patients (8 with CAD and 4 VV controls) had both in-vivo and *ex vivo* measures of endothelial function. In these 12 participants there was no relationship between the two different assessments of endothelial function, r=-0.27, P=0.4 (figure 4.8).

Table 4.6 Demographics and clinical characteristics for patients with CAD and healthy controls in *in vivo* vascular function study

	CAD (n=22)	Control (n=30)	*P*-value
Age (years)	66.6±10.8	60.8±12.8	**NS**
Male (%)	18(81.8)	17(56.7)	**NS**
Systolic BP (mm Hg)	139.8±19.8	142.0±20.5	**NS**
Diastolic BP (mm Hg)	76.8±8.6	82.4±12.2	**NS**
Pulse pressure (mm Hg)	63.0±17.3	59.6±13.8	**NS**
Heart rate (beats/min)	64.4±8.7	72.0±13.2	**0.015**
BMI, kg/m^2	30.0±3.2	26.0±3.3	**<0.0001**
Current Smokers (%)	1(4.5)	2(6.7)	**NS**
Type 2 DM (%)	5(22.7)	0	**n/a**
Hypertension (%)	17(77.3)	8(26.7)	**0.002**
Myocardial Infarction (%)	11(50)	0	**n/a**
TIA/CVA (%)	1(4.5)	1 (3.3)	**NS**
Chronic renal failure (%)	1(4.5)	0	**n/a**
Heart failure (%)	1(4.5)	0	**n/a**
Aspirin (%)	19(86.4)	3(10)	**<0.0001**
Other antiplatelet agent (%)	7(31.8)	1(3.3)	**0.007**
Statin (%)	22(100)	5(16.7)	**<0.0001**
ACEi/ARB (%)	15(68.5)	2(6.7)	**<0.0001**
Beta-blocker (%)	16(72.7)	2 (6.7)	**<0.0001**
Calcium channel blocker (%)	9(40.9)	1 (3.3)	**0.001**
Nitrate (%)	16(72.7)	0	**<0.0001**
Diuretic (%)	5(22.7)	4(13.3)	**NS**
Oral hypoglycaemic agent (%)	2(9.1)	0	**n/a**
Insulin (%)	1(4.5)	0	**n/a**

Continuous variables are mean±standard deviation. Discrete variables are absolute numbers and percentage (%) TIA; transient ischaemic attack, CVA, cerebrovascular accident, BP, Blood pressure, ACEi, Angiotensin-converting enzyme inhibitor, ARB, Angiotensin receptor blocker.

Table 4.7 Biochemical results for patients with CAD and healthy controls in *in vivo* vascular function study

	CAD (n=22)	Control (n=30)	*P*-value
Cholesterol (mmol/L)	4.01±0.84	5.69±1.09	<0.0001
Triglycerides (mmol/L)	1.96±0.75	1.68±0.83	NS
LDL (mmol/L)	1.93±0.71	3.36±1.04	<0.0001
HDL (mmol/L)	1.18±0.29	1.53±0.37	0.001
CRP (mg/L)	3.15±4.42	1.96±2.67	0.039
HbA_{1c} (%)	5.95±0.84	5.55±0.33	0.027
Urinary ACR (mg/mmol)	4.14±10.7	1.40±0.84	NS

All variables mean ± Standard deviation. LDL, low density lipoprotein; HDL, high density lipoprotein; CRP, C reactive protein;HbA_{1c}, glycated haemoglobin; urinary ACR, urinary albumin:creatinine ratio.

Table 4.8 Response to salbutamol in patients with CAD and healthy controls

	Healthy Control (n=30)	***P*-value**	**CAD (n=22)**	***P*-value**
Heart rate (beat/min)				
0	63.9±10.5		58.5±6.9	
5	66.3±10.8	0.001	59.6±7.2	0.006
10	66.4±10.6	0.01	60.5±7.4	0.03
15	64.7±10.1	0.013	60.2±7.4	0.018
20	66.2±10.3	0.005	60.0±7.4	NS
Max. change in Heart rate	2.6±3.8		2.0±5.2	
SBP (mmHg)				
0	136.4±17.9		134.9±18.4	
5	139.3±20.9	NS	139.7±17.3	0.006
10	137.0±18.1	NS	137.0±18.0	NS
15	136.1±20.5	NS	138.6±19.6	NS
20	135.6±18.5	NS	134.8±19.3	NS
Max. change in SBP	3.3±14.9		2.9±15.3	
DBP (mmHg)				
0	80.1±11.5		73.0±8.0	
5	82.7±11.4	NS	75.3±7.4	0.01
10	76.2±17.5	NS	74.5±8.2	NS
15	78.7±9.7	NS	76.8±8.2	0.01
20	78.1±10.1	NS	74.4±7.9	NS
Max change in DBP	2.2±14.9		4.5±8.2	
Peripheral AIx (%)				
0	85.7±18.5		84.7±12.7	
5	82.7±17.7	0.005	86.3±12.7	0.032
10	81.3±18.8	<0.0001	82.7±11.3	NS
15	80.9±19.0	0.001	86.0±14.1	NS
20	80.7±17.9	<0.0001	83.8±16.2	NS
Max change peripheral AIx(%)	-7.32±8.1		-0.6±9.4*	

BP, blood pressure; AIx, augmentation index, P-values indicate significant change from baseline. *Significant difference between CAD and controls (*P*=0.008).

Table 4.9. Response following GTN in healthy controls and patients with CAD

	Healthy Control (n=30)	***P*-value**	**CAD (n=22)**	***P*-value**
Heart rate (beats/min)				
0	65.2±9.8		59.5±7.8	
5	68.3±9.7	<0.0001	63.3±7.1	<0.0001
Change in heart rate	3.2±3.5		3.9±2.6	
SBP(mmHg)				
0	138.6±21.3		136.7±19.0	
5	138.1±18.4	NS	134.7±19.3	NS
Change in SBP	-0.5±9.5		-3.2±8.0	
Diastolic BP(mmHg)				
0	80.3±9.7		75.1±8.4	
5	78.1±13.0	NS	74.6±7.9	NS
Change in DBP	-2.1±10.2		-0.8±5.5	
Peripheral Aix (%)				
0	81.8±20.9		84.1±10.9	
5	64.6±20.2	<0.0001	70.2±19.6	<0.0001
Change in peripheral AIx	-16.8±8.7		-11.9±7.1*	

BP, blood pressure; AIx, augmentation index, *P*-values indicate significant change from baseline

*Significant difference between CAD and controls (*P*=0.05).

Figure 4.5. Maximum change in peripheral AIx post salbutamol in patients with CAD and controls

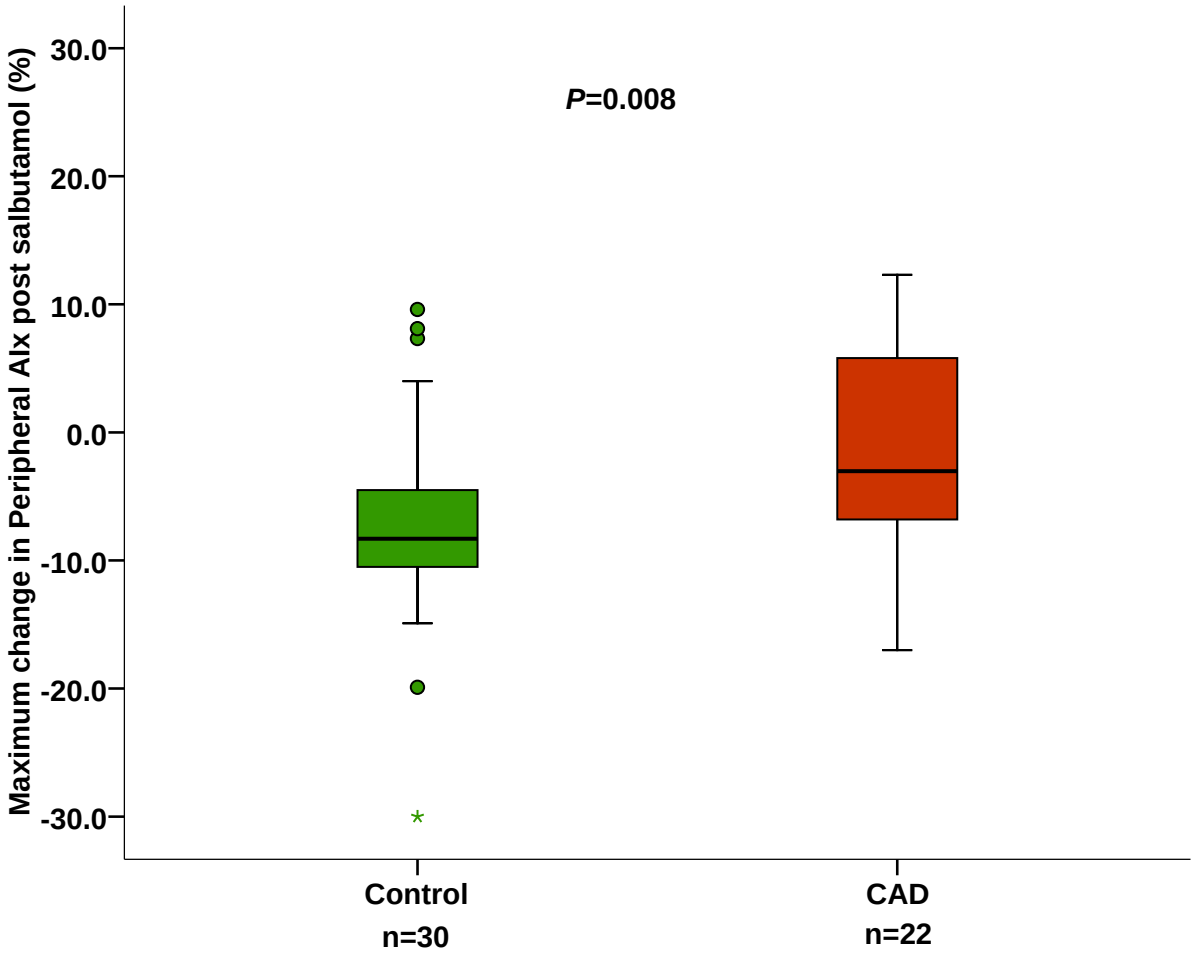


Figure 4.6. Maximum change in peripheral AIx post GTN in patients with CAD and controls

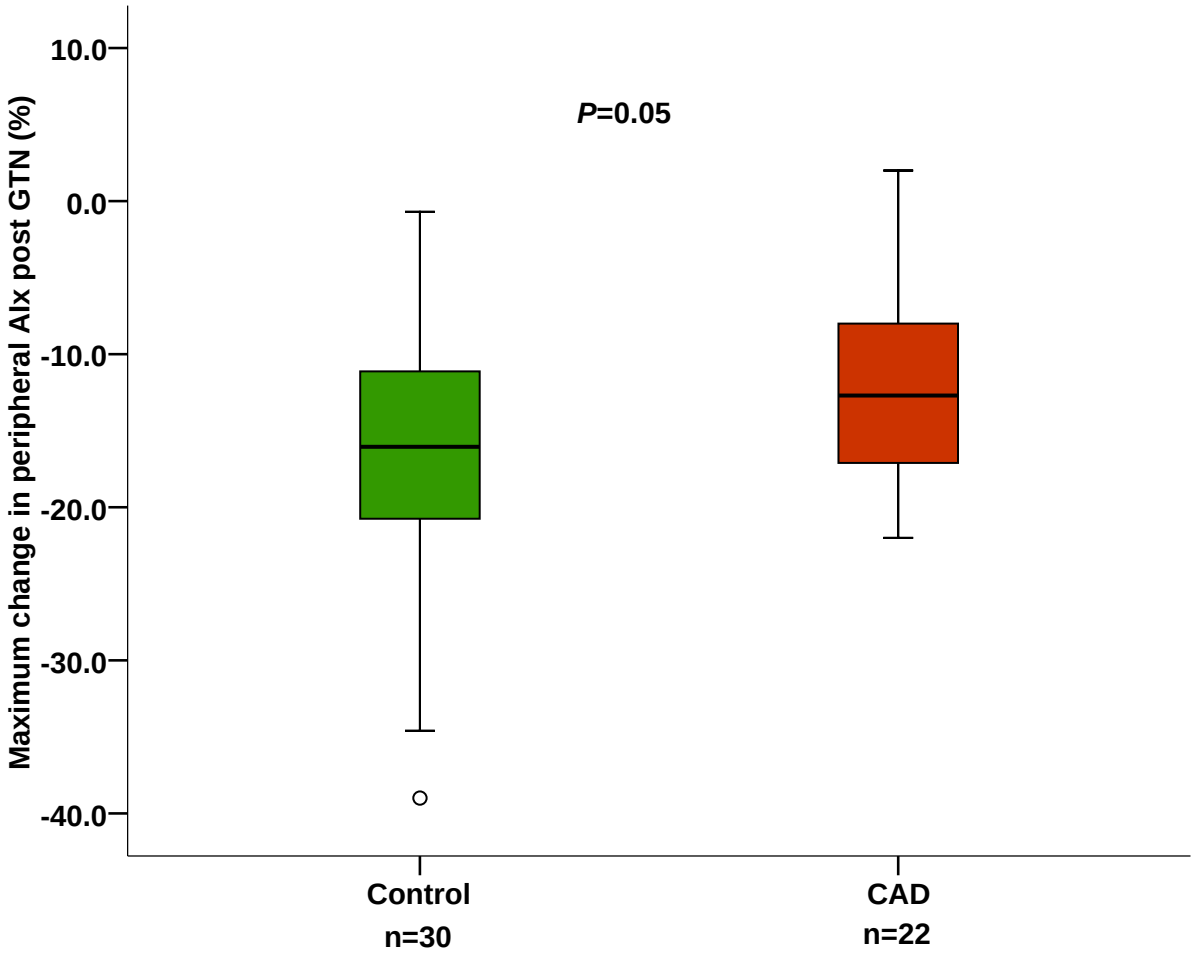
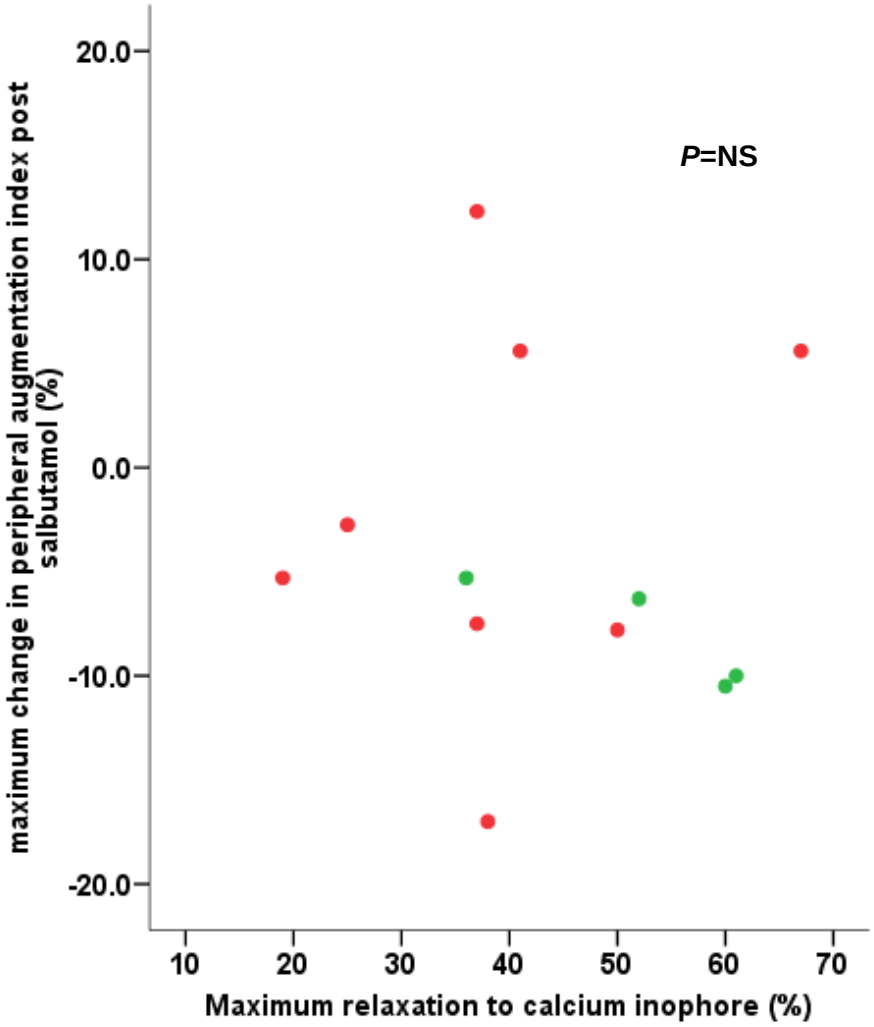


Figure 4.7. Scatter plot of maximum change in peripheral AIx post salbutamol and maximum relaxation to calcium inophore



4.5 Discussion

Using an *ex vivo* method the presence of endothelial dysfunction was confirmed in patients with CAD compared to healthy controls. There was no impairment in endothelium independent vasodilation. These results are consistent with a plethora of published data[87;109;179;182;187;188]. Twenty eight per cent of the CAD patients had type 2 DM; this is representative of all patients undergoing CABG in Scotland[99]. Endothelial function was significantly impaired in patients

with type 2 DM and CAD compared to patients with CAD alone. There was no difference in endothelium independent vasodilation. These findings are in keeping with a number of previously published studies[110;195]. Endothelial function has been found to be an important prognostic factor in patients with established CAD[187;188]. The greater degree of endothelial impairment in patients with type 2 DM may explain the worse cardiovascular outcomes seen in these patients.

Despite a number of interventions that have been associated with improved endothelial function, endothelial dysfunction persisted in patients with CAD, with and without type 2 DM. Previous studies have shown LDL levels to be one of the major determinants of endothelial function[179]. LDL levels were significantly lower in the CAD group compared to controls. LDL levels were also well controlled in patients with type 2 DM, indeed there was a trend to lower LDL levels although this was not statistically significant. Statin therapy was widespread in all patients with CAD. Statins therapy has been shown to improve endothelial function through both a reduction in LDL levels and lipid independent effects[206-208]. In some studies ACEi/ARBs have been associated with improvements in endothelial function[222]. Use of ACEi/ARB was unsurprisingly high in patients with CAD compared to controls. In addition a higher proportion of patients with type 2 DM were on ACEi/ARBs although this was not statistically significant.

Interestingly diabetes status was the only significant determinant of *ex vivo* endothelial function. Although this only accounted for approximately 16% of the variability observed, suggesting that factors other than those commonly known to alter endothelial function (Age, CAD status, LDL and HDL) are important. A number of mechanisms could account for the endothelial dysfunction seen in patients with type 2 DM, including insulin resistance, hyperglycaemia, increased oxidative stress and dyslipidaemia. These same mechanisms may also partly contribute to the endothelial dysfunction observed in patients with CAD alone.

Hyperglycaemia as assessed by HbA_{1c} was significantly higher in patients with type 2 DM compared to controls. Hyperglycaemia may contribute to endothelial dysfunction through a number of mechanisms including increased oxidative stress, advanced glycation end products and increased flux through the hexosamine biosynthetic pathway[91;225;226]. Blood glucose levels may have also partly contributed to the impaired endothelial function observed in CAD patients without type 2 DM. Blood glucose is continuous variable therefore dividing in to diabetes yes/no somewhat arbitrary. It is likely that a proportion of CAD patients without diabetes will have had impaired glucose tolerance. HbA_{1c} with a cut off of 6.5% was accepted in January 2011 by the WHO for the diagnosis of diabetes [3]. At present the use of HbA_{1c} for the diagnosis of impaired glucose tolerance is not recommended[3]. Although HbA_{1c} was higher in patients with type 2 DM compared to controls, glycaemic control was reasonable in patients with type 2 DM (mean HbA_{1c} 7.4±1.35%) and as highlighted by recent clinical trials tight glycaemic control may actually be harmful in some groups of patients [3;27]. Therefore even if hyperglycaemia is contributing to endothelial dysfunction further reductions in blood glucose levels may not be appropriate in this group of patients.

Insulin resistance has also be shown to be associated with endothelial dysfunction and may partly explain the worse endothelial function in patients with type 2 DM[197]. Insulin resistance like blood glucose levels is a continuous variable therefore may have also contributed to endothelial dysfunction seen in CAD patients without type 2 DM. Insulin resistance was not assessed in this study however type 2 DM is by definition an insulin resistant state. It has been shown in patients with established CAD without diabetes insulin resistance is also common, illustrated by the high prevalence of the metabolic syndrome[227]. A similar pattern is likely in this study. Previous studies have shown that insulin resistance is associated with endothelial function [228;229]. What degree this is a direct consequence of insulin resistance and how much can is the result of coexisting factors such as dyslipidaemia is not clear. Some but not all studies have

shown that insulin resistance is an independent predictor of endothelial function. In healthy individuals without diabetes or other cardiovascular risk factors fasting insulin levels as a surrogate for insulin resistance were related to brachial FMD[229]. However Hamburg et al.[228] showed that the impaired brachial FMD associated with insulin resistance was due to components of the metabolic syndrome rather than independent effect of insulin resistance. Further evidence for the role of insulin resistance in the development of endothelial dysfunction comes from studies looking at the effects of improving insulin sensitivity. Insulin sensitizing agents have been shown to improve endothelial function. Rosiglitazone improves endothelial function assessed during venous plethysmography in patients with type 2 DM[230]. Metformin also improves endothelial function in patients with type 2 DM and in individuals with the metabolic syndrome but normal glucose tolerance[231;232] Metformin was used in 47% of patients with type 2 DM in this study, glitazones in 11%. Ongoing use of glitazones and in particular rosiglitazone in patients with CVD is not recommended due to studies suggesting increased CV events [32]. Indeed since October 2010 rosiglitazone has been withdrawn in Europe and has restrictions placed on its use within the USA. Metformin is however safe and well tolerated. Other strategies that are known to improve insulin sensitivity including weight loss and increased physical activity have also been shown to improve endothelial function[233-235].

Lipid abnormalities in addition to LDL levels may also contribute to endothelial dysfunction. HDL levels have been shown to be correlated with endothelial function[209]. HDL was significantly lower in patients with CAD compared to controls furthermore there was a trend towards lower HDL levels in the patients with type 2 DM although this was not significant. However in multivariate analysis HDL was not a significant determinant of endothelial function. Insulin resistant states are associated with changes in LDL subfractions with increased levels of small dense LDL particles[236]. Small dense LDL has been associated with endothelial dysfunction[237].

Increased oxidative stress has been reported in patients with type 2 DM[177]. Increased oxidative stress is thought to be one of the major mechanisms underlying endothelial dysfunction in patients with CAD[84]. Antioxidants such as vitamin C improves endothelial function in patients with type 2 DM, however large clinical trials have not shown improved cardiovascular outcomes with anti-oxidants[238-240]. The role of oxidative stress in the development of endothelial dysfunction warrants further investigation.

Using a non-invasive pulse wave analysis technique for assessing *in vivo* endothelial function the presence of endothelial dysfunction in patients with CAD compared to healthy controls was confirmed. In this method there was also evidence for impaired endothelial independent vasodilation. Previous reports using this PWA technique in patients with CAD have reported normal responses to GTN[109]. Our results may simply be due to small numbers in this study. There were insufficient numbers of patients in the PWA endothelial function group to assess the effect of type 2 DM although there was a trend to worse endothelial function in this group.

Use of the PWA based technique for assessment of endothelial function has not been widely reported to date. Endothelial dysfunction using the PWA based technique was found in patients with established CVD and patients with hypercholesterolaemia [108;109]. Wilkinson et al [108] have shown that response to salbutamol is consistent in healthy volunteers examined over a week with a mean difference of -2.3±3.0%. Repeatability of response to salbutamol was not further examined in this study however the within observer reproducibility of AIx index measurement in this study was similar to previously reported data [241].

The two methods for assessing endothelial function both detected endothelial dysfunction in patients with CAD compared to controls. There was however no correlation between the two methods. This is not surprising given the small numbers of patients in whom both methods were performed. Furthermore the two methods assess endothelial function in different vascular beds.

Rambaran et al.[186] compared endothelial function assessed by change in wave reflection following salbutamol and FMD. They found that although endothelial function as assessed by these two methods was correlated this relationship was not particularly strong. Only a subgroup of the all the participants recruited for the study had endothelial function data available reflecting some of the difficulties with the two methods. Organ bath data was limited by the availability of vessels which cannot be easily controlled for. More disappointing were the number of patients in whom *in vivo* endothelial function data was available. The PWA technique was relatively easy to perform and well tolerated by participants. The major factor limiting completion of in vivo endothelial function studies was time. The protocol took approximately 1 hour which was excessive for many of the CAD patients and was a major limitation of the technique. Patients were seen immediately prior to admission to hospital and time was limited due to admission procedures and distance many of the patients had to travel. This was a deficiency of the study design.

The presence of impaired endothelial function in patients with type 2 DM and CAD despite well controlled LDL levels, widespread use of statins and ACEi/ARBs and reasonable glycaemic control highlights the need for further cardiovascular management strategies specifically targeted at improving endothelial function. Insulin resistance, increased oxidative stress, inflammation and dyslipidaemia may all contribute to endothelial dysfunction seen in patients with CAD with and without overt diabetes. Elucidating the relative contribution of these mechanisms should help identify novel strategies for tackling endothelial dysfunction in these patients. Since endothelial dysfunction is an important prognostic factor in patients with established CAD it is hoped that improved endothelial function would lead to improved cardiovascular outcomes. Large prospective studies are required to confirm this hypothesis. To date large trials assessing endothelial function have been limited by the availability of simple non-invasive techniques. The PWA technique used in this study would be suitable for larger

trials. It was simple to perform and well tolerated by the majority of patients. The major drawback was the time required for completion of the protocol. Furthermore it is unclear as to the sensitivity of this test and the ability to detect the small changes in endothelial function that might be expected in an intervention study.

There are a number of limitations to this study. The design of the study limited the time available to examine patients and therefore reduced the numbers of patients who had *in vivo* assessment of endothelial function performed. The small number of patients in the study, particularly with *in vivo* results limited the further analysis of the results. The cross-sectional design, limits the conclusions that can be drawn from this study. The VV control group was significantly younger than patients with CAD and contained more women. Age has previously been shown to be an important determinant of endothelial function and results need to be interpreted in the context of the age difference [81]. However the results of the *ex vivo* endothelial function studies were confirmed using an *in vivo* method in a healthy control group that was more closely matched for age to the patients with CAD.

4.6 Chapter summary

This study has shown patients with established CAD have impaired endothelial function compared to healthy controls. Type 2 DM is associated with significantly impaired endothelial function compared to patients with CAD alone. The impairment in endothelial function persists despite good control of cardiovascular risk factors and widespread use of secondary prevention therapies. Novel strategies targeting endothelial dysfunction are therefore required. A non-invasive PWA technique for assessing endothelial function is feasible for use in larger clinical trials however a major limitation is the time for required to complete studies.

5 Oxidative stress

5.1 Introduction

Increased production of reactive oxygen species (ROS) and in particular superoxide (O_2^-)is understood to be pivotal the development of endothelial dysfunction and cardiovascular disease (CVD)[84]. Sources of O_2^- include both vascular cells and circulating cells such as phagocytes[92;178]. Within the vasculature there are a number of enzymatic sources of O_2^-. These include NAD(P)H oxidase, xanthine oxidase, endothelial nitric oxide synthase (eNOS) and the mitochondrial electron transport system[84]. Of these NAD(P)H oxidase is understood to be one of the principle sources[87]. The enzyme eNOS under normal conditions produces nitric oxide (NO). However under certain circumstances the enzyme becomes uncoupled and can switch to being a net producer of O_2^-[177]. This may occur when the availability of the cofactor tetrahydrobiopterin is limited[177]. Circulating cells such as phagocytes also contribute to the burden of oxidative stress both whilst in circulation and as they infiltrate atherosclerotic plaques[92]. Phagocytes produce O_2^- via NAD(P)H oxidase system[242].

5.1.1 Assessing oxidative stress

The term oxidative stress refers to complex interactions between ROS and antioxidant systems. Levels of ROS can be measured directly or assessed using indirect markers. Direct measurement of ROS in the mainstay focuses on measurement of superoxide as this is considered to be the principle ROS. O_2^- is highly reactive and unstable making assessment difficult. O_2^- production can be measured directly in tissues affected by the CVD process for example vascular tissue, cardiac tissue and also in cells that are involved in the pathogenesis of CVD such as mononuclear cells or endothelial progenitor cells[116;178;243;244]. Primary cells such as vascular smooth muscle cells can also be cultured and ROS species measured[245]. Using the above methods for assessing oxidative stress in humans is clearly limited by the availability of suitable samples. Furthermore the results need to be interpreted cautiously as due the *ex vivo* nature of the methods processes involved may differ from those occurring *in situ*. Techniques suitable for

direct measurement of O_2^- include chemiluminescence-based techniques and electron spin resonance spectroscopy[113].

Indirect methods involve the quantification of products produced as a result of oxidative damage. These include oxidised low density lipoprotein (LDL), isoprostanes and thiobarbituric acid-reacting substances. These indirect measures are attractive as samples of blood or urine are easily obtained. In addition the techniques required for these assays tend to be more straightforward than those employed for the direct measurement of ROS. The sensitivity and specificity of these techniques has been questioned however[246]. Oxidised LDL can be measured using enzyme linked immunosorbant assays. There are a number of different assays that measure slightly different changes in LDL particles that comprise the oxidised LDL entity. The results are therefore not necessarily comparable between different assays[247]. Oxidised LDL has been shown to be a predictor of severity of coronary artery disease (CAD)[248;249]. However some assays are not independent of LDL levels[247] There are as yet no prospective studies of oxidised LDL and cardiovascular outcomes. Isoprostanes are stable compounds formed following the reaction of ROS with the phospholipid domain of cell membranes and can be measured in urine or plasma [250].Urinary isoprostanes have been shown to be an independent predictor of CAD[251]. Prospective studies are once again needed. Thiobarbituric acid reacting substances are products of lipid peroxidation and had previously been used in a number of studies[246]. However ongoing use of these in the assessment of oxidative stress has been questioned due to low accuracy of these methods[246;250].

An ideal tool for assessing oxidative stress would be simple, specific, non-invasive and repeatable. At present such a test does not exist restricting the detailed assessment of oxidative stress in humans to *ex vivo* experiments.

5.1.2 Oxidative stress and CAD

There is a wealth of data implicating increased oxidative stress, and in particular increased O_2^- levels, as a major underlying mechanism underlying cardiovascular disease in animal models[79]. This is supported by evidence from studies in humans although this is more limited due to the inherent difficulties with assessing oxidative stress *in vivo*.

Vascular O_2^- is increased in saphenous veins of patients with CAD compared to controls[179]. In patients with CAD NAD(P)H oxidase is thought to be the predominant source of O_2^-[87]. There is evidence that hypertension, hypercholesterolemia and diabetes mellitus (DM) all contribute to the increased levels of oxidative stress observed in patients with CAD. Diabetes status and hypercholesterolaemia are both independently associated with levels of NAD(P)H oxidase activity[87]. Levels of O_2^- are increased in circulating phagocytes in patients with hypertension and cardiovascular risk factors[116;252]. Levels of mononuclear O_2^- production has been shown to be associated with carotid–intima media thickness, a surrogate marker of atherosclerosis[253]. Less is known regarding mononuclear O_2^- production *in vivo* in patients with established CAD. Mononuclear cell O_2^- production was increased in patients with stable angina and these cells exhibited greater adhesiveness in *ex vivo* studies[254].

5.1.3 Oxidative stress and DM

The role of oxidative stress in patients with type 2 DM is of particular interest. Increased oxidative stress has been implicated as a key mechanism underlying many of the complications of diabetes including CVD[255]. In addition increased oxidative stress may contribute to insulin resistance and β-cell dysfunction driving the development of type 2 DM[225]. Increased oxidative stress in patients with type 2 DM may partly explain the endothelial dysfunction and increased cardiovascular risk associated with DM.

Vascular O_2^- is increased in vessels of patients with CAD and type 2 DM compared to patients with CAD alone[177].In these patients important sources included NAD(P)H oxidase and uncoupled eNOS[177]. Mononuclear NAD(P)H oxidase O_2^- production is increased in phagocytes of patients with metabolic syndrome and this appeared to be related to hyperinsulinaemia[252]. Mononuclear cells from patients with type 2 DM secrete increased levels of O_2^- compared to controls[256]. Mononuclear cells incubated in hyperglycaemic conditions also produce increased O_2^-[257] Other markers of increased oxidative stress such as increased circulating markers and decreased antioxidant capacity abnormal in patients with type 2 DM [91].

The mechanisms underlying increased oxidative stress in type 2 DM are not fully understood. Hyperglycaemia appears to be one of the driving factors. Hyperglycaemia can cause increased oxidative stress through a number of mechanisms including glucose autoxidation, through the formation of advanced glycation end products (AGE) and activation of polyol pathway[91]. Important enzymes are thought to include NAD(P)H oxidases, mitochondrial electron transport chain and uncoupled eNOS [177;258].

5.1.4 Strategies to reduce oxidative stress

Increased intake of fruit and vegetables protects against CVD [259]. The epidemiological data together with evidence implicating increased oxidative stress in the pathogenesis of CVD disease led to the hope that oral antioxidants would be beneficial in reducing CVD. Both vitamin C and vitamin E improve endothelial dysfunction in patients with cardiovascular risk factors[223;224]. Large randomised control trials have however shown no improvement in cardiovascular outcomes. The HOPE trial randomised patients to either vitamin E or placebo and showed no reduction in cardiovascular events after 5 years[260]. Furthermore subgroup analysis showed no benefit of vitamin E in terms of CVD in patients with type 2 DM[239]. This is despite evidence that patients with type 2 DM are thought to have higher levels of oxidative stress. Similar results were found in the heart protection study[240]. The heart protection study

showed no improvement in cardiovascular outcomes with a combination of vitamin E, vitamin C and β carotene[240] It is not clear why the use of antioxidants failed to improve cardiovascular outcomes in these large trials. Due to the inherent difficulties of assessing oxidative stress these trials did not quantify the changes in levels of oxidative stress. Therefore it is unknown whether the administration of oral antioxidants had the desired effect on oxidative stress. The negative results seen in large clinical trials do not refute the importance of oxidative stress in the development of cardiovascular disease. Various explanations have been proposed for the failure of antioxidants to improve cardiovascular outcomes. Possible reasons include selection of the wrong antioxidants, the wrong dose of antioxidants and targeting patients too far along the cardiovascular continuum[261].

In view of the disappointing results with oral vitamins and antioxidants other strategies for reducing oxidative stress have been considered. There is evidence that use of Angiotensin converting enzyme inhibitors (ACEi) and Angiotensin receptor blockers (ARBs) can reduce vascular NAD(P)H oxidase O_2^- production[262]. Statins have been shown in improve both endothelial dependent vasodilation and reduce endothelial O_2^- production[263]. Exercise training has also been shown to reduce oxidative stress in patients with CAD[264].

5.1.5 Summary

Increased oxidative stress appears to be pivotal in the development of cardiovascular disease. Increased oxidative stress can contribute to endothelial dysfunction by reducing the bioavailability of NO. Increased oxidative stress may be particularly important in the development of cardiovascular disease in patients with type 2 DM. However the failure of oral antioxidants in large clinical trials highlights the need for further understanding of the mechanisms underlying oxidative stress in CVD and diabetes and better tools for assessing oxidative stress clinically.

5.2 Aims

The hypotheses were firstly that patients with CAD would have increased oxidative stress compared to healthy controls. Secondly patients with CAD and type 2 DM would have further elevations in oxidative stress compared to patients with CAD alone.

The aims of this study were:

1. To assess O_2^- levels in the vasculature, mononuclear cell and whole blood of patients with CAD compare to healthy controls
2. To assess the impact of type 2 DM on O_2^- levels in patients with CAD
3. To assess the use of whole blood O_2^- production as a simple marker of oxidative stress.

5.3 Methods

Vascular O_2^- production was assessed in saphenous veins in patients with CAD and VV controls recruited as part of the VASCAB study. Mononuclear and whole blood O_2^- levels were assessed using EPR spectroscopy in patients with CAD and controls recruited as part of the VASCAB study. Detailed methods are provided in chapter 2.

5.4 Results

5.4.1 Vascular O_2^- studies

5.4.1.1 Vascular O_2^- studies in patients with CAD

Vascular O_2^- measurements were performed in saphenous veins from 71 patients with CAD undergoing CABG and in 13 control veins from patients undergoing VV surgery. Participant characteristics and demographics are shown in table 5.1. VV controls were significantly younger than CAD group with greater proportion of females. There was no significant difference in blood pressure. As expected there was high usage of cardiovascular secondary prevention therapies in the CAD group. Biochemistry results are shown in table 5.2. Lipid profiles reflect the widespread use of statins in the patients with CAD with lower total cholesterol and LDL

cholesterol levels compared to controls. HDL was significantly lower in patients with CAD compared to controls. Triglyceride levels were significantly higher in patients with CAD. The patients in whom vascular O_2^- levels were available did not differ from the whole study cohort in terms of clinical examination and biochemistry results (see supplementary data tables 10.1 to 10.4).

Levels of O_2^- in saphenous veins from patients with CAD were significantly higher compared to varicose vein controls, 0.75±0.48 vs.0.43±0.33 nmol/mg/min *P*=0.012 (figure 5.1).The 95% confidence intervals for the difference in mean vascular O_2^- in patients with CAD compared to controls was 0.09 to 0.54 nmol/mg/min.

Table 5.1 Demographics and clinical characteristics for patients with CAD and control patients with VV in vascular O_2^- studies

	CAD (n=71)	VV Controls (n=13)	*P*-value
Age (years)	64.8±10.1	47.3±18.0	<0.0001
Male (%)	58(81.7)	5(38.5)	0.003
Systolic BP (mm Hg)	139.4±25.2	134.8±22.8	NS
Diastolic BP(mm Hg)	78.2±11.8	85.3±12.4	NS
Heart rate (beats/min)	63.6±12.7	73.5±17.7	NS
Body Mass Index (kg/m^2)	29.6±5.0	26.5±2.0	NS
Current Smoker (%)	3(4.2)	2(15.4)	NS
Type 2 diabetes mellitus (%)	22(31)	0	n/a
Hypertension (%)	41(57.7)	3(23.1)	0.015
Myocardial Infarction (%)	38(53.5)	0	n/a
TIA/CVA (%)	4(5.6)	1(7.7)	NS
Chronic renal failure (%)	3(4.2)	0	NS
Heart failure (%)	12(16.9)	0	n/a
Aspirin (%)	61(85.9)	2(15.4)	<0.0001
Other antiplatelet agent (%)	20(28.2)	1(7.7)	NS
Statin (%)	63(88.7)	2(15.4)	<0.0001
ACEi/ARB (%)	45(63.4)	1(7.7)	<0.0001
Beta-blocker (%)	53(74.6)	1(7.7)	<0.0001
Calcium channel blocker (%)	22(31.0)	1(7.7)	NS
Nitrate (%)	36(50.7)	0	<0.0001
Diuretic (%)	21(29.6)	2(15.4)	NS
Oral hypoglycaemic agent (%)	11(15.5)	0	n/a
Insulin (%)	4(5.6)	0	n/a

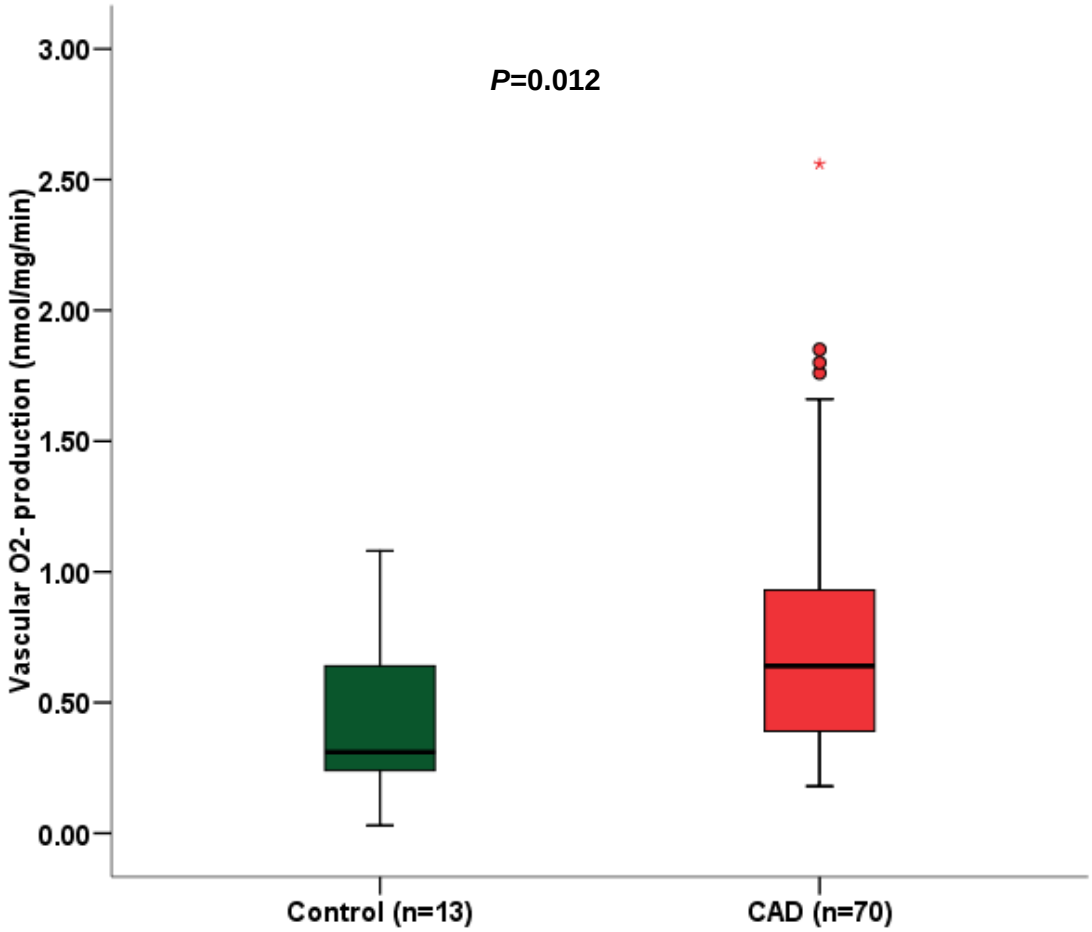
Continuous variables are mean± standard deviation. Discrete variables are absolute numbers and percentage (%) BP, blood pressure, TIA; transient ischaemic attack, CVA, cerebrovascular accident. ACEi, Angiotensin-converting enzyme inhibitor, ARB, Angiotensin receptor blocker.

Table 5.2. Biochemistry results in patients with CAD and control patients with VV for vascular O_2^- studies

	CAD (n=71)	**VV controls (n=13)**	***P*-value**
Cholesterol (mmol/L)	4.07±1.04	4.96±0.99	0.034
Triglycerides (mmol/L)	2.22±1.37	1.17±0.47	0.005
LDL (mmol/L)	1.95±0.82	2.69±1.11	0.03
HDL (mmol/L)	1.14±0.26	1.72±0.30	<0.0001
CRP(mg/L)	4.45±9.27	3.55±4.17	NS
HbA_{1c} (%)	6.08±1.07	5.42±0.17	<0.0001
Urinary ACR(mg/mmol)	3.06±7.50	1.19±0.46	NS

All variables mean ± Standard deviation. LDL, low density lipoprotein; HDL, high density lipoprotein; CRP, C-reactive protein; HbA_{1c}, glycated haemoglobin; urinary ACR, urinary albumin:creatinine ratio.

Figure 5.1 Vascular O_2^- production in saphenous veins from patients with CAD and control patients with VV.



5.4.1.2 Vascular O_2^- in patients with CAD with and without type 2 DM

Twenty two (31%) of the CAD patients had type 2 DM. Demographics and clinical characteristics for CAD patients with and without type 2 DM are shown in table 5.3. Body mass index was significantly higher in the patients with type 2 DM compared to those without. There was a higher prevalence of hypertension in the patients with type 2 DM. There was significantly higher usage of diuretics in the patients with diabetes. Use of modifiers of renin-angiotensin

system (ARB/ACEi) was also more widespread in patients with type 2 DM although this was not statistically significant.

Biochemistry results are shown in table 5.4. HbA_{1c} was significantly higher in the patients with Type 2 DM. There was a trend to lower levels of both LDL and HDL in patients with type 2 DM although this was not statistically significant.

There was no significant difference in vascular O_2^- production in patients with CAD and type 2 DM compared to patients with CAD alone, 0.64±0.43 vs 0.79±0.50 nmol/mg/min, P=0.164 (Figure 5.2).The 95% confidence intervals for the difference in mean vascular O_2^- production in patients with and without type 2 DM was -0.39 to 0.09 nmol/mg/min.

Table 5.3 Demographics and clinical characteristics in CAD patients with and without type 2 DM in vascular O_2^- study.

	CAD alone (n=49)	CAD and type 2 DM (n=22)	*P*-value
Age (years)	64.8±9.6	64.9±11.3	NS
Male (%)	39(79.6)	19(86.4)	NS
Systolic BP(mm Hg)	139.7±26.5	138.8±22.2	NS
Diastolic BP(mm Hg)	79.7±11.0	74.4±13.2	NS
Heart rate (beats/min)	63.0±12.1	64.9±14.2	NS
Body Mass Index, kg/m^2	28.7±4.7	32.2±5.1	0.023
Current smokers (%)	2(4.1)	1(4.5)	NS
Hypertension (%)	20(40.8)	21(95.5)	<0.0001
Myocardial Infarction (%)	24(49)	14(63.6)	NS
TIA/CVA (%)	2(4.1)	2(9.1)	NS
Chronic renal failure (%)	2(4.1)	1(4.5)	NS
Heart failure (%)	5(10.2)	7(31.8)	NS
Aspirin (%)	41(83.7)	20(90.2)	NS
Other antiplatelet agent (%)	16(32.7)	4(18.2)	NS
Statin (%)	43(87.8)	20(90.9)	NS
ACEi/ARB (%)	28(57.1)	17(77.3)	NS
Beta-blocker (%)	35(71.4)	18(81.8)	NS
Calcium channel blocker (%)	15(30.6)	7(31.8)	NS
Nitrate (%)	27(55.1)	9(40.9)	NS
Diuretic (%)	10(20.4)	11(50)	0.026
Oral hypoglycaemic agent (%)	n/a	11(50)	n/a
Meformin		8(36)	
Glitazones		2(9)	
Sulphonylureas		6(27)	
Insulin (%)	n/a	4(18.2)	n/a

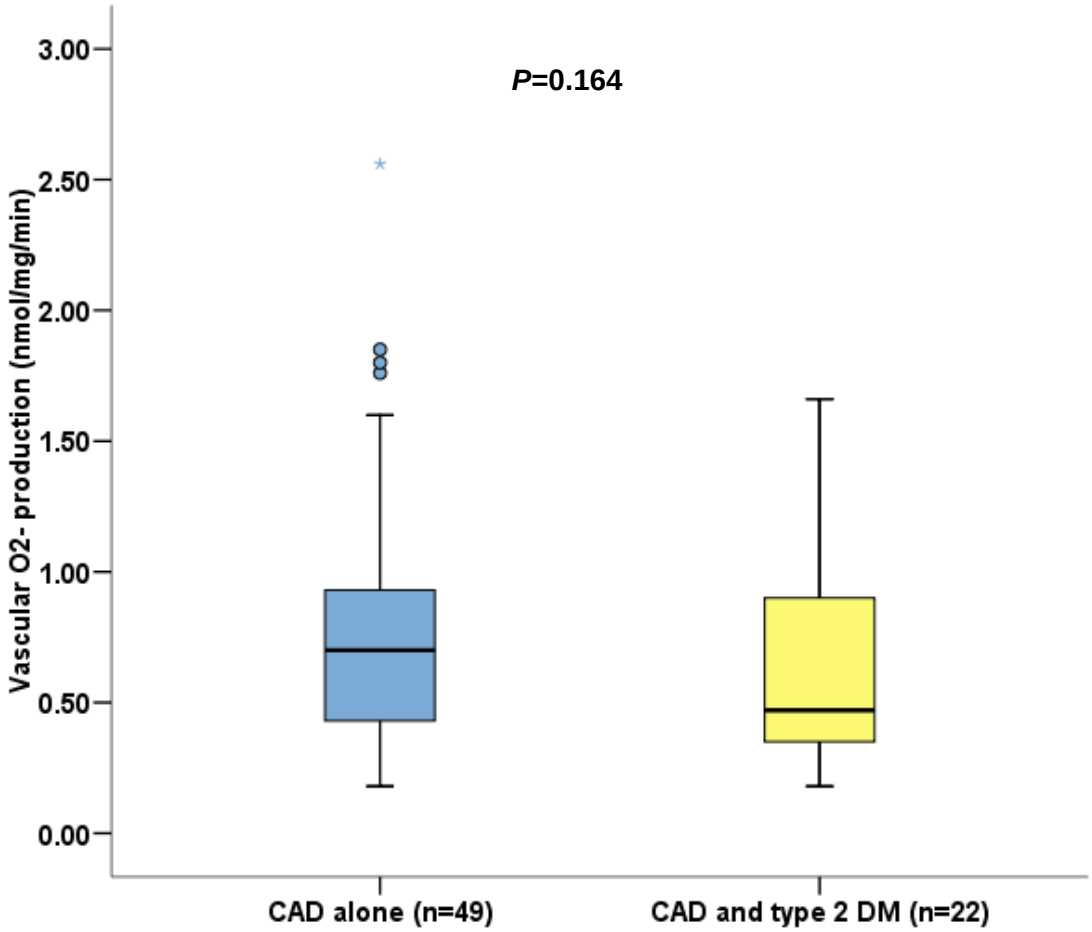
Continuous variables are mean± standard deviation. Discrete variables are absolute numbers and percentage (%) BP, blood pressure, TIA; transient ischaemic attack, CVA, cerebrovascular accident. ACEi, Angiotensin-converting enzyme inhibitor, ARB, Angiotensin receptor blocker.

Table 5.4. Biochemistry results for vascular O_2^- studies in CAD patients with and without type 2 DM

	CAD alone (n=49)	CAD and type 2 DM (n=22)	*P*-value
Cholesterol (mmol/L)	4.10±1.01	3.99±1.11	NS
Triglycerides (mmol/L)	2.04±0.98	2.63±1.97	NS
LDL (mmol/L)	2.01±0.80	1.81±0.85	NS
HDL (mmol/L)	1.16±0.28	1.10±0.22	NS
CRP(mg/L)	5.27±10.9	2.59±2.91	NS
HbA_{1c} (%)	5.6±0.42	7.2±1.26	<0.0001
Urinary ACR (mg/mmol)	3.19±8.95	2.79±2.69	NS

All variables mean ± Standard deviation. LDL, low density lipoprotein; HDL, high density lipoprotein; CRP, C-reactive protein; HbA_{1c}, glycated haemoglobin; urinary ACR, urinary albumin:creatinine ratio.

Figure 5.2. Vascular O_2^- production in saphenous veins in patients with CAD alone and CAD with type 2 DM.



5.4.2 EPR spectroscopy studies

5.4.2.1 EPR spectroscopy studies in patients with CAD

O_2^- release from mononuclear cell and whole blood was assessed in 89 patients with CAD and 68 healthy controls. Participant demographics and clinical characteristics are shown in table 5.5. Controls were younger than patients with CAD and included more women. Diastolic blood pressure was lower in patients with CAD compared to controls there was no significant

difference in systolic blood pressure. There was widespread use of cardiovascular secondary prevention strategies in patients with CAD.

Biochemistry results are shown in table 5.6. As would be expected given the prevalence of statin therapy total cholesterol and LDL cholesterol levels were lower in patients with CAD. HDL was lower in and triglyceride levels higher in patients with CAD. HbA_{1c}, CRP and urinary ACR were all higher in the CAD group.

Mononuclear cell O_2^- release is shown in figure 5.3. Basal O_2^- release from mononuclear cells was significantly greater in patients with CAD compared to controls 70.0±52.68 vs. 49.7±30.98 AU, P=0.01. In addition PMA stimulated O_2^- levels were also higher in CAD patients compared to controls (773.4±371.5 vs. 416.6±189.4 AU, P<0.0001).

O_2^- levels in whole blood were also increased in CAD patients compared to controls 54.7±57 x 10^3 AU vs. 31.4±14 x10^3AU, P=0.043 (figure 5.4).The 95% confidence intervals for the difference in mean whole blood O_2^- between patients with CAD compared to controls were 14.3 to 45.8 x10^3 AU.

Table 5.5. Demographics and clinical characteristics in patients with CAD and controls in EPR spectroscopy studies

	CAD (n=89)	Controls (n=68)	*P*-value
Age (years)	67.0±9.1	60.8±10.5	<0.0001
Male (%)	69(77.5)	41(60.3)	0.023
Systolic BP, mm Hg	139.2±23.6	137.6±18.8	NS
Diastolic BP, mm Hg	77.4±11.3	81.3±10.6	0.03
Heart rate (beats/min)	63.2±11.1	68.2±12.7	0.01
Body Mass Index, kg/m^2	29.2±4.8	26.0±3.6	NS
Current Smokers (%)	8(9.0)	4(5.9)	NS
Type 2 diabetes mellitus (%)	25(28.1)	0	n/a
Hypertension	49(55.1)	18(27.3)	0.001
Myocardial Infarction	42(47.2)	0	n/a
TIA/CVA	6(6.7)	1(1.5)	NS
Chronic renal failure	5(5.6)	0	NS
Heart failure	14(15.7)	0	n/a
Aspirin	76(85.4)	9(13.2)	<0.0001
Other antiplatelet agent	23(25.8)	1(1.5)	<0.0001
Statin	80(89.9)	8(11.8)	<0.0001
ACEi/ARB	52(58.4)	(8.8)	<0.0001
Beta-blocker	68(76.4)	5(7.4)	<0.0001
Calcium channel blocker	32(36.0)	4(5.9)	<0.0001
Nitrate	56(62.9)	0	<0.0001
Diuretic	21(23.6)	5(7.4)	0.008
Oral hypoglycaemic agent	16(18.0)	0	n/a
Insulin	5(5.6)	0	n/a

All variables mean ± Standard deviation. LDL, low density lipoprotein; HDL, high density lipoprotein; CRP, C-reactive protein; HbA_{1c}, glycated haemoglobin; urinary ACR, urinary albumin:creatinine ratio.

Table 5.6.Biochemistry results in patients with CAD and controls in EPR spectroscopy studies

	CAD (n=89)	Controls (n=68)	*P*-value
Cholesterol (mmol/L)	4.04±0.86	5.67±1.16	<0.0001
Triglycerides (mmol/L)	1.99±0.72	1.51±0.79	0.001
LDL(mmol/L)	1.96±0.72	3.45±1.04	<0.0001
HDL(mmol/L)	1.17±0.27	1.53±0.40	<0.0001
CRP(mg/L)	4.25±8.94	1.96±2.37	0.002
HbA_{1c}(%)	6.19±1.09	5.52±0.32	<0.0001
Urinary ACR(mg/mmol)	2.79±6.03	1.34±0.94	0.046

All variables mean ± Standard deviation. LDL, low density lipoprotein; HDL, high density lipoprotein; CRP, C-reactive protein; HbA_{1c}, glycated haemoglobin; urinary ACR, urinary albumin:creatinine ratio.

Figure 5.3. Basal and stimulated mononuclear cell O_2^- production in patients with CAD and controls (mean+standard deviation)

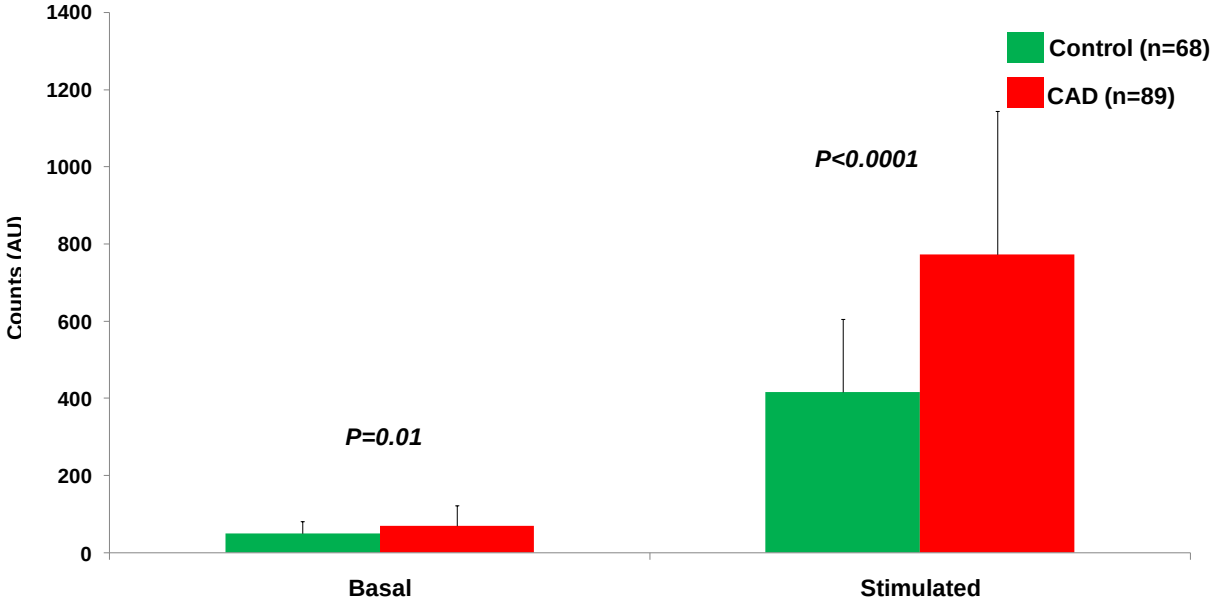
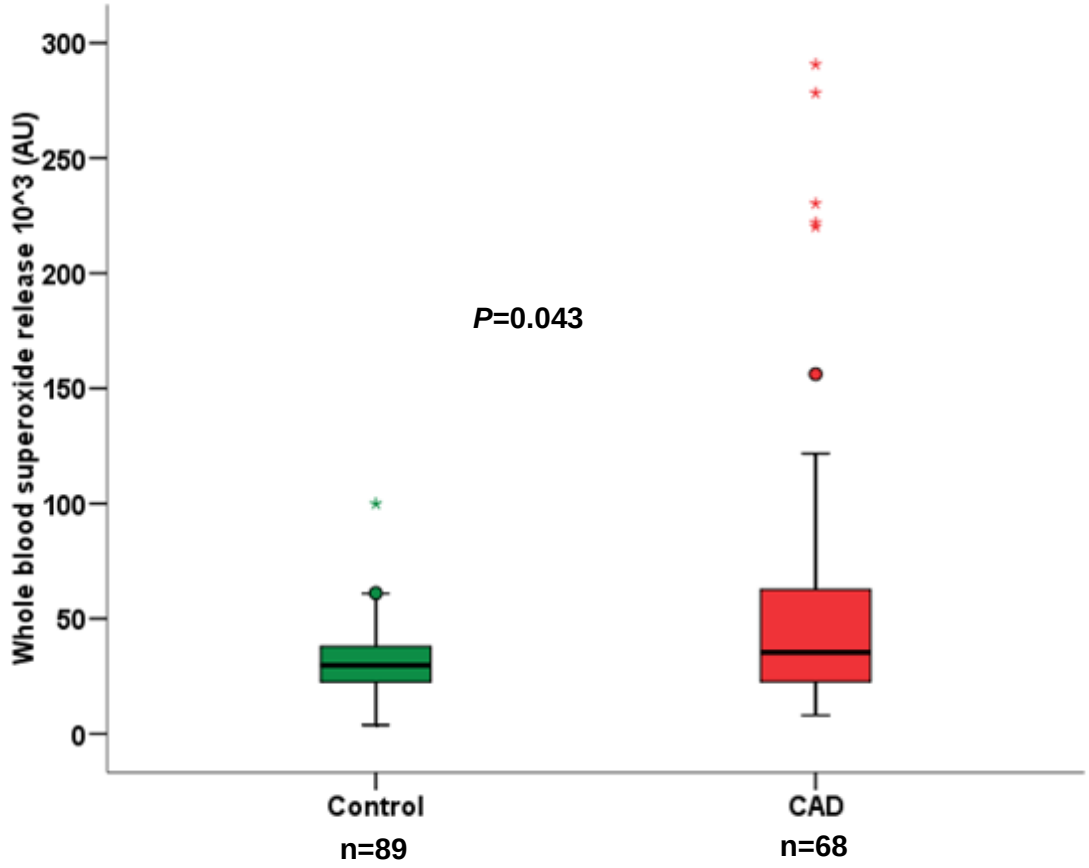


Figure 5.4. Whole blood O_2^- production in patients with CAD compared to controls



5.4.2.2 EPR spectroscopy studies in patients with CAD, with and without type 2 DM

EPR spectroscopy O_2^- studies were performed in 25 patients with CAD and type 2 DM and 64 patients with CAD alone. Demographics and clinical characteristics are shown in table 5.7.There was a higher prevalence of hypertension in the patients with type 2 DM. Blood pressure was not difference between the two groups there was however higher usage of ARB/ACEi, calcium channel blockers and diuretics in patients with type 2 DM.

Biochemistry results are shown in table 5.8.Unsurprisingly patients with type 2 DM had higher HbA_{1c} levels compared to patients with CAD alone. There were no other significant differences between the two groups.

Basal mononuclear cell O_2^- was not increased in patients with type 2 DM compared to those without diabetes, rate 53.7±32.5 vs. 75.7±57.3 AU, P=0.12. In fact there was a trend towards lower O_2^- levels in patients with type 2 DM. There was no difference in stimulated mononuclear cell O_2^- release between patients with and without type 2 DM, 30.2±21.5 vs.34.7±21.4 AU p=0.38 (Figure 5.5). There was also no difference in whole blood O_2^- release between the 2 groups. 60.8±68.2 x 10^3 AU in patients with type 2 DM vs 52.4±53 x10^3 AU in patients without diabetes *P*=0.8 (figure 5.6).The 95% confidence intervals for the difference in mean whole blood superoxide between patients with and without type 2 DM were -35.2 to 33.3 x10^3 AU.

There was a strong correlation between whole blood O_2^- release and basal mononuclear cell O_2^- release, r=0.689, *P*<0.0001 (figure 5.7).

Table 5.7. Demographics and clinical characteristics for patients with CAD with and without type 2 DM in the EPR spectroscopy study

	CAD alone (n=64)	**CAD and type 2 DM (n=25)**	***P*-value**
Age (years)	66.1±9.8	69.1±6.8	NS
Male (%)	48(75)	21(84)	NS
Systolic BP (mm Hg)	138.5±25.4	141.1±18.2	NS
Diastolic BP(mm Hg)	78.5±11.6	74.7±9.9	NS
Heart rate (beats/min)	63.5±12.2	62.4±7.9	NS
Body Mass Index (kg/m^2)	28.8±4.9	30.5±4.2	NS
Current Smoker (%)	5(7.8)	3(12)	NS
Hypertension (%)	28(43.8)	21(84)	0.004
Myocardial Infarction (%)	28(43.8)	14(56)	NS
TIA/CVA (%)	2(3.1)	4(16)	NS
Chronic renal failure (%)	3(4.7)	2(8)	NS
Heart failure (%)	8(12.5)	6(24)	NS
Aspirin (%)	53(82.8)	23(92)	NS
Other antiplatelet agent (%)	16(25)	7(28)	NS
Statin (%)	57(89.1)	23(92)	NS
ACEi/ARB (%)	33(51.6)	19(76)	0.042
Beta-blocker (%)	48(75)	20(80)	NS
Calcium channel blocker (%)	18(28.1)	14(56)	0.026
Nitrate (%)	42(65.6)	14(56)	NS
Diuretic (%)	11(17.2)	10(40)	0.05
Oral hypoglycaemic agent (%)	0	15(60)	n/a
Insulin (%)	0	5(20)	n/a

Continuous variables are mean± standard deviation. Discrete variables are absolute numbers and percentage (%) BP, blood pressure, TIA; transient ischaemic attack, CVA, cerebrovascular accident. ACEi, Angiotensin-converting enzyme inhibitor, ARB, Angiotensin receptor blocker.

Table 5.8 Biochemical analysis for patients with CAD, with and without type 2 DM in the EPR spectroscopy study

	CAD alone (n=64)	**CAD and type 2 DM (n=25)**	***P*-value**
Cholesterol (mmol/L)	4.07±0.87	3.95±0.87	NS
Triglycerides (mmol/L)	1.89±0.82	2.22±1.23	NS
LDL(mmol/L)	2.00±0.74	1.83±0.66	NS
HDL(mmol/L)	1.20±0.28	1.10±0.24	NS
CRP(mg/L)	4.16±9.4	4.45±7.78	NS
HbA_{1c} (%)	5.7±0.35	7.4±1.3	<0.0001
Urinary ACR (mg/mmol)	2.46±6.61	3.6±4.3	NS

All variables mean ± Standard deviation. LDL, low density lipoprotein; HDL, high density lipoprotein; CRP, C-reactive protein; HbA_{1c}, glycated haemoglobin; urinary ACR, urinary albumin:creatinine ratio.

Figure 5.5 Mononuclear cell O_2^- prodction in patients with CAD, with and without type 2 DM (mean+standard deviation)

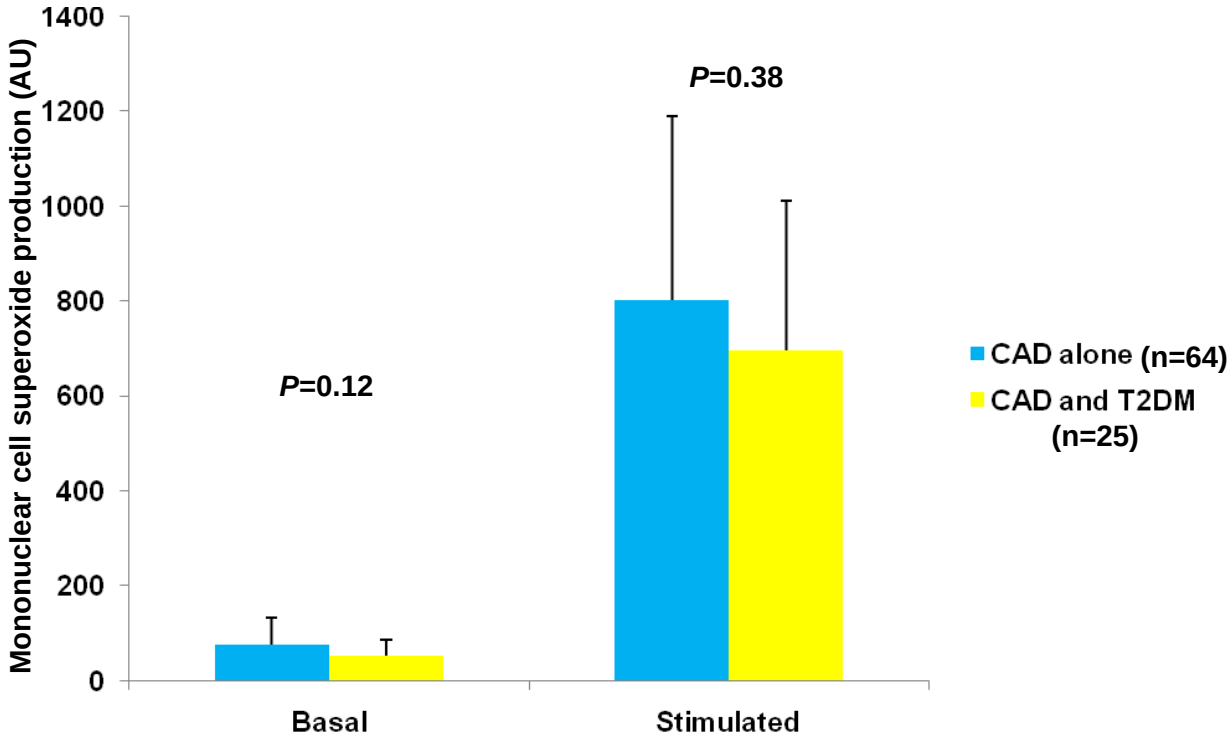


Figure 5.6. Whole blood O_2^- release in patients with CAD with and without type 2 DM.

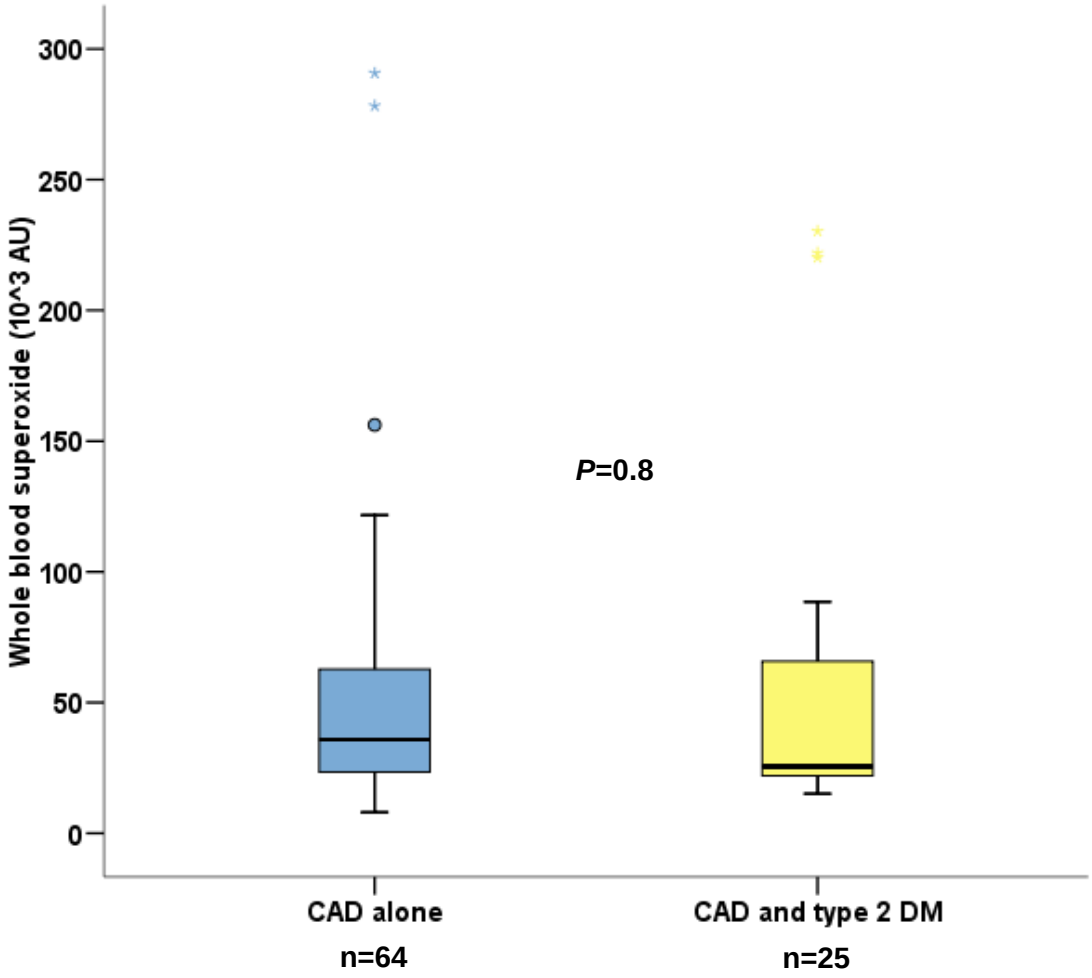
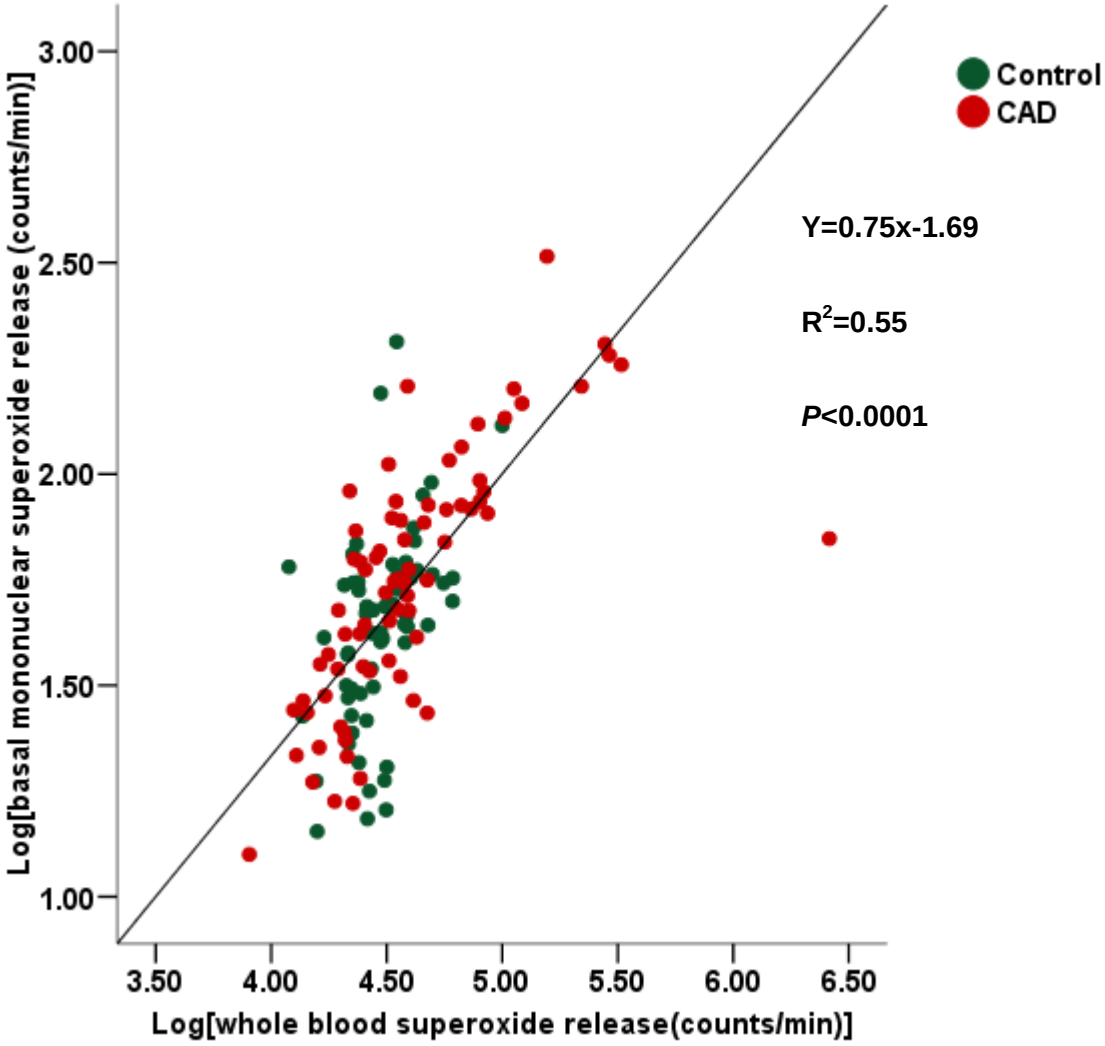


Figure 5.7. Basal mononuclear cell O_2^- release and whole blood O_2^- release.



5.5 Discussion

In this study vascular, mononuclear and whole blood O_2^- was increased in patients with CAD compared to healthy controls. However patients with type 2 DM had similar levels of oxidative stress compared to those without diabetes. Whole blood O_2^- levels are a simple measure of oxidative stress and were strongly correlated with mononuclear O_2^- production. Whole blood O_2^- levels may therefore be a measure of oxidative stress that can be applied to larger studies in the future.

The finding of increased vascular O_2^- production in patients with CAD is in keeping with previous other studies[87;179]. It is understood this is the first study to use EPR spectroscopy techniques to assess mononuclear cell O_2^- production in patients with established CAD. Basal mononuclear cell O_2^- levels may modify oxidative status of blood as it circulates and in addition reduce NO bioavailability contributing to endothelial dysfunction[116]. Stimulated mononuclear cell O_2^- production indicates the responsiveness of the cells to stimuli. Mononuclear cells have been shown to have higher stimulated levels of O_2^- in patients with cardiovascular risk factors[252]. Increased responsiveness may contribute to increased ROS production in vascular tissues. Little was previously known regarding mononuclear production of O_2^- in patients with established CAD. However our finding of increased levels compared to controls is in keeping with other studies that have shown increased phagocyte O_2^- in patients with cardiovascular risk factors[116;252;253]. To the best of knowledge this is the first study to show that whole blood O_2^- levels are increased in patients with CAD and that these levels correlate with phagocyte O_2^- levels. Whole blood O_2^- production may provide information about both systemic levels of oxidative stress and white cell O_2^- release.

It is of interest that patients with CAD have increased levels of oxidative stress despite the use of ACEi/ARBs and statins which have been shown to reduce O_2^- production[262;263]. Elevated levels of oxidative stress are thought to be pivotal in the development of cardiovascular disease

and in particular the development of endothelial dysfunction[84]. The endothelial dysfunction observed in patients with CAD may be partly a consequence of these elevated O_2^- levels. Increased O_2^- levels seen in patients with CAD may be a target for therapy but larger studies are needed to show the prognostic significance of O_2^- and whether lowering these improves cardiovascular outcomes.

Both hyperglycaemia and diabetes have previously been shown to be associated with increased levels of oxidative stress[91;177] However in contrast to other studies the presence of type 2 DM was not associated with increased O_2^- in the vasculature, mononuclear cells or whole blood. There are a number of possible explanations for these findings. Pharmacological management of patients with type 2 DM may be one explanation. ACEi/ARBs, statins and metformin have all been shown to reduce O_2^- production[262;263;265]. In this study a high proportion of patients with type 2 DM were on ACEi/ARBs, in contrast in the study by Guzik et al.[87] where use of ACEi/ARBs was similar between patients with and without diabetes. In this study 36% of patients were taking metformin. It is possible that the patients with type 2 DM had higher levels of vascular superoxide that were controlled by medication.

As has been previously discussed the categorisation of patients in to diabetes or no diabetes is somewhat arbitrary. Hyperglycaemia and insulin resistance are continuous variables. Impaired glucose tolerance and insulin resistance were not assessed formally in our study; however given the elevated body mass index in the control group it is likely that a proportion of these patients had impaired glucose tolerance and insulin resistance. Fortuno et al.[252] showed that in patients with metabolic syndrome mononuclear O_2^- production was related to insulin resistance Patients with CAD without diabetes but with insulin resistance may have attenuated the difference between the two groups.

In chapter 4 it was shown that patients with type 2 DM and CAD had greater degrees of endothelial impairment compared to patients with CAD alone. Increased oxidative stress in patients with type 2 DM was previously believed to explain the impairment in endothelial function[84]. However in this study increased oxidative stress does not explain the greater degrees of endothelial dysfunction found in patients with type 2 DM. This data does not refute the importance of oxidative stress in the development of cardiovascular disease and endothelial dysfunction in patients with type 2 DM. These results do however show that patients with CAD and type 2 DM, on secondary prevention therapies with well controlled cardiovascular risk factors and reasonable glycaemic control do not have elevated oxidative stress. The finding that patients with type 2 DM have greater degree of endothelial dysfunction despite comparable levels of oxidative stress suggests that other mechanisms underlying endothelial dysfunction in this group of patients are important. Furthermore these mechanisms are not being addressed by current cardiovascular management strategies.

Future studies are required to further understand the role of oxidative stress in cardiovascular disease. This includes the prognostic value of O_2^- levels and the impact of decreasing superoxide levels on surrogate endpoints such as endothelial dysfunction but more importantly on cardiovascular outcomes. The use of whole blood O_2^- levels could be applied to larger studies to try and answer these questions. In patients with type 2 DM further studies are required to identify mechanisms underlying impaired endothelial function.

This study clearly has a number of limitations. It is an observational study so although O_2^- levels are elevated in patients with CAD this gives use no information on the consequences of this effect. Furthermore results are only available for a small group of patients making further subgroup analysis limited. Our control groups were not well matched in terms of sex or age. This was partly a consequence of using patients with VV as controls. Healthy controls were recruited form health clubs and this may have biased us to recruiting more female patients. Age

has been associated with increased levels of oxidative stress[266;267]. Gender may also have effects on levels of oxidative stress[79]. This study focused only on one ROS and although O_2^- is felt to be the key ROS others may also be important.

5.6 Chapter summary

This study has found increased levels of oxidative stress in patients with CAD compared to controls. Levels of oxidative stress are however similar between patients with type 2 DM compared to those without. Although oxidative stress may be important in the development of endothelial function and CVD, increased levels of oxidative stress do not explain the greater degree of endothelial impairment seen in patients with CAD and type 2 DM. Whole blood O_2^- levels may be a simple direct measure of ROS suitable for large scale clinical studies.

6 Dyslipidaemia in type 2 diabetes mellitus

6.1 Introduction

Abnormal lipids are a major risk factor for cardiovascular disease (CVD). Type 2 diabetes mellitus (DM) and other insulin resistant states are associated with a characteristic dyslipidaemia. This dyslipidaemia may partly explain the increased endothelial dysfunction in patients with type 2 DM described in chapter 4.

6.1.1 Diabetic dyslipidaemia

In insulin resistant states and type 2 DM abnormalities in lipoprotein metabolism result in a dyslipidemia characterised by high triglyceride levels and low, high density lipoprotein cholesterol (HDL)[40]. Low density lipoprotein cholesterol (LDL) levels are not significantly increased; there is however a preponderance of small dense LDL particles[268]. This has been called the atherogenic lipoprotein phenotype[269]. This phenotype may be partly genetically determined, however insulin resistance appears to play a key role[236].

6.1.2 Hypertriglyceridaemia

The role of elevated triglycerides in the development of CVD is complex. Triglyceride levels are associated with increased risk of CVD[270]. The risks associated with hypertriglyceridaemia decrease after adjustment for HDL levels but remain significant[270]. Much of the increased risk associated with elevated triglycerides appears to be due to various effects and interactions with other lipoproteins[268]. Hypertriglyceridemia and altered metabolism of triglyceride rich lipoproteins is pivotal in the development of the dyslipidemia seen in insulin resistant states[236].

Triglycerides are transported in the circulation as chylomicrons and very low density lipoprotein (VLDL). Type 2 DM and insulin resistance is associated with increased levels of VLDL[40]. Insulin resistance increases three main sources of triglycerides for VLDL assembly in the liver. These are increased mobilisation of fatty acids from the adipose tissue, increased hepatic uptake of VLDL, intermediate LDL (ILDL) and chylomicron remnants, and increased de novo

lipogenesis[271]. Overproduction of VLDL results in low levels of HDL and generation of small dense LDL. This arises through in part to cholesterol transfer protein (CETP)-mediated triglyceride enrichment of LDL and HDL particles and the action of hepatic lipase on these particles[271].

6.1.3 Small dense LDL particles

The structure of LDL is not homogenous. Usually 3 peaks are seen in the LDL density profile termed LDL-I, LDL-II and LDL-III[272]. LDL-I has lowest density and LDL-III, also called small, dense LDL has the highest density. In humans LDL shows a bimodal distribution and can be separated in to two phenotypes. Phenotype A consists of larger more buoyant LDL and pattern B where smaller denser LDL predominates[272]. An increase in small dense LDL particles is a feature of insulin resistance[273]. LDL size correlates positively with HDL levels and negatively with triglyceride levels[274]. Triglyceride is a major determinant of appearance of small, dense LDL particles. Small dense LDL is seen infrequently in patients with plasma triglyceride levels of less than 1.3mmol/L[275]. When triglyceride levels increase above this level small dense LDL increases in proportion to rise in triglycerides. The formation of small dense LDL particles in patients with hypertriglyceridaemia appears in part to be related to hepatic lipase activity[236]. Hepatic lipase activity is increased in insulin resistant states[271]. Hepatic lipase converts triglyceride enriched LDL to small dense LDL It is suggested that raised triglyceride levels are a key determinant of hepatic lipase activity[236].

A number of studies have shown an increased risk of CVD with small dense LDL[276;277]. However in many of these studies after multivariate adjustment small dense LDL is no longer an independent predictor of cardiovascular risk[277]. Small dense LDL may simply be a marker of a cluster of a broader pathology occurring as part of insulin resistant states rather than the driving factor underlying increased atherosclerosis.

Despite the above concerns small dense LDL exhibits a number of features that are proatherogenic. The susceptibility to oxidation increases with decreasing LDL size[278]. Small dense LDL is taken into arterial tissues more readily than larger LDL and resides in the sub-endothelial space for longer due to increased proteoglycan[279]. Small dense LDL is also associated with impaired endothelial function[237].

6.1.4 HDL

HDL levels are low in type 2 DM and other insulin resistant states[40]. Like LDL, HDL is heterogeneous with multiple subclasses of different diameter and density existing[280]. In type 2 DM and other insulin resistant states there are reductions in the larger less dense species of HDL with relative increases in levels of smaller denser HDL [268]. The causes of low HDL in type 2 DM are likely to be multiple. One major factor appears to be transfer of cholesterol from HDL to triglyceride rich lipoproteins and transfer of triglyceride to HDL. Triglyceride rich HDL is hydrolysed by hepatic lipase and cleared from the plasma [268]. As has already been discussed hepatic lipase is increased in insulin resistance [236].

HDL levels are inversely correlated with cardiovascular risk [49;50]. HDL mediates reverse transport of cholesterol. In addition HDL activates eNOS and may be associated with improved endothelial function [211;281].

6.1.5 Managing dyslipidaemia in DM

To date much of the focus on lipid management for CV prevention in type 2 DM has concentrated on the management of LDL. Levels of LDL are an important risk factor for CVD and statin therapy is generally safe and well tolerated [44]. Concentrating on LDL alone will neglect to address important lipid disorders in type 2 DM and may hinder the further prevention of cardiovascular disease in patients with type 2 DM.

6.1.6 Lifestyle interventions

Weight loss through calorie restriction has a beneficial effect on dyslipidaemia in type 2 DM [282]. Low carbohydrate diets may be more effective than other weight loss diets. In a small randomised control study a low carbohydrate diet was associated with greater reductions in triglyceride levels and increase in HDL levels compared to a conventional low calorie, high carbohydrate diet. At 12 months there was no significant difference in weight loss achieved or LDL levels [283] .

Dietary modification with increased intake of omega -3 fatty acids may also be beneficial. Fish oil increased HDL and reduced triglycerides but had no effect on levels of small dense LDL in patients with type 2 DM [53].

Exercise can improve lipoprotein profiles in patients with insulin resistance. In a study of 111 overweight individuals increasing the amount and intensity of exercise increased LDL particle size, HDL levels and reduced triglyceride levels. There were no changes in LDL levels [284] .

6.1.7 Statins

The evidence for the use of statins in the prevention of CVD is extensive [44]. Statin therapy is therefore the mainstay of treatment for the management of dyslipidaemia in patients with type 2 DM. Statins predominantly lower LDL levels through the inhibition of hepatic cholesterol synthesis and up-regulation of LDL receptors. Statins have a lesser effect on triglyceride levels and HDL levels [44]. The effect of statins on levels of small dense LDL is variable with some classes increasing LDL size to a greater degree although results have been variable [277].

6.1.8 Fibrates

The mechanism of action of fibrates is not fully understood however one effect of fibrates is to decrease VLDL levels by increasing lipoprotein lipase activity [271]. Fibrate therapy is therefore associated with increased HDL levels, decreased triglyceride levels and a decrease in small

dense LDL [285]. There have been a number of fibrate studies assessing surrogate markers of atherosclerosis progression that showed promising results. The Diabetes Atherosclerosis study assessed the effect of fenofibrate on atherosclerosis progression as assessed by angiography [286]. Fibrate therapy was associated with a reduction in the progression of angiographic markers of atherosclerosis. This effect appears to be partly mediated by changes in LDL size associated with fenofibrate [287].

The results from large prospective clinical outcome trials of fibrates have been mixed however. The Veterans Affairs High-Density Lipoprotein Intervention study trial (VA-HIT) showed that in men with established coronary artery disease (CAD) and low HDL levels gemfibrozil reduced the risk of major cardiovascular events [288]. The use of other lipid lowering medication in particular statins were extremely low in this study. The Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study looked at fenofibrate in over 9000 patients with type 2 DM [289]. Compared to placebo fenofibrate did not reduce the primary endpoint of cardiovascular events there was however a significant reduction in non-fatal myocardial infarction and revascularisation [289]. In the FIELD study a significantly higher proportion of patients in the placebo group commenced statin therapy compared to those in the treatment group. This may account for the apparent lack of benefit seen with fenofibrate. Most recently the lipid arm of the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial aimed to address whether intensive lipid management would be associated with further cardiovascular risk reduction [290]. Over 5000 patients were randomised to either simvastatin and fenofibrate or simvastatin alone. There were no significant differences in LDL or HDL cholesterol between the two groups. Fibrate therapy was associated with a significant reduction in triglyceride levels. Combination therapy was not associated with a reduction in cardiovascular risk compared to statin therapy alone. Subgroup analysis however showed there appeared to be benefit in patients with a high baseline triglyceride and low baseline HDL levels[290]. At present there is no evidence supporting

the widespread use of fibrates in patients with type 2 DM. Fibrate therapy may however be beneficial in selected groups, and further research is required.

6.1.9 Nicotinic acid

Nicotinic acid predominantly raises HDL levels but in addition reduces triglyceride, LDL and small dense LDL levels [285]. Nicotinic acid is associated with reduction of atherosclerosis assessed during angiography and significant regression of carotid intima media thickness [291;292]. There is however only one cardiovascular outcome study, Coronary Drug Project [293]. This study showed a reduction in myocardial infarction with niacin. Results of large clinical trials with niacin are awaited. There is concern regarding the use of niacin in patients with type 2 DM as it is associated with impaired glycaemic control. This effect however appears to be minimal when niacin is used in low doses.

6.1.10 Summary

Type 2 DM is commonly associated with a mixed dyslipidaemia of raised triglycerides, low HDL and increased small dense LDL levels. This dyslipidaemia appears to be a consequence of insulin resistance and may explain the increased cardiovascular risk. These lipid abnormalities may also partly account for the impaired endothelial function seen in patients with type 2 DM and other insulin resistant states. Strategies that specifically tackle these lipid abnormalities may be beneficial in preventing CVD in these groups, although evidence from clinical trials to support this hypothesis is limited at present.

6.2 Aims

In chapter 4 patients with type 2 DM and CAD were shown to have impaired endothelial function compared to patients with CAD alone. In this cohort oxidative stress does not appear to account for this difference in endothelial function. Type 2 DM is associated with a typical dyslipidaemia. These lipid abnormalities may persist in patients with type 2 DM and established

CAD and may partially account for the greater impairment in endothelial function. The hypothesis was therefore that patients with type 2 DM and CAD would continue to have elevated triglyceride levels, low HDL and a preponderance of small dense LDL particles compared to patients without diabetes. Furthermore that this lipid abnormality may explain the greater endothelial dysfunction found in patients with CAD and diabetes compared to patients with CAD alone.

The aims of this study were to perform detailed lipid analysis including LDL and HDL subfraction analysis in patients with CAD undergoing CABG with and without type 2 DM to try and explain the difference in endothelial function that had previously been observed.

6.3 Methods

Patients with established CAD with and without diabetes were recruited as part of the VASCAB study. Detailed lipid analysis was performed in these patient according to the methods described in chapter 2.

6.4 Results

In total 90 patients with CAD alone and 36 patients with type 2 DM and CAD were recruited as part of the VASCAB study. Demographics and clinical characteristics are shown in table 6.1. Patients with type 2 DM had significantly higher body mass index (BMI) compared to those without diabetes. In patients with type 2 DM prevalence of hypertension was significantly higher as was the use of modifiers of the renin-angiotensin system and diuretics.

There was no difference in LDL levels in patients with type 2 DM compared to those without. There was however a preponderance of small dense LDL particles in patients with type 2 DM. LDL peak particle diameter (25.6±2.24 *vs* 25.8±0.53 nm; *P*=0.010) and mean particle diameter (25.8±2.24 *vs* 26.0±0.44 nm, *P*=0.035) were significantly smaller (table 6.2). The proportion of

LDL present in fraction 3 (small LDL-III) was greater in subjects with diabetes (53±2 *vs.* 47±1%, *P*=0.041) (Figure 6.1). Levels of HDL were significantly lower in patients with diabetes compared to those without diabetes (1.07±0.05 *vs.* 1.27±0.04 mmol/L, *P*=0.015, table 6.2). There was a trend towards smaller dense particles (measured as mean particle diameter) although this was not significant. There was a trend to greater triglyceride levels in patients with type 2 DM although this was not statistically significant (Table 6.2).

Table 6.1. Demographics and clinical characteristics of patients with CAD and type 2 DM compared to CAD alone

	CAD alone (n=90)	CAD and Type 2 DM (n=36)	*P*-value
Age (years)	65.7±9.2	66.9±10.0	NS
Male (%)	67(77)	31 (83.8)	NS
Systolic BP (mm Hg)	139.0±24.8	140.3±19.7	NS
Diastolic BP (mm Hg)	78.8±11.3	74.7±12.7	NS
Heart rate (beats/min)	64.0±12.6	64.6±11.6	NS
Body Mass Index, kg/m^2	28.7±4.8	31.3±4.6	0.014
Current smokers (%)	8(9.2)	3(8.1)	NS
Hypertension (%)	36(41.4)	33(89.2)	0.0001
Myocardial Infarction (%)	41(47.1)	22(59.5)	NS
TIA/CVA (%)	5(5.7)	4(10.8)	NS
Chronic renal failure (%)	5(5.7)	2(5.4)	NS
Heart failure (%)	11(12.6)	11(29.7)	NS
Aspirin (%)	72(82.8)	33(89.2)	NS
Other antiplatelet agent (%)	26(29.9)	9(24.3)	NS
Statin (%)	76(87.4)	35(94.6)	NS
ACEi/ARB (%)	45(51.7)	30(81.1)	0.007
Beta-blocker (%)	64(73.6)	31(83.8)	NS
Calcium channel blocker (%)	25(28.7)	17(45.9)	NS
Nitrate (%)	51(58.6)	19(51.4)	NS
Diuretic (%)	14(16.1)	18(48.6)	0.001
Oral hypoglycaemic agent (%)	0	23(62.2)	n/a
Insulin (%)	0	8(21.6)	n/a

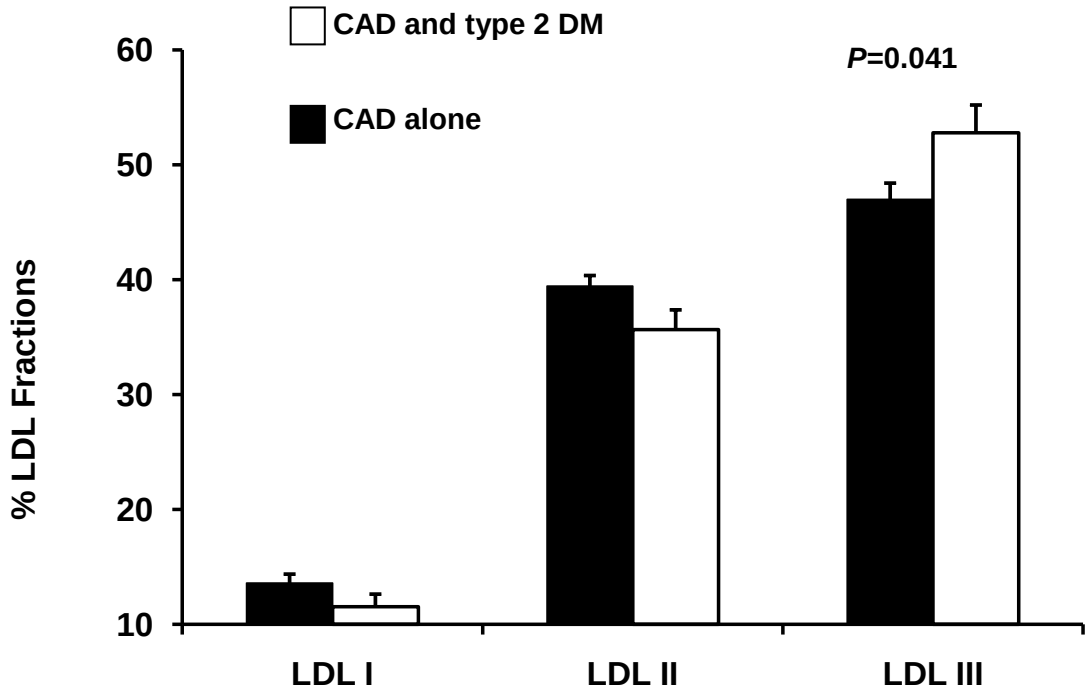
Continuous variables are mean± standard deviation. Discrete variables are absolute numbers and percentage (%) BP, blood pressure, TIA; transient ischaemic attack, CVA, cerebrovascular accident. ACEi, Angiotensin-converting enzyme inhibitor, ARB, Angiotensin receptor blocker.

Table 6.2 Lipid results for patients with type 2 DM and CAD compared to CAD alone

		Type 2 diabetes		No diabetes	*P*-value
	n	mean±SEM	n	mean±SEM	
Total Cholesterol (mmol/L)	36	3.83±0.17	90	4.09±0.10	**NS**
LDL-cholesterol (mmol/L)	34	1.79±0.12	90	2.00±0.08	**NS**
LDL MPD (nm)	28	25.78±0.08	79	25.98±0.05	**0.035**
LDL PPD(nm)	28	25.56±0.08	79	25.83±0.06	**0.010**
HDL-cholesterol (mmol/L)	36	1.07±0.05	90	1.21±0.04	**0.015**
HDL MPD (nm)	27	9.57±0.03	80	9.65±0.02	**0.075**
Triglycerides (mmol/L)	36	2.38±0.29	84	1.92±0.10	**NS**

SEM standard error of the mean; LDL low density lipoprotein; HDL, high density lipoprotein; MPD, mean particle diameter; PPD, peak particle diameter.

Figure 6.1. LDL particle size in patients with CAD and type 2 DM compared to CAD alone



6.5 Discussion

This study has shown that LDL levels are similar in patients with and without type 2 DM but there was a preponderance of small dense LDL particles. In addition HDL levels were lower in patients with type 2 DM. Triglyceride levels were increased in patients with type 2 DM but were not significantly different from levels in patients without diabetes.

Low HDL levels and increased small dense LDL particles have been reported by other groups as a feature of type 2 DM and other insulin resistant states [117;273]. Hypertriglyceridaemia is thought to be an important factor driving this abnormality [271]. Although triglyceride levels were elevated in patients with type 2 DM, the difference was only moderate and not statistically significant. Other groups have reported that triglyceride levels above 1.3mmol/L are associated with increase in small dense LDL particles; triglyceride levels that are within the range seen in patients with type 2 DM in this study [275].

Small dense LDL particles are associated with endothelial dysfunction [237]. The finding of increased small dense LDL in this study may explain the greater endothelial dysfunction associated with type 2 DM described in chapter 2. The precise mechanisms underlying small dense LDL and endothelial dysfunction are not fully understood. However small dense LDL has greater susceptibility to oxidation and oxidised LDL reduces endothelium dependent vasodilation [278;294]. Although small dense LDL is associated with increased CV risk the effect of LDL size as a predictor is often lost during multivariate adjustment further more at present the effect of modifying LDL particle size is unclear [277].

The finding of significantly lower HDL levels in patients with type 2 DM may also explain the greater impairment of endothelial function described in patients with type 2 DM. HDL levels are a determinant of endothelial function and may be more important than LDL particle size [193;209].

HDL can activate and increase expression of eNOS which may partly account for the association between HDL levels and endothelial function [211;281].

The patients in this study all had established CAD and were undergoing elective CABG. These patients should therefore be on optimal secondary prevention therapies. Use of statins was high in patients with and without diabetes reflected by the low LDL levels found in both groups. Indeed patients with type 2 DM had a trend towards lower LDL levels. The fact that patients with type 2 DM in this study continued to have low HDL levels and a preponderance of small dense LDL highlights the limitations of current secondary prevention strategies. Statin therapy will only have some impact on LDL particle size and HDL levels [44;236]. This study was not powered to analyse the effect of different statins on lipid profiles although other studies have suggested that different statins may have different effects on LDL particle size [277]. Currently there is no evidence from clinical trials to support the use of one statin above another independent of LDL levels [44]. More information on the effect of modifying LDL size on clinical outcomes is needed.

The dyslipidemia found in patients with type 2 DM compared to those without may partly explain the impaired endothelial dysfunction found in these patients. Future studies are needed to show whether addressing LDL particle size and HDL levels improves endothelial function and cardiovascular outcomes. Current management strategies are limited. There is currently no evidence to support the widespread use of fibrates in patients with type 2 DM, although the use in selected groups may be beneficial [290]. Other options for improving dyslipidaemia associated with insulin resistant states include weight loss, dietary modification and increasing levels of physical activity. However with all of these interventions it will be difficult to separate whether modifying LDL size or increasing HDL levels are most important as the two variables are so inextricably linked.

This study has a number of limitations. Patient with type 2 DM were found to have both impaired endothelial function and increased small dense LDL and low HDL but since this is a cross-sectional study conclusions regarding causation cannot be made. The small numbers of the study limited subgroup analysis. The impact of addressing the specific lipid abnormalities in patients with type 2 DM on endothelial function is not known. The relative importance on the different aspects of diabetes related dyslipidaemia can also not be determined.

6.6 Chapter summary

In patients with type 2 DM and CAD there is a preponderance of small dense LDL particles and low HDL levels compared to patients with CAD alone. These lipid abnormalities may account for the impaired endothelial function associated with type 2 DM. Strategies to specifically address these abnormalities may improve endothelial function and cardiovascular outcomes in patients with type 2 DM.

7 Strategies for managing cardiovascular disease in type 2 diabetes mellitus; the role of low density lipoprotein lowering

7.1 Introduction

7.1.1 Low density lipoprotein and cardiovascular disease

Low density Lipoprotein (LDL) cholesterol is an important risk factor for cardiovascular disease (CVD) [41]. Lowering LDL significantly reduces rates of CVD. Although lowering LDL by any means, including surgical methods is effective in reducing CVD the majority of the evidence comes from statin trials [42;43]. The Cholesterol trialists collaboration showed a 20% reduction in cardiovascular events per mmol/L reduction in LDL [44]. This benefit was seen across the range of LDL concentrations. Guidelines for the management of cardiovascular disease in high risk patients are therefore recommending increasingly low LDL-cholesterol targets in patients at high cardiovascular risk [22-24]. Although patients with type 2 diabetes mellitus (DM) do not have elevated LDL levels compared to those without diabetes, considering the increased cardiovascular risk in patients with type 2 DM aggressive LDL lowering is one strategy for reducing CVD in these patients.

LDL-cholesterol contributes to the development of CVD through a number of mechanisms. LDL-cholesterol is a key component of atherosclerotic plaques; however the role in the development of CVD is much more complex than simple accumulation of lipid within the vessel wall. LDL-cholesterol contributes to oxidative stress and endothelial dysfunction that underlie the atherosclerotic process [87;179].

LDL-cholesterol levels are an important determinant of endothelial function[179]. Lowering LDL improves endothelial function. This is seen in statin therapy and by removal of LDL by apheresis [207;295;296]. LDL can impair endothelial function through a number of mechanisms. LDL-cholesterol levels are an important determinant of oxidative stress [87;179]. Furthermore Oxidised LDL-cholesterol can impair endothelial nitric oxide synthase (eNOS) activity by a

depletion of caveolae cholesterol, a redistribution of eNOS away from caveolae and a diminished capacity to activate eNOS [297;298].

7.1.2 Statin therapy

Statins are effective at lowering LDL levels, are generally safe and well tolerated and therefore provide the mainstay of cholesterol lowering therapy. It can be difficult to differentiate between lipid effects and statin effects. Clearly achieving lower LDL targets will usually require more intensive statin therapy so LDL levels may be a surrogate marker for the intensity of statin therapy. Previous studies have shown that lipid lowering with statins is associated with improvements in endothelial function [207]. Endothelial function improves rapidly following the initiation of statin therapy and is not related to the degree of LDL lowering [208]. Statins have a number of effects that can contribute to improved endothelial function independent of lipid effects. Statins can increase eNOS expression; phosphorylation and activation [299]. Arginase competes with eNOS for L-arginine and has been implicated in development of endothelial dysfunction [202]. Statins inhibit a number of small signalling molecules for example inhibition of RhoA with consequent inhibition of arginase activity [299]. Statins also reduce superoxide production through inhibition of vascular NAD(P)H oxidise expression and recoupling of eNOS [263;300].

The clinical significance of the apparent lipid independent effects of statins is not certain. Meta-analysis of the large statin trials shows no evidence that statins either as a class or individually provided additional cardiovascular risk reduction above that expected from degree of LDL lowering achieved [301]. Indeed lowering LDL surgically by ileocecal bypass associated with similar risk reductions to those seen in statin prevention trials [43].

Statin therapy is generally safe and well tolerated. Statin therapy can be associated with rhabdomyolysis although this is rare. The cholesterol trialists collaboration showed there was no

significant difference in rhabdomyolysis in patients on long term statins compared to controls [44]. This analysis did not contain many high dose statin trials; and as rate of rhabdomyolysis is dose dependent high dose statin therapy may alter this risk. More recently concerns were raised regarding risk of DM with statin therapy. A meta-analysis by Sattar et al.[302] found an increased risk of newly diagnosed DM with statin therapy. The risk of diabetes was small and the clinical significance is unclear. Furthermore the possible mechanisms underlying this association are not known. The lower LDL targets that are increasingly recommended are likely to increase the use of higher doses of statin therapy. It is therefore important as with all preventative therapy to reconsider both the risks and benefits of this strategy.

There is a limit to the reduction in LDL levels that can be achieved with statin therapy alone. Combining other lipid lowering agents with statins are therefore of interest. Recent studies however have questioned the benefit of achieving lower LDL levels with combination therapy. In patients with familial hypercholesterolaemia there was no additional benefit on carotid intima media thickness with the addition of ezetimibe despite lower LDL levels [303] . More recently the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial showed no additional benefit on cardiovascular outcomes in patients with type 2 DM following the addition of fibrates to statin therapy, although LDL levels were not different between the two groups [290]. The value of intensive lipid lowering and the choice of drugs to achieve this are still under debate.

7.1.3 Summary

In summary LDL cholesterol is an important factor in the development of CVD and a key determinant of endothelial function. Lowering cholesterol levels by any means improves cardiovascular outcomes. Statin therapy appears to have a number of lipid independent effects that may further contribute to the improvements in cardiovascular outcomes, however the clinical significance of these effects are uncertain. More intensive lipid lowering is recommended for high risk groups. The effect of these recommendations on the processes

underlying cardiovascular disease, such as endothelial function and oxidative stress is currently uncertain.

7.2 Aims

The hypothesis was that aggressive control of LDL cholesterol levels in patients with established coronary artery disease (CAD) in line with current secondary prevention protocols will lead to improvement in endothelial function and reduced oxidative stress.

The aims of this study were therefore to compare lipid profiles, endothelial function, and levels of vascular superoxide (O_2^-) in a group of patients who underwent coronary artery bypass graft surgery in 2003 with patients who underwent surgery in 2007.

7.3 Methods

Patients undergoing CABG for severe CAD were recruited as part of the VASCAB study and comprised the 2007 CAD cohort. The 2007 cohort of patients was compared to a cohort undergoing CABG in 2003. Clinical examination, routine biochemistry, vascular O_2^- and ex vivo endothelial function was performed in both 2003 and 2007 cohorts. VV control patients recruited in 2003 and 2007 were recruited to confirm consistency of assessments for vascular O_2^- and *ex vivo* endothelial function. Detailed methods are provided in chapter 2.

7.4 Results

7.4.1 Clinical characteristics and cholesterol levels

The 2007 group was slightly older and contained more males than the 2003. Diastolic blood pressure was lower in the 2007 group (Table.7.1). Control subjects undergoing VV surgery were similar between 2003 (n=19; age 48±13 years) and 2007 (n=19; age 48±13 years). In patients with CAD, statin use increased from 88% in 2003 to 94% in 2007 (P=0.038). Average statin dose increased from 26±16 mg/d in 2003 to 37±17 mg/d in 2007 (P<0.001). Lower total

(4.0±0.9 *vs.* 4.8±1.0 mmol/L; *P*<0.001) and LDL cholesterol levels (2.0±0.7 *vs.* 3.0±0.9 mmol/L; *P*<0.001) in 2007 reflect this increased statin usage (figure 7.1). HDL cholesterol levels were unchanged between 2007 and 2003 (1.1 [0.9: 1.3] *vs.* 1.2 [1.0; 1.4] mmol/L; *P*=0.124; Figure 7.1).

7.4.2 Endothelial function

In patients with CAD endothelium dependent vasodilation, measured as the maximum relaxation of saphenous veins to calcium ionophore A23187, was significantly greater in the 2007 compared to the 2003 group (44±15% *vs.* 28±12%, *P*<0.001, n=36 each; Figure 7.2). Endothelium-dependent vasodilation remained unchanged in saphenous vein from control subjects between 2003 and 2007(maximum response to calcium ionophore A23187 in 2003: 60±11 %, in 2007: 65±15 %; *P*=0.252; n=14).

There was an inverse correlation between LDL cholesterol and endothelial function (r=-0.482, *P*<0.0001 figure 7.3) Linear regression analysis was performed with endothelial function (vasodilatory response to calcium inophore) as the dependent variable and characteristics that were different between 2003 and 2007 groups (age, sex, diabetes status, smoking status, LDL-C levels, diastolic blood pressure, statin dose and ACEI/ARB usage) together with a variable indicating year of study as predictors (table 7.2). Only LDL cholesterol contributed significantly to endothelial function explaining 15.6% of its variability.

7.4.3 Vascular O_2^- generation

Levels of total vascular O_2^- were not significantly different between the 2003 and 2007 groups (0.77 [0.53; 1.08] *vs.* 0.50 [0.37; 0.85] nmol/mg/min; *P*=0.053; n=33 each) although there was a trend towards lower levels in the 2007 group (Figure 7.4). Vascular O_2^- production remained unchanged in control subjects between 2003 and 2007 (0.47±0.26 *vs.* 0.43±0.32 nmol/mg/min, *P*=0.697; n=14).

Table 7.1 Demographics and clinical characteristics for patients with CAD in 2003 and 2007

	2003 n=121	**2007 n=105**	***P*-value**
Age (years)	62 ± 9	65 ± 10	**0.028**
Male (%)	77 (64%)	84 (80%)	**0.003**
BMI (kg/m²)	28.7 ± 4.9	29.5 ± 5.0	**0.243**
Systolic BP (mmHg)	140 ± 14	140 ± 24	**0.999**
Diastolic BP (mmHg)	82 ±11	78 ± 12	**0.009**
Type 2 DM (%)	27 (22%)	28 (27%)	**0.092**
Active smokers* (%)	28 (23%)	18 (17%)	**0.071**
Aspirin (%)	103 (85%)	88 (84%)	**0.140**
Beta blocker (%)	75 (62%)	67 (64%)	**0.106**
ACEI or ARB (%)	58 (48%)	55 (52%)	**0.085**
Statin (%)	107 (88%)	99 (94%)	**0.038**
Simvastatin	68	58	**0.148**
Atorvastatin	19	30	**0.073**
Pravastatin	11	4	**0.069**
Rosuvastatin	0	2	**n/a**
Fluvastatin	0	1	**n/a**

*Current smokers and recent history of smoking. Continuous variables are mean± standard deviation. Discrete variables are absolute numbers and percentage (%) BP, blood pressure, ACEi, Angiotensin-converting enzyme inhibitor, ARB, Angiotensin receptor blocker.

Table 7.2 Determinants of endothelial function in the combined 2003 and 2007 cohort

	Full model (R^2=0.275)		**Stepwise model (R^2=0.156)**	
	β	*P*-value	β	*P*-value
Age	0.062	0.558	_	_
Sex (0=female;1=male)	0.005	0.965	_	_
Type 2 DM (0=no;1=yes)	-0.061	0.562	_	_
Active smoking (0=no,1=yes)	0.128	0.245	_	_
Diastolic BP	-0.163	0.140	_	_
LDL cholesterol	-0.0286	0.022	-0.395	<0.001
ACEI/ARB (0=no,1=yes)	-0.025	0.856	_	_
Statin dose	-0.058	0.597	_	_
Year of study	0.343	0.027	_	_

In the full model all variables were forced into the model. The stepwise model was developed using probabilities of *F* to enter and remove variables of ≤0.05 and ≥0.10 respectively. β indicates the partial correlation coefficients, ACEI, Angiotensin-converting enzyme inhibitor; ARB, Angiotensin receptor blocker.

Figure 7.1 Total cholesterol, LDL and HDL in patients with CAD in 2003 and 2007

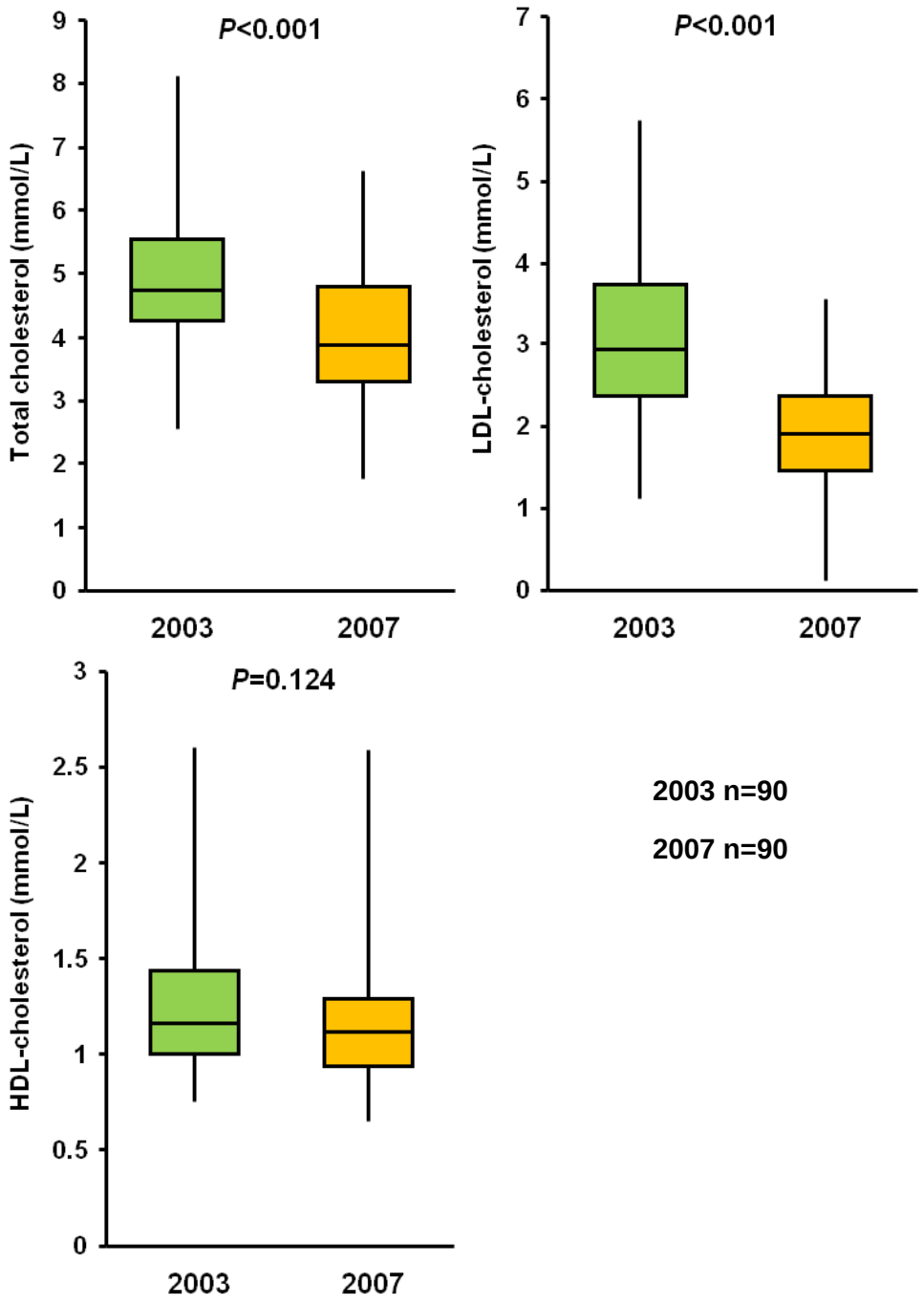


Figure 7.2 Endothelial function in patients with CAD in 2003 and 2007

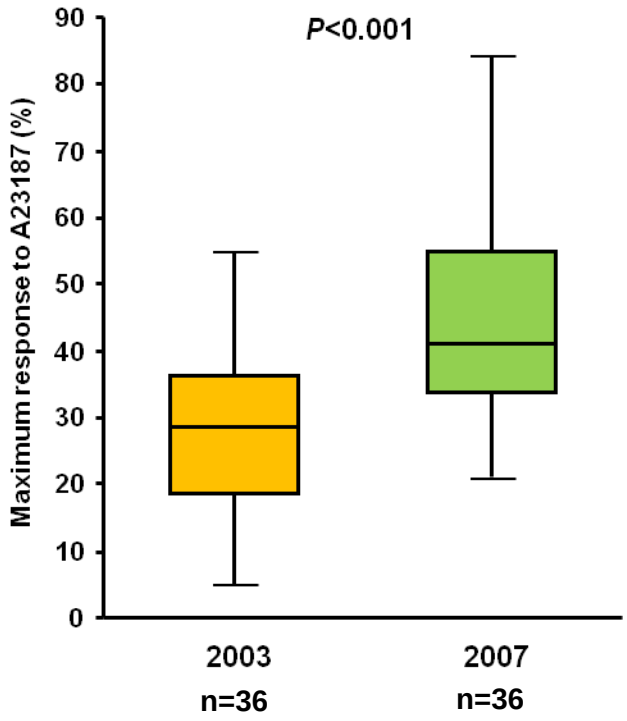


Figure 7.3 Scatterplot of maximum response to calcium ionophore and LDL-cholesterol levels

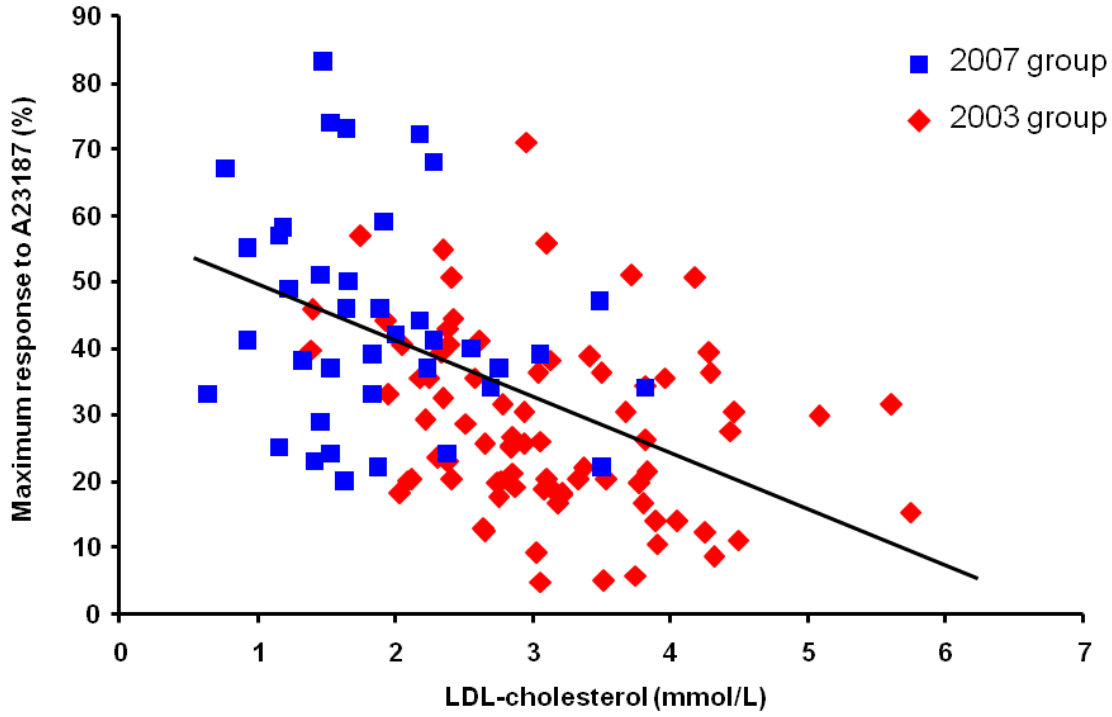
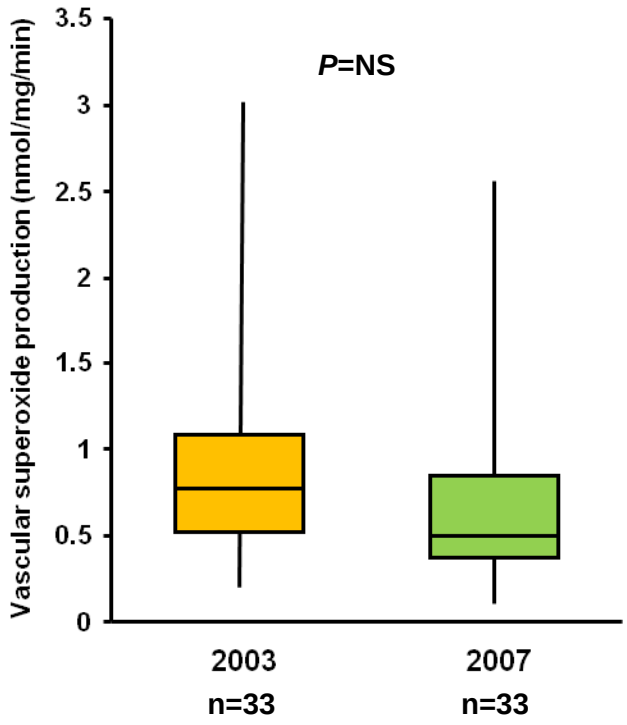


Figure 7.4.Vascular O_2^- levels in patients with CAD in 2003 and 2007



7.5 Discussion

The results of this study show that between 2003 and 2007 statin use increased and LDL levels decreased. This was paralleled by improved endothelial function in patients with CAD. Endothelial function did not change in control subjects between 2003 and 2007. Endothelial function is an independent predictor of morbidity and mortality in patients with cardiovascular disease [187-189]. The improvement in endothelial function may provide surrogate evidence for improved cardiovascular outcomes as a result of increased targeting of LDL-cholesterol levels.

LDL was a significant determinant of endothelial function. This is in keeping with previous studies [108;204;205]. LDL levels can contribute to endothelial dysfunction through a number of different mechanisms. LDL-cholesterol can impair functioning of eNOS and increase oxidative

stress [297;298]. This will reduce the bioavailability of NO and result in impaired endothelium dependent vasodilation.

LDL levels only accounted for 16% of the variability in endothelial function. Clearly other factors in addition to LDL are important in determining endothelial function. Increased oxidative stress has frequently been implicated as a major mechanism underlying endothelial dysfunction. Both LDL and oxidised LDL increase superoxide production through xanthine oxidase, NAD(P)H oxidase and uncoupling of eNOS [304-306]. Interestingly, despite improvements in endothelial function and reduced levels of LDL-cholesterol there were no changes in levels of O_2^- between 2007 and 2003. In this study changes in levels of oxidative stress do not account for the improvement in endothelial function seen in the 2007 group. Other determinants of endothelial function that differed between 2003 and 2007 included use of ACEi/ARBs, and diastolic blood pressure. However these were not significant predictors of endothelial function in this cohort of patients.

Statin dose and the choice of statins differed between the two groups. Statins may have a lipid independent effect on endothelial function [208]. This effect may be different between different statins. Statin dose was significantly different between the two groups but was not a predictor of endothelial function. The study was too small to analyse the effects of different statins.

A number of other factors that have been associated with endothelial function were not analysed in this study. LDL particle size may be of particular relevance. LDL particle size is associated with endothelial function, with small dense LDL being associated with greater impairment of endothelial function [237]. Statin therapy can modulate LDL size causing a shift to larger less dense species [307]. There is however wide variation between the different statins. Fluvastatin and atorvastatin are reported most frequently as having the greatest beneficial effect on LDL size, pravastatin the least [277].

Although lower LDL levels in this study were associated with improved endothelial function there is clearly other factors that are important in determining endothelial function. Targeting LDL levels can only therefore partly correct endothelial dysfunction. Future studies to identify these other determinants are required if further improvements in endothelial function and cardiovascular outcomes are to be achieved. It would be of interest to see how LDL-cholesterol particle size compared between the 2003 and 2007 groups. With regards to statin and LDL lowering future studies are required to try and distinguish between statin and LDL effects. Finally outcome studies are required to assess whether the improvements in endothelial function are associated with improvements in cardiovascular outcomes.

In chapter 4 diabetes status was the major determinant of endothelial function. Lack of HbA_{1c} measurement in the 2003 cohort limited the direct comparison of endothelial function in patients with diabetes in 2007 cohorts. From case note review HbA_{1c} was available for a proportion of the patients recruited in 2003. The HbA_{1c} levels were however not taken at similar time prior to surgery. Although HbA_{1c} gives an indication of blood glucose levels over the preceding 3 months, HbA_{1c} is a weighted average of blood glucose levels over this time. This means that blood glucose levels over the preceding 30 days contribute significantly more to HbA_{1c} levels compared to blood glucose levels over days 90-120. It was therefore felt that using HbA_{1c} levels that had been obtained over a range of weeks and months was not appropriate. Although the effects of LDL lowering would not be expected to be any less in patients with type 2 DM the specific effect on patients with type 2 DM remains unclear and cannot be addressed by this study.

Clearly the major limitation of this study is that it is observational rather than a randomised controlled trial. It is therefore not possible to make any conclusions as to whether LDL levels and statin therapy cause the differences in endothelial function observed or are merely a markers for other changes that exist between the two groups. Furthermore this study cannot distinguish

relative contributions between LDL levels and statin dose and individual statins. The study initially aimed to investigate the effect of aggressive management of LDL-cholesterol levels in patients with and with type 2 DM. A number of features of the study design prevented this analysis. The small numbers in this study limited sub group analysis. Furthermore as already discussed measures of hyperglycaemia such as HbA_{1c} were not available for the 2003 cohort.

7.6 Chapter summary

In summary this study has found a significant improvement in endothelial function in patients with established CAD in 2007 compared to 2003. This is paralleled by lower LDL cholesterol levels and increased statin usage. This improvement in endothelial function was not associated with a reduction in levels of oxidative stress. Endothelial function is an independent predictor of morbidity and mortality and may provide surrogate evidence for improved survival of patients with CAD as a consequence of current secondary prevention guidelines. LDL-cholesterol levels however only explain a small proportion of the variability in endothelial function. Further studies are therefore required to elucidate the other mechanisms underlying endothelial dysfunction in patients with CAD.

8 Strategies for managing cardiovascular disease; the role of physical activity

8.1 Introduction

8.1.1 Sedentary lifestyles and cardiovascular disease

For over 50 years it has been recognised that sedentary lifestyles are an important risk factor for cardiovascular disease (CVD). In 1953 the now classic epidemiological study by Morris et al.[58] showed that bus conductors compared to bus drivers had lower rates of coronary artery (CAD). Furthermore when conductors were diagnosed with CAD it tended to be later in life with a lower mortality. This difference was partly attributed to the conductors being more physically active at work compared to bus drivers. Since then numerous studies have confirmed a reduction in levels of CVD with increased levels of physical activity [69;71]. Increased levels of physical activity also reduce the risk of further cardiovascular events in patients with established CVD [71]. For the prevention of CVD it is recommended that individuals take 30 minutes of regular physical activity most days of the week [22]

Part of the reduction in cardiovascular events can be explained by the effect on conventional risk factors. Increasing levels of physical activity increased high density lipoprotein (HDL) levels by approximately 3% in both men and women in Heritage study [51]. Meta-analysis of exercise studies on total cholesterol and low density lipoprotein (LDL) levels showed more variable results with some but not all studies reporting moderate reductions of total and LDL cholesterol following interventions aimed at increasing physical activity [308]. Increasing physical activity reduces blood pressure on average by 3.4/2.4 mmHg however greater reductions can be achieved in hypertensive individuals [309] Not all the risk reduction can be explained by reductions in conventional risk factors, there appears to be an independent effect of exercise [69]. Other mechanisms that may contribute to the beneficial effects of increased physical activity include reduced levels of oxidative stress, improved endothelial function, reduced insulin resistance, reduced inflammation and improved lipoprotein subclass distribution [310].

8.1.2 Sedentary lifestyles and type 2 diabetes mellitus

Increasing physical activity is important in both the prevention and management of type 2 diabetes mellitus (DM). Three large studies the Diabetes Prevention Study (DPS) Diabetes prevention project (DPP) and The Da Qing IGT and Diabetes study looked at the role of physical activity in the prevention of type 2 DM in high risk populations [311-313]. All studies showed a large and significant reduction in the progression to type 2 DM in individuals randomised to exercise programmes. In patients with established type 2 DM meta-analysis has shown that increasing physical activity reduces HbA_{1c} by approximately 0.66% [314]. This is a clinically significant result with HbA_{1c} reductions comparable to those achieved in UKPDS and of a magnitude that would be expected to result in a reduction of diabetes related complications.

8.1.3 Physical activity as a strategy for tackling CVD in patients with type 2 DM

Physical activity may reduce the excess cardiovascular risk associated with insulin resistant states such as type 2 DM. The Whitehall study looked at the relationship between levels of physical activity and CAD mortality in men with impaired glucose tolerance/diabetes and normal glycaemia [315]. In men with impaired glucose tolerance/diabetes the relationship between levels of physical activity and CAD mortality was steeper compared to the normoglycaemic men. The risk reduction associated with increased physical activity was therefore greater in patients with impaired glucose tolerance/diabetes compared to normoglycaemic individuals [315]. Similar results have been observed in other studies. The aerobic centre longitudinal study found unfit men with the metabolic syndrome had cardiovascular mortality rates of 31.0/10000 years of observation compared to 11.9/10000 years of observation in fit men with the metabolic syndrome[316]. Rates in unfit and fit men without the metabolic syndrome were 19.0 and 5.0 deaths/10 000 years respectively[316].

8.1.4 Cardiac rehabilitation; a strategy for increasing physical activity

Across the general population levels of activity are low. In Scotland only 38% of men and 27% of women met the recommended weekly levels of physical activity [72]. There is evidence that levels of activity in individuals with type 2 DM may be even lower [317]. Cardiac rehabilitation is defined as a "programme that aims to enable patients with established cardiovascular disease maintain optimal physical and psychosocial health" [318]. One of the key aspects of cardiac rehabilitation is a structured 10-12 week exercise programme. The typical comprehensive cardiac rehabilitation programme consists of 4 phases, phase 1 starting whilst the person is inpatient, phase 2 immediately following discharge. Phase 3 takes the form of a structured exercise programme together with advice and support on risk factors. Phase 4 is the long term maintenance of physical activity and lifestyle change e.g. membership of a local gym. In some instances exercise only cardiac rehabilitation may be offered.

A Cochrane review of 32 trials found that exercise only cardiac rehabilitation reduced all cause mortality by 27%, cardiac death by 31% and the combined end point of mortality, non fatal MI and revascularization by 19% [319]. There was no additional benefit from comprehensive programmes; indeed comprehensive cardiac rehabilitation had a smaller effect on all cause mortality. It was unclear why there was the discrepancy between the exercise only programmes and comprehensive cardiac rehabilitation and this may simply be a reflection of study design.

There are few large randomised trials looking at the impact of cardiac rehabilitation programmes on cardiac risk factors. In the Cochrane review only a minority of studies analysed lipid levels [319]. In the studies of exercise only cardiac rehabilitation there was no effect on total cholesterol, LDL, HDL or triglycerides. In the comprehensive cardiac rehabilitation studies there was a significant reduction in total cholesterol and LDL with no significant changes in HDL and inconsistent effects on triglyceride levels. Of the minority of studies that reported blood pressure as an outcome there were no significant changes following rehabilitation [319]. There is little

information regarding cardiac rehabilitation in patients with type 2 DM or the effects on glycaemic control.

8.1.5 Cardiac rehabilitation and endothelial function

Exercise programmes have been shown to improve endothelial function in patients with CAD [233;235;264]. Edwards et al.[233] measured endothelial function using brachial FMD before and after 12 weeks of exercise only cardiac rehabilitation. A control group were patients who did not have access to cardiac rehabilitation due to lack of insurance or distance from the rehabilitation class. Patients attending cardiac rehabilitation had improved endothelial function compared to the control group. This was a small study with only 9 patients in each group, no comment was made on diabetes status of patients and there was no assessment of conventional risk factors before and after cardiac rehabilitation. Hambrecht et al.[235] also reported improvements in endothelial function in patients with established CVD following increased physical activity. Endothelial function assessed during coronary artery angiography improved following 4 weeks of exercise training. The mechanisms underlying this improvement were not clear. The patients in this group were highly selected, both diabetes and hypercholesterolaemia were exclusion factors. Finally Adams et al.[264] randomized patients either to an exercise group or control group prior to surgical revascularisation. Segments of left internal mammary artery obtained at time of bypass showed improved endothelial function in the exercise group. This study looked at the effects of an in hospital exercise training program rather than more standard cardiac rehabilitation programme and excluded patients with type 2 DM and hypercholesterolaemia. Blood pressure fell significantly in the training group but there were no significant differences in lipids or other cardiovascular risk factors.

8.1.6 Cardiac rehabilitation and oxidative stress

Physical activity may modify levels of oxidative stress. In patients following surgical revascularisation circulating markers of oxidative stress; lipid peroxidation products

(thiobarbituric acid reactive substances, diene conjugates and lipid hydroperoxide) and markers of blood antioxidant levels (total antioxidative capacity and glutathione markers) were reduced following a 12 week exercise programme [320]. In this study the greatest improvements were seen in the patients with lowest levels of fitness.

In both the studies by Edwards et al.[233] and Adams et al.[264] the improvements in endothelial function were associated with improvements in markers of oxidative stress. Edwards et al.[233]. assessed levels of oxidative stress using total plasma SOD activity, glutathione peroxidase activity and levels of isoprostanes. In the cardiac rehabilitation group there was a reduction in the levels of oxidative stress. Adams et al.[264] showed that exercise training was associated with reduced expression of NAD(P)H oxidase subunits and NAD(P)H oxidase activity and ROS (total superoxide production as measured by SOD inhibitable reduction of ferricytochrome C in segments of internal mammary artery obtained at the time of surgery.

8.2 Aims

The hypothesis was that phase 3 of the cardiac rehabilitation programme would improve endothelial function, reduce oxidative stress and improve HDL levels in patients with established CAD. In patients with type 2 DM who have more marked endothelial dysfunction it was hypothesised that increasing physical activity would be of greater benefit.

The aims of this study were using cardiac rehabilitation as a strategy for increasing levels of physical activity:

1. To investigate the effect of phase 3 cardiac rehabilitation on conventional cardiovascular risk factors.
2. To investigate the effect of phase 3 cardiac rehabilitation on endothelial function.
3. To measure mononuclear and whole blood superoxide (O_2^-) levels as an indicator of degree of oxidative stress before and after cardiac rehabilitation.

8.3 Methods

Patients who had undergone CABG and were attending cardiac rehabilitation were recruited to assess the effect of increased exercise on endothelial function and levels of O_2^-. Patients were assessed before and after a 10 week programme of education and exercise classes provided as part of phase 3 of cardiac rehabilitation. Detailed clinical examination and routine biochemistry was performed at both visits. Endothelial function was assessed using a PWA based technique. Whole blood and mononuclear cell O_2^- was assessed using EPR. A detailed description of the methods used is provided in chapter 2.

8.4 Results

Thirty four patients attending cardiac rehabilitation following coronary artery by-pass were recruited. Of these patients 29 completed the study and attended for visits both before and after cardiac rehabilitation. Demographics for these patients are shown in table 8.1. Three patients were unable to attend for the second visit. Reasons included moving from the area, ill health and not being available to attend the unit due to a return to work. Two participants were not willing to attend for a second visit.

Clinical examination recordings pre and post cardiac rehabilitation are shown in table 8.2. Diastolic blood pressure and heart rate fell significantly following cardiac rehabilitation. There was a trend to reduced body mass index and waist circumference but this was not significant.

Levels of physical activity were assessed by taking total leisure time activity recorded in the SPAQ. Levels of physical activity increased significantly following cardiac rehabilitation from 317.5 [207.5; 567.5] minutes per week to 540[362.5; 1316.3] minutes per week, $P<0.0001$. Functional capacity significantly increased following cardiac rehabilitation from 4.87±0.92 Metabolic Equivalent of Task (METS) to 5.93±0.98 METS, $P<0.0001$.

Biochemistry results are shown in table 8.3. Total cholesterol and HDL cholesterol increased following cardiac rehabilitation. There was a small but significant rise in HbA_{1c}. There were no other significant changes.

Endothelium dependent vasodilation was improved following cardiac rehabilitation. Maximum change in peripheral AIx following salbutamol was -8.3±8.2% at visit 2 compared to -1.1±7.5% at visit 1, *P*=0.003(figure 8.1). In 4 patients endothelial function was worse at visit 2. No clear reason for this finding could be identified. This aberrant response following exercise did not appear to be accounted for by number of exercise classes attended, change in weight, HDL, HbA_{1c} or blood pressure. There was no significant difference in endothelium independent vasodilation between the two visits; -18.1±8.8% vs.-19.9±8.7% *P*=NS (figure 8.2).

There were no significant changes in basal mononuclear cell superoxide release (66.6 [35.3;102.2] AU vs 53.5 [38.3;102.7] AU *P*=0.93, figure 8.3), stimulated mononuclear cell superoxide release (529.3 [422.2;1056.6] AU vs 441.6 [340.3,829.9] AU, *P*=0.094,figure 8.4) or whole blood superoxide release following cardiac rehabilitation (61.4[30.0;80.8] x 10^3AU vs 37[22.8;58.6] x 10^3 AU, *P*=0.44,figure 8.5).

Table 8.1. Demographics of patients attending for cardiac rehabilitation

	Recruited (n=34)	**Completed study (n=29)**
Age (years)	66.4±8.4	67.0±8.6
Male (%)	30(81.1)	25(86.2)
Current smokers (%)	2(5.4)	0
Type 2 DM (%)	5(13.5)	4(13.8)
Hypertension (%)	19(51.4)	15(51.7)
Myocardial Infarction (%)	20(54.1)	16(55.2)
Cerebrovascular disease (%)	3(8.1)	2(6.9)
Chronic renal failure (%)	1(2.7)	0
Heart failure (%)	3(8.1)	3(10.3)
Aspirin (%)	36(97.3)	29(100)
Other antiplatelet agent (%)	4(10.8)	1(3.4)
Statin (%)	32(86.5)	25(86.2)
ACEi/ARB (%)	22(59.5)	17(58.6)
Beta-blocker (%)	27(73.0)	22(75.9)
Calcium channel blocker (%)	1(2.7)	0
Nitrate (%)	3(8.1)	2(6.9)
Diuretic (%)	9(24.3)	6(20.7)
Oral hypoglycaemic agent (%)	2(5.4)	1(3.4)
Insulin (%)	0	0

Continuous variables are mean± standard deviation. Discrete variables are absolute numbers and percentage (%) BP, blood pressure, TIA; transient ischaemic attack, CVA, cerebrovascular accident. ACEi, Angiotensin-converting enzyme inhibitor, ARB, Angiotensin receptor blocker.

Table 8.2 Clinical examination before and after phase 3 cardiac rehabilitation

	Pre rehabilitation (n=29)	**Post rehabilitation (n=29)**	***P*-value**
Systolic BP (mm Hg)	137.8±21.8	135.8±22.2	NS
Diastolic BP (mm Hg)	81.2±12.5	76.6.3±10.8	0.007
Heart rate (beats/min)	72.3±10.8	64.9±8.9	0.008
Body Mass Index (kg/m^2)	29.3±6.39	28.4±3.80	NS
Waist circumference (cm)	100.9±9.8	99.5±7.8	NS

Mean±Standard deviation, BP, blood pressure.

Table 8.3 Basic biochemistry and white cell count results before and after phase 3 cardiac rehabilitation

	Pre rehabilitation (n=29)	**Post rehabilitation (n=29)**	***P*-value**
Cholesterol (mmol/l)	3.54±0.74	3.84±0.8	0.016
Triglycerides (mmol/l)	1.38±0.54	1.48±1.0	NS
LDL (mmol/l)	1.79±0.69	1.93±0.78	NS
HDL(mmol/l)	1.11±0.3	1.23±0.34	0.004
CRP(mg/L)	6.21±8.8	4.68±7.0	NS
HbA_{1c} (%)	5.52±0.38	5.86±0.46	0.04
Urinary ACR(mmol/l)	4.25±6.21	3.85±6.52	NS
Fasting Glucose(mmol/l)	5.51±0.17	5.75±0.15	NS
Total white cell count (10^9/L)	7.03±1.88	6.48±1.38	NS
Lymphocytes(%)	31.4±8.2	33.7±8.1	NS
Mononuclear cell (%)	8.2±2.9	5.9±2.3	NS
Granulocytes(%)	60.3±9.1	60.4±9.1	NS

All variables mean ± Standard deviation. LDL, low density lipoprotein; HDL, high density lipoprotein; CRP C-reactive protein; HbA_{1c}, glycated haemoglobin; urinary ACR, urinary albumin:creatinine ratio.

Figure 8.1. Endothelium dependent vasodilation pre and post cardiac rehabilitation

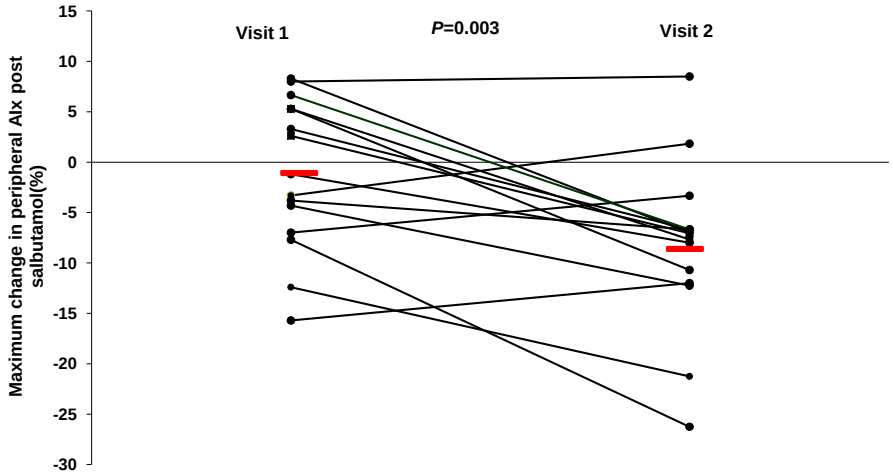


Figure 8.2. Endothelium independent vasodilation following cardiac rehabilitation

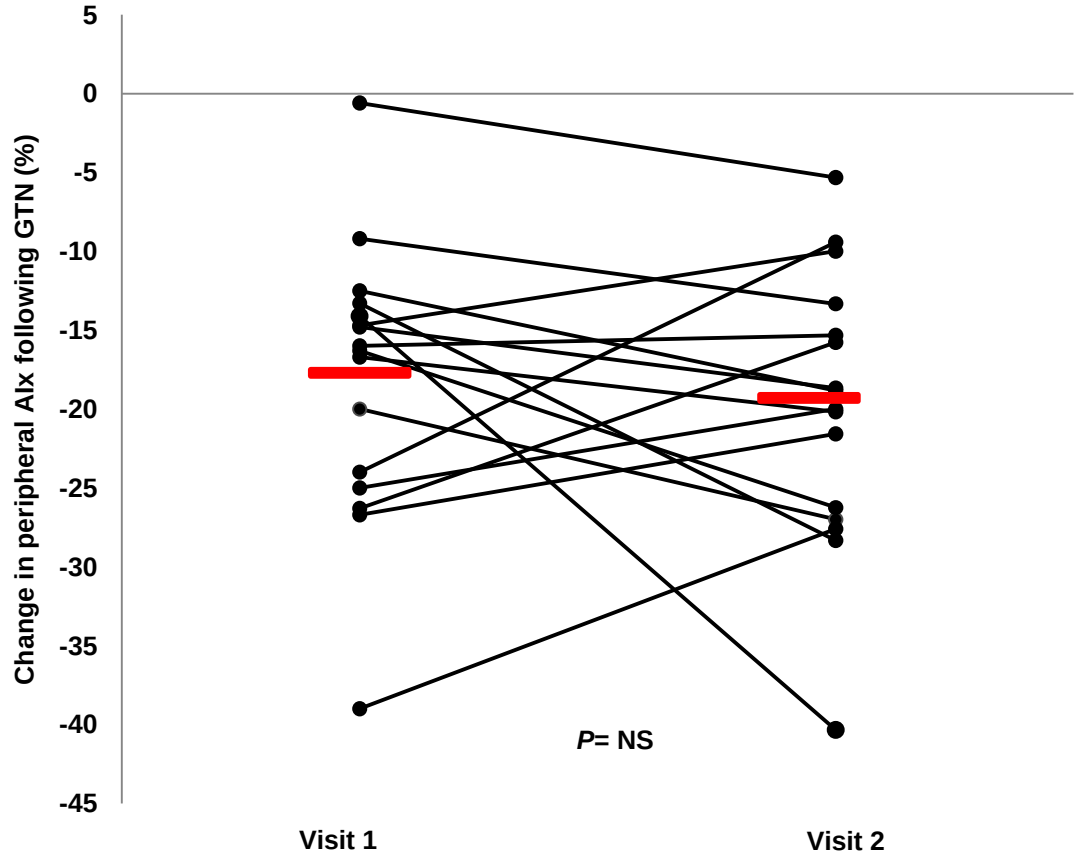


Figure 8.3 Basal mononuclear cell O_2^- production before and after cardiac rehabilitation

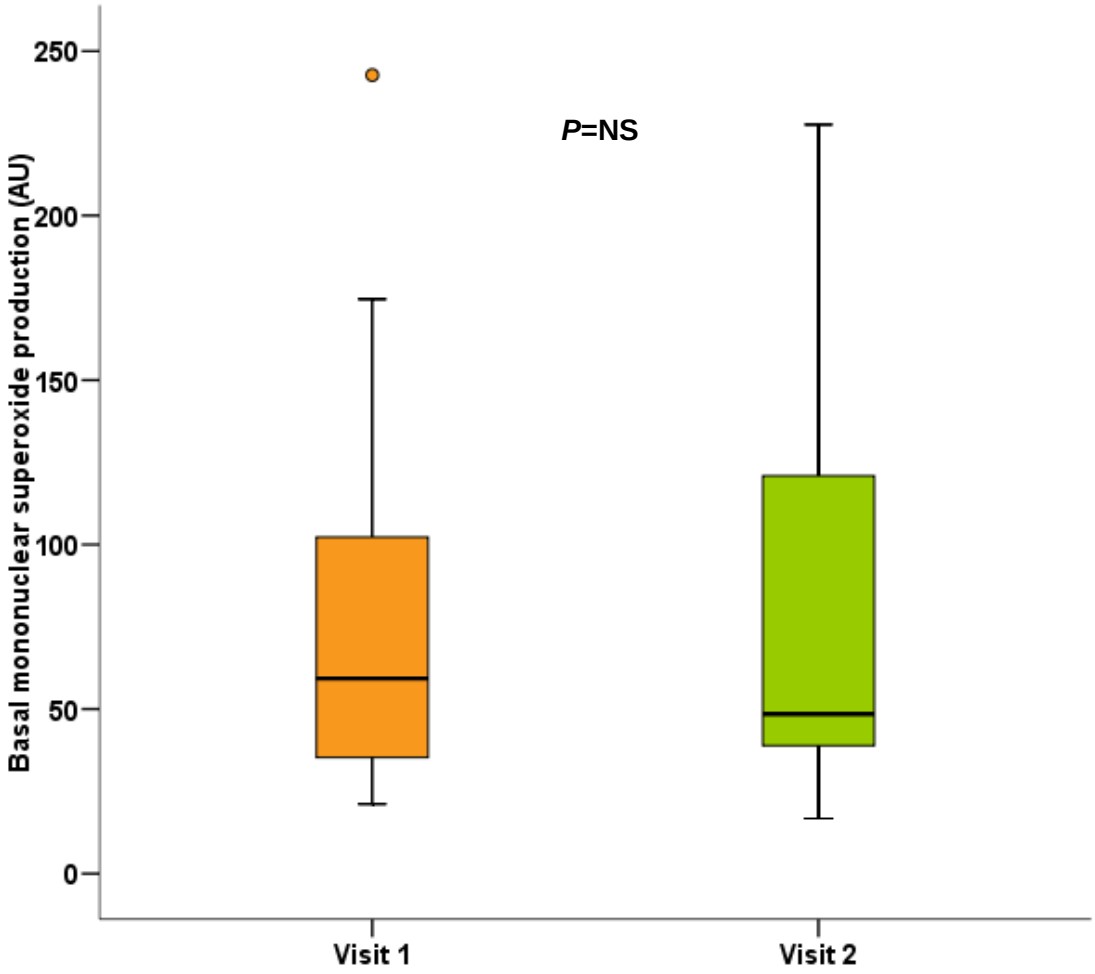


Figure 8.4 Stimulated mononuclear cell O_2^- production before and after cardiac rehabilitation

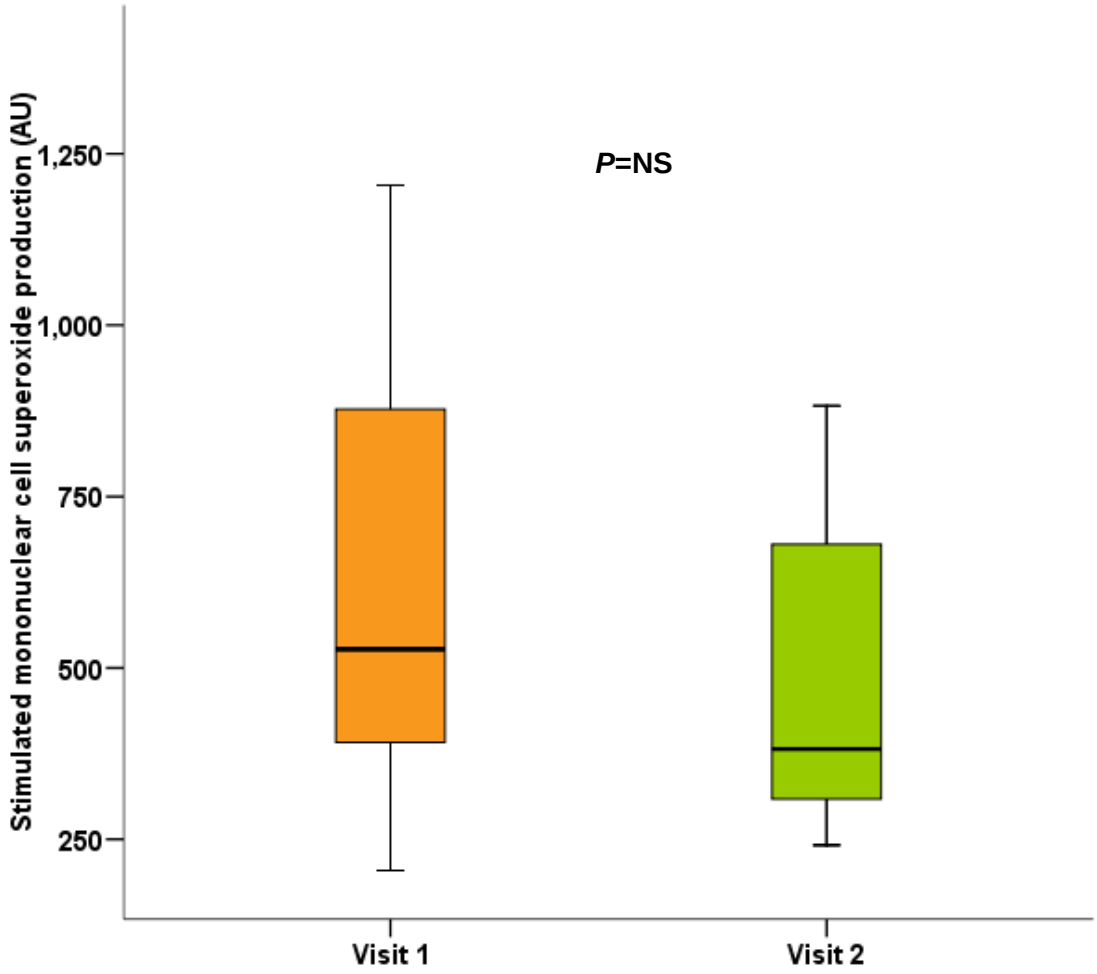
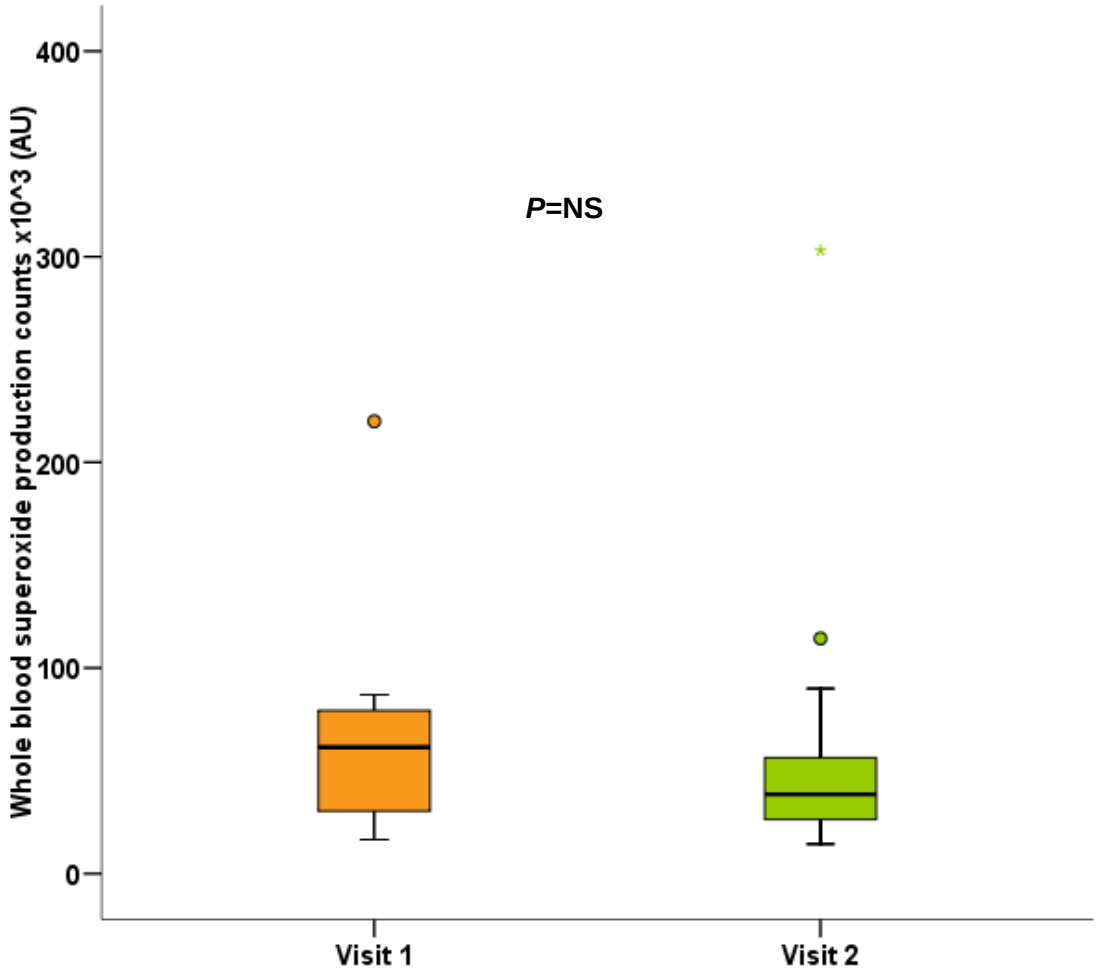


Figure 8.5 Whole blood O_2^- production before and after cardiac rehabilitation



8.5 Discussion

Endothelial function improved significantly following completion of the phase 3 stage of cardiac rehabilitation. Levels of oxidative stress as assessed by mononuclear and whole blood O_2^- levels were not altered by cardiac rehabilitation program. There was a significant increase HDL-cholesterol following cardiac rehabilitation.

The finding of improved endothelial function following cardiac rehabilitation is in keeping with a number of other studies[233;235;264]. Endothelial function is an important predictor of

cardiovascular morbidity and mortality. The improvements in endothelial function seen in this study may result in improved cardiovascular outcomes. In the studies by Adams et al.[264] and Edwards et al.[233] reductions in levels of oxidative stress occurred alongside the improvements in endothelial function. Reduced levels of oxidative stress were therefore thought to be the mechanism underlying improved endothelial function. In this study there were no changes in mononuclear cell or whole blood O_2^- levels in our patients following cardiac rehabilitation, suggesting that reduced levels of oxidative stress did not underlie the improvements in endothelial function observed.

Low HDL levels are a strong risk factor for CVD [48-50]. Previous studies have shown HDL cholesterol levels are correlated with endothelial function [209]. It is well established that HDL can be increased by increasing levels of physical activity [51]. Some but not all previous studies have shown an improvement in levels of HDL following cardiac rehabilitation [319]. In this study there was a significant increase in HDL cholesterol following 10 weeks of exercise training as part of cardiac rehabilitation. The increase in HDL levels in our study may partly account for the improvement in endothelial function observed. HDL can contribute to improvements in endothelial function through a number of mechanisms including activation of endothelial nitric oxide synthase (eNOS) [281].

HDL levels may simply be a marker for increased levels of physical activity with other mechanisms driving the improvements in endothelial function observed. In addition to increasing HDL cholesterol levels increased physical activity can alter lipoprotein composition. Exercise increases the mean size of HDL and LDL particles [284] . Small dense LDL particles have a number of features that are atherogenic and are associated with endothelial dysfunction [237].

Increased insulin sensitivity is likely to partly account for the reduction in cardiovascular mortality associated with increased levels of physical activity [321]. Insulin resistance is associated

with endothelial dysfunction [197]. Insulin resistance may contribute to impaired endothelial function through impairment of insulin stimulated nitric oxide (NO) production [197;199]. Improving insulin sensitivity pharmacologically with thiazolidinediones or metformin has been shown in small studies to improve endothelial function [230;231]. Insulin resistance was not formally assessed in this study. There was a small although non-significant reduction in waist circumference measurements. Even small reductions in central obesity may be associated with improvements in insulin sensitivity [60].

Increasing levels of physical activity may be a strategy for tackling endothelial dysfunction in patients with established CVD. This strategy may be particularly beneficial in patients with type 2 DM who have a greater degree of endothelial impairment and higher cardiovascular risk compared to patients without diabetes. In these patients insulin resistance is thought to be a key mechanism underlying increased cardiovascular risk and endothelial dysfunction [15;18;199]. The improvements in endothelial function seen following cardiac rehabilitation in this study may be partly mediated by improvements in HDL levels. There are currently no pharmacological agents that specifically increase HDL, lifestyle changes such as increasing physical activity are therefore the main strategy for addressing low HDL levels. Four patients in this study had worse endothelial function following exercise. The reasons for this aberrant response were not clear.

Future studies are required to assess the frequency and intensity of exercise required for improvements in endothelial function. In addition long term follow-up studies are required to assess whether improved endothelial function is associated with improved cardiovascular outcomes. It will also be important to investigate the role of other mechanisms such as LDL particle size and insulin resistance that may contribute to improved endothelial function following increased physical activity.

There are a number of limitations to this study. This study was a cross-sectional observational study. The lack of a control group clearly limits the conclusions that can be drawn from this study with regard to the causal link between increased physical activity and improvements in endothelial function. When designing the study the lack of a control group was of concern. Cardiac rehabilitation is a standard part of a patients management post cardiac events that is known to reduce morbidity and mortality. It would therefore have been unethical to recruit a control group who were not offered cardiac rehabilitation. When designing the study it had been hoped to partially compensate for a lack of control group by looking at the number of exercise classes attended however the final number of patients recruited made this analysis impossible. One of the aims of this study was to assess the effect of increased physical activity on endothelial function in patients with type 2 DM however due to the small numbers of patients recruited were not able to look at this group separately. Increased insulin sensitivity is thought to be an important mechanism of the improved endothelial function and reduced cardiovascular risk associated with increased physical activity. Insulin sensitivity was not formally assessed in this study so it is not possible to comment on this mechanism.

9 Final discussion

9.1 Discussion

Type 2 diabetes mellitus (DM) is associated with 2-4 fold increase in risk of cardiovascular disease (CVD) compared to patients without diabetes [9]. Type 2 DM is dramatically increasing in prevalence due to a combination of an ageing population, obesity epidemic and increasingly sedentary lifestyles[4]. Currently management of cardiovascular risk in patients with type 2 DM focuses on control of blood pressure, low density lipoprotein (LDL) and hyperglycaemia. Recent studies such as ADVANCE and ACCORD have highlighted the limitations of this approach[26;27]. New strategies are needed particularly in light of the predicted worldwide rise in cases of type 2 DM. Changes in the mechanical and functional properties of arteries are key in the development of atherosclerotic plaques that cause coronary artery disease (CAD) [74;82;93]. Understanding these process and the underlying mechanisms should help identify new management strategies.

I have shown using *ex vivo* and *in vivo* techniques that endothelial function is impaired in patients with CAD compared to healthy controls. This finding is in keeping with a plethora of previously published work [87;109;179;182;187;188]. Furthermore using *ex vivo* techniques I have confirmed that endothelial function is significantly worse in patients with CAD and type 2 DM compared to patients with CAD alone [110;194;195]. Endothelial dysfunction is an important prognostic factor in patients with established CVD [187;188]. It is hoped that improving endothelial function will improve cardiovascular outcomes. The majority of patients in this study were well treated with established cardiovascular secondary prevention therapies. Cardiovascular risk factors including blood pressure, LDL levels and hyperglycaemia were also well controlled. That endothelial dysfunction persists in these patients highlights the need for novel therapeutic strategies. Type 2 DM was the only significant determinant of endothelial dysfunction. The mechanisms underlying endothelial dysfunction are not fully understood but may include hypergylcaemia, insulin resistance, dyslipidaemia and oxidative stress [84;87;89;108;197;204;205].

Understanding the development of endothelial dysfunction in patients with type 2 DM may identify novel cardiovascular prevention strategies.

Previous evidence supported increased oxidative stress as the major mechanism underlying endothelial dysfunction in type 2 DM [87]. In my study superoxide (O_2^-) levels were similar in patients with and without type 2 DM, suggesting that increased oxidative stress did not account for the increased endothelial dysfunction associated with type 2 DM. The reasons underlying the different findings in this study compared to earlier reports of elevated oxidative stress in type 2 DM are not clear. It is hypothesised that the pharmacological management of the patients with type 2 DM may have lowered O_2^- levels to those of patients without diabetes. Use of Angiotenisn-converting enzyme inhibitors (ACEi) and Angiotensin receptor blockers (ARBs) are more widespread in patients with CAD and type 2 DM compared to CAD alone. These agents have been shown to lower NAD(P)H oxidase vascular O_2^- production[262]. Statins have also been shown to reduce O_2^- production[263]. The use of statins was high in patients with and without type 2 DM. Finally approximately half of patients with type 2 DM were taking metformin. Metformin has been shown to reduce oxidative stress in cultured cells [265]. The results of this study do not refute the role of oxidative stress in the development of endothelial dysfunction but does suggest that oxidative stress does not explain endothelial dysfunction associated with type 2 DM in this group of patients.

Type 2 DM and other insulin resistant states are associated with low high density lipoprotein (HDL) levels and a preponderance of small dense LDL particles[40;268]. This pattern of dyslipidaemia was confirmed in patients with type 2 DM and CAD in this study. Small dense LDL particles have been associated with increased cardiovascular risk and endothelial dysfunction[237;276]. Low HDL levels are also an important cardiovascular risk factor and associated with endothelial dysfunction[49;50;209]. It is proposed that the persisting dyslipidaemia seen in patients with type 2 DM may partly explain the endothelial dysfunction that was

observed. The optimal treatment for the specific lipid abnormalities associated with insulin resistance is complex. Fibrates reduced triglyceride levels increase HDL and increase LDL particle size. In theory fibrates are an optimal therapy however results from the ACCORD study question this approach [290] . In ACCORD fenofibrate added to statin therapy was not associated with improved cardiovascular outcomes. Whether fibrate therapy may be of benefit in selected patients is currently unclear. Insulin resistance is understood to be the major process driving lipid abnormalities associated with type 2 DM [268;271]. Strategies that improve insulin sensitivity such as increased physical activity are therefore of interest in the management of the dyslipidaemia associated with type 2 DM.

Structural changes in the vasculature such as increased arterial stiffness are understood to play an important role in the development of cardiovascular disease[80;93;119]. Age and hypertension are the key determinants of arterial stiffness[122]. The relative contribution of other factors such as type 2 DM and hypercholesterolaemia is currently uncertain[122]. Furthermore the optimal methods for assessing arterial stiffness in routine clinical care unclear. Aortic pulse wave velocity (PWV) is currently the gold standard method for assessing arterial stiffness although this method is associated with a number of limitations[121]. This study has shown increased aortic PWV in patients with CAD compared to control in keeping with other studies. The problems associated with measurement of aortic PWV are well recognised and were highlighted in this study. The difficulties associated with recording aortic PWV make simpler methods attractive. Other surrogate markers of arterial stiffness including augmentation index (AIx), and brachial PWV were not increased in this cohort of patients with CAD. The use of these surrogate markers of arterial stiffness has been previously called in to question by the results of other studies [127;137;155]. This study adds further weight to the evidence against these other measures of arterial stiffness in preference to aortic PWV. Time to reflected wave (TR) assessed by PWA is a simple measure that correlates with PWV[176]. TR was significantly reduced in patients with

CAD compared to controls. The use of TR as a simple surrogate measure of arterial stiffness warrants further investigation. Although there is a wealth of evidence for increased arterial stiffness in patients with type 2 DM this study was unable to confirm this observation. This finding is in keeping with the meta-analysis by Cecelja et al.[122]. showing diabetes status accounts for only a small proportion of variability in arterial stiffness. The role of assessing arterial stiffness and the optimal techniques for doing this in routine clinical care remain unclear.

Intensive cholesterol lowering has increasingly been a strategy for preventing CVD in high risk patients such as those with diabetes or established CVD. Intensive statin therapy may improve endothelial function through both reductions in LDL levels and lipid independent effects[206-208]. This study found that in 2007 compared to 2003 LDL levels were significantly lower, statin usage increased and endothelial function improved. Endothelial function is an important prognostic factor in patients with established CVD [187;188]. The increased endothelial function seen in this study may therefore provide indirect evidence for improved cardiovascular outcomes as the result of intensive lipid lowering strategies. Oxidative stress has been proposed as one mechanism by which LDL levels contribute to endothelial dysfunction [87;179]. The reduced LDL levels and improved endothelial function observed in the 2007 cohort of patients was not associated with reduced levels of oxidative stress. This finding adds further weight to the findings of chapter 3 that suggest oxidative stress may not be a key contributor to the endothelial dysfunction in this group of patients. LDL levels only accounted for less than a fifth of the variability in endothelial function. Other mechanisms are clearly important and remain to be elucidated. The specific effect of intensive LDL lowering in patients with type 2 DM could not be determined by this study.

Insulin resistance is also thought to be an important factor underlying both development of CVD and endothelial dysfunction[15;197]. Type 2 DM is by definition an insulin resistant state. Insulin resistance is also common in patients with established CAD without overt diabetes[227]. Increased

physical activity is associated with reduced cardiovascular risk and this is likely to be in part due to improved insulin sensitivity [69]. This study showed increased physical activity following the cardiac rehabilitation programme improved endothelial function in patients with type 2 DM, possibly mediated through increased HDL levels. A "prescription" of regular supervised exercise classes, similar to the cardiac rehabilitation programme may be a strategy for reducing the cardiovascular risk associated with type 2 DM. Exercise classes as a cardiovascular management strategy are attractive as the risks/side effects are low and there are likely to be multiple additional benefits. Increased physical activity may help to address a number of cardiovascular risk factors such as HDL levels, LDL particle size, insulin resistance that are not well controlled with currently available drugs[51;310].

9.2 Study limitations

Clearly there are a number of limitations with this study. The major limitations are discussed below.

Firstly the number of patients and controls recruited in to this study was relatively small. This may have been of particular importance when comparing the differences between patients with and without type 2 DM in whom the numbers were further still. The effect of type 2 DM on arterial stiffness in a group of patients with established CAD are likely to be small. This study is likely to have been under powered to detect such small differences within groups of patients. However despite the small numbers of patients within this study a number of clearly significant differences such as endothelial function were identified between patients with and without type 2 DM.

One of the major themes of this study was to assess the effect of type 2 DM on cardiovascular phenotypes. In a number of the sub-studies in particular the arterial stiffness and cardiac rehabilitation studies that number of patients with diabetes was smaller than expected. This

limited the conclusions that can be drawn specifically regarding the impact of diabetes and will further studies are required to address this shortcoming.

All the sub-studies that form this thesis are observational. Although a number of important associations have been identified as part of this study causation cannot clearly be attributed. For example prospective interventional studies would be required to ascertain whether there is a direct causative role between low HDL levels and endothelial dysfunction.

The main technique employed to assess endothelial dysfunction was an *ex vivo* technique. *Ex vivo* techniques are clearly limited by the availability of tissue but the results also need to be interpreted carefully as the samples may behave differently compared to when *in vivo*. Alternative *in vivo* measures of endothelial function were used when possible and confirmed the *ex vivo* results.

Due to the nature of the *ex vivo* tests used (endothelial function testing and vascular O_2^- assessment) the characteristics of the control group was significantly different to the patient group in terms of age and gender. This limits some of the conclusions that can be drawn with regards to mechanisms underlying differences between the two groups. A further control group for *in vivo* studies was recruited that was better matched in terms of age and sex and partially compensated for the limitations of the *ex vivo* control group.

9.3 Future directions

One of the key findings of this study is that endothelial dysfunction remains a major problem in patients with type 2 DM which is not adequately addressed by current cardiovascular prevention strategies. This endothelial dysfunction may partly account for the worse cardiovascular outcomes associated with type 2 DM. Results from this study have shown some potential therapeutic targets that may result in improved endothelial function. Correcting the specific lipid abnormalities associated with insulin resistant states, namely low HDL and small dense LDL,

may be of particular benefit. Furthermore use of structured exercise programmes to increase levels of physical activity may be beneficial by targeting cardiovascular risk factors such as low HDL levels that are not presently well addressed by currently available cardiovascular prevention therapies. Future studies to assess the impact on cardiovascular outcomes in addition to surrogate markers such as endothelial function are required.

A large proportion of the variability in endothelial function found was not explained by factors measured as part of this study. Understanding these mechanisms may identify more targeted approaches than those discussed above. The role of mitochondrial dysfunction in the development of insulin resistant states such as type 2 DM and complications of diabetes is of particular interest. Mitochondrial function has been shown to be impaired in insulin resistant states[322]. This impairment in mitochondrial function is thought to be due to smaller and fewer mitochondria[322]. Mitochondrial dysfunction is thought to be an important mechanism underlying insulin resistance in skeletal muscle[322]. Mitochondrial dysfunction is also thought to play an important role in the development of atherosclerotic lesions and endothelial dysfunction[323;324]. Endothelial mitochondrial dysfunction may contribute to endothelial dysfunction through a number of mechanisms including increased oxidative stress, altered intracellular calcium signalling and endothelial cell apoptosis amongst others[323;325]. Factors thought to contribute to mitochondrial dysfunction include aging, genetic factors, oxidative stress[322-324]. Strategies that improve mitochondrial function may therefore reduce insulin resistance and improve cardiovascular outcomes. Both thiazolidinediones and metformin have been shown to increase mitochondrial biogenesis [265]. Aerobic exercise has also been shown to increase mitochondrial biogenesis[322]. Novel agents that target mitochondrial oxidative stress are of particular interest. Ongoing work in the VASCAB cohort of patients by Dr Ruth Mackenzie has suggested increased levels of mitochondrial oxidative stress in patients with CAD and type 2 DM compared to patients with CAD alone (data submitted for PhD thesis to University of Glasgow,

2010). $MitoQ_{10}$ is a mitochondrial targeted antioxidant that is currently the focus of the cardiovascular research group lead by Professor Dominiczak. Work from this group has shown $MitoQ_{10}$ is associated with improved endothelial function in animal studies [326].

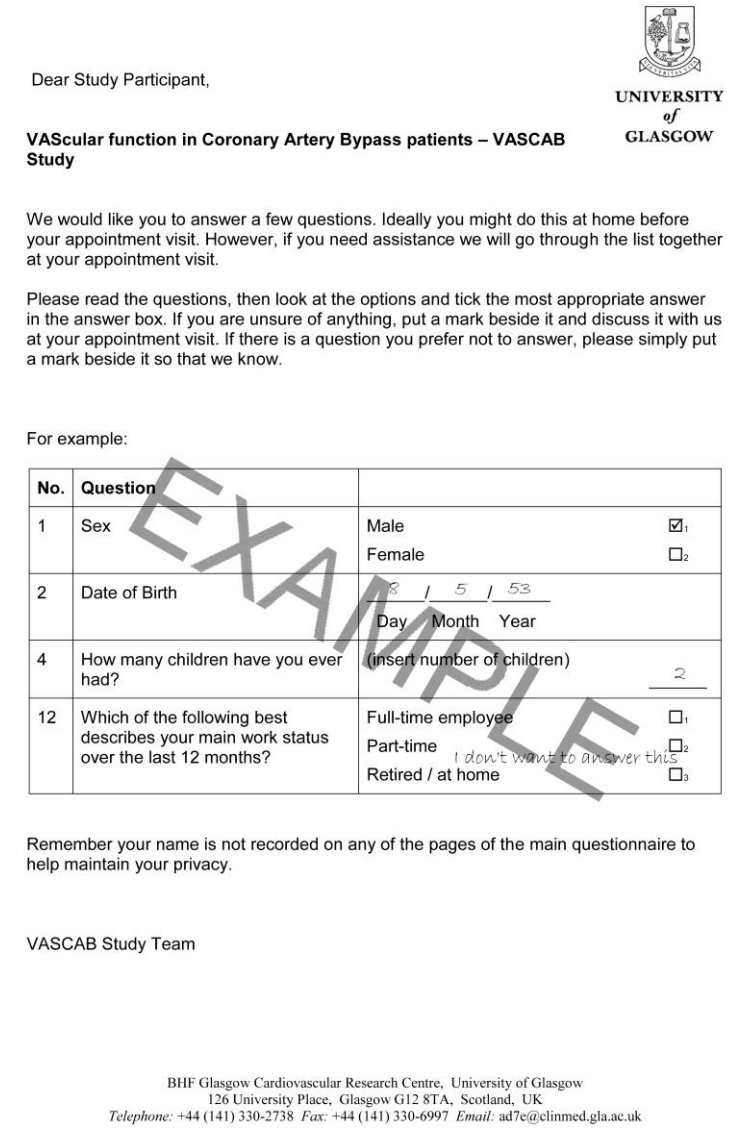
In summary based on the work in this thesis future directions should include studies to investigate the effect of improving endothelial function (through the management of dyslipidaemia and insulin resistance) on cardiovascular outcomes in patients with type 2 DM. Further studies are also required to elucidate the additional mechanisms that are contributing to endothelial dysfunction in patients with type 2 DM as only a proportion of the variability to be explained by conventional cardiovascular risk factors.

10 Supplementary data

10.1 Participant questionnaire

All participants recruited to the VASCAB study completed a questionnaire to provide detailed information regarding lifestyle and medical history.

Figure 10.1. Participant questionnaire



UNIVERSITY of GLASGOW

Dear Study Participant,

VAScular function in Coronary Artery Bypass patients – VASCAB Study

We would like you to answer a few questions. Ideally you might do this at home before your appointment visit. However, if you need assistance we will go through the list together at your appointment visit.

Please read the questions, then look at the options and tick the most appropriate answer in the answer box. If you are unsure of anything, put a mark beside it and discuss it with us at your appointment visit. If there is a question you prefer not to answer, please simply put a mark beside it so that we know.

For example:

No.	Question	
1	Sex	Male ☑1 Female ☐2
2	Date of Birth	8 / 5 / 53 Day Month Year
4	How many children have you ever had?	(insert number of children) 2
12	Which of the following best describes your main work status over the last 12 months?	Full-time employee ☐1 Part-time ☐2 Retired / at home ☐3 I don't want to answer this

EXAMPLE

Remember your name is not recorded on any of the pages of the main questionnaire to help maintain your privacy.

VASCAB Study Team

BHF Glasgow Cardiovascular Research Centre, University of Glasgow
126 University Place, Glasgow G12 8TA, Scotland, UK
Telephone: +44 (141) 330-2738 *Fax:* +44 (141) 330-6997 *Email:* ad7e@clinmed.gla.ac.uk

Study Number _____M / F

Participant Main Questionnaire

Section 1 - Demographics and Family

The background of a person has a substantial effect on an individual's risk of heart disease. In this first section we would like to find out a bit about you, your living circumstances and your family.

No.	Question	
1	Sex	Male ☐1 Female ☐2
2	Date of Birth	_____/_____/_____ Day Month Year
3	Marital status	Single (never married) ☐1 Married ☐2 Living with partner ☐3 Divorced or separated ☐4 Widowed ☐5
4	How many children have you ever had?	(insert number of children) _____
5	How many children are alive now?	(insert number) _____
6	Are you one of a twin?	No ☐1 Yes, identical ☐2 Yes, non-identical ☐3
7	How many brothers do you have (all live births)?	(insert number of brothers) _____
8	How many brothers are alive now?	_____
9	How many sisters do you have? (all live births)	(insert number of sisters) _____
10	How many sisters are alive now?	_____

Study Number _____M / F

The next questions are about your family.

No.	Question				
F1	Have any of your relatives had a **heart attack**?	Yes			☐1
		No (skip the next question and go to question F3)			☐2
F2	If **yes** in question F1, **who** has had a heart attack and **how old** were they at heart attack? (Please enter "not known" if you are not sure. Please indicate if you know approximate ages but not exact ages.)	Mother	Yes ☐1	No ☐2	Age ___
		Father	Yes ☐3	No ☐4	Age ___
		Sister	Yes ☐5	No ☐6	Age ___
		Brother	Yes ☐7	No ☐8	Age ___
		Son/Daughter	Yes ☐9	No ☐10	Age ___
		Other	Yes ☐11	No ☐12	Age ___
F3	Have any of your relatives had a **stroke**?	Yes			☐1
		No (skip the next question and go to question F5)			☐2
F4	If **yes** in question F3, **who** has had a **stroke** and **how old** were they at stroke? (Please enter "not known" if you are not sure. Please indicate if you know approximate ages but not exact ages.)	Mother	Yes ☐1	No ☐2	Age ___
		Father	Yes ☐3	No ☐4	Age ___
		Sister	Yes ☐5	No ☐6	Age ___
		Brother	Yes ☐7	No ☐8	Age ___
		Son/Daughter	Yes ☐9	No ☐10	Age ___
		Other	Yes ☐11	No ☐12	Age ___
F5	Is there any relative in your family who has or had **high blood pressure**?	Yes			☐1
		No (skip the next question and go to question F7)			☐2
F6	If **yes** in question F5, **who** has or had **high blood pressure** and **how old** were they when high blood pressure was diagnosed? (Please enter "not known" if you are not sure. Please indicate if you know approximate ages but not exact ages.)	Mother	Yes ☐1	No ☐2	Age ___
		Father	Yes ☐3	No ☐4	Age ___
		Sister	Yes ☐5	No ☐6	Age ___
		Brother	Yes ☐7	No ☐8	Age ___
		Son/Daughter	Yes ☐9	No ☐10	Age ___
		Other	Yes ☐11	No ☐12	Age ___

Study Number _____M / F

No.	Question		
F7	Is there any relative in your family who has or had **diabetes** (high blood sugar)?	Yes No (skip the next question and go to the next section)	☐1 ☐2
F8	If **yes** in question F5, **who** has or had **diabetes** and **how old** were they when diabetes was diagnosed? (Please enter "not known" if you are not sure. Please indicate if you know approximate ages but not exact ages.)	Mother Yes ☐1 No ☐2 Age ___ Father Yes ☐3 No ☐4 Age ___ Sister Yes ☐5 No ☐6 Age ___ Brother Yes ☐7 No ☐8 Age ___ Son/Daughter Yes ☐9 No ☐10 Age ___ Other Yes ☐11 No ☐12 Age ___	

Section 2 - Life Style Factors

In this section, there are questions about your lifestyle. A person's lifestyle can give us important clues as to the cause of their heart disease.

The first questions are about how much alcohol you drink.

No.	Question		
A1	Have you ever consumed a drink that contains alcohol?	Yes No (skip this section and go to the next section)	☐1 ☐2
A2	Have you consumed alcohol in the **past 12 months**?	Yes No (skip this section and go to the next section)	☐1 ☐2
A3	In the past 12 months, **how frequently** have you had at least one drink?	Daily 3 to 4 days per week Weekly Fortnightly Monthly or on special occasions only	☐1 ☐2 ☐3 ☐4 ☐5
A4	When you drink alcohol, **on average,** how many drinks do you have during one day?	Number of drinks per day: (A drink is equal to 1 small glass of wine, a half pint of beer, 1 shot of spirits or liqueur.)	_____

VASCAB Version MQ.2.0 – 22 February 2007

Study Number _____M / F

These questions are about smoking and use of tobacco.

No.	Question		
S1	Have you **ever smoked** any tobacco products?	Yes, currently smoke Yes, but stopped within past 12 months Yes, but stopped more than 12 months ago No (skip this question and go to the next section)	☐1 ☐2 ☐3 ☐4
S2	How **old** were you when you **first started** smoking daily?	(Give age in years)	_____
S3	What is the **maximum number** you have smoked per day for as long as a year	(insert number of cigarettes / cigars / hand made cigarettes per week / oz. of tobacco)	_____
S4	**PAST SMOKERS – only** Why did you give up smoking?	On doctor's advice Other reason	☐1 ☐2
S5	**PAST SMOKERS – only** How **long ago** did you **stop** smoking daily?	Years ago Months ago Weeks ago	☐1 ☐2 ☐3

These questions are about your diet.

No.	Question		
D1	In a typical week, on **how many days** do you eat **fruit**?	(Insert number of days)	_____
D2	Approximately **how many pieces/ servings** of fruit do you eat on one of those days?	(Insert number of servings/ pieces)	_____
D3	In a typical week, on **how many days** do you eat **green leafy vegetables**? (e.g. spinach, salad leaves)	(Insert number of days)	_____
D4	Approximately **how many servings/ meals** would you have **green leafy vegetables** on one of those days?	(Insert number of servings/ meals)	_____

These questions are about your regular exercise and physical activity.

VASCAB Version MQ.2.0 – 22 February 2007

Study Number _____M / F

No.	Question		
P1	On average, how much physical activity do you do each day **during working hours**? (if retired or at home, this refers to during the day)	Lots (e.g. heavy lifting, digging, going up & down stairs) Medium (e.g. light lifting, walking, light house-work, shopping, painting) Light activity (e.g. standing, occasional working) Almost none (e.g. desk job, sitting, driving)	☐1 ☐2 ☐3 ☐4
P2	On average, how much physical activity do you do each day **after working hours**? (if retired, this refers to evenings and weekends)	Lots (e.g. competitive sports, aerobics, multiple times a week) Medium (e.g. Casual sports, going to gym, regular walks 1-2 times per week) Light activity (e.g. occasional working or bowls) Almost none (e.g. Watching TV, listening to music, cooking, driving)	☐1 ☐2 ☐3 ☐4

Section 3 - Current Medical conditions and risk factors

This final section is about your medical conditions and treatments.

No.	Question		
M1	Have you ever been told by a doctor or other health worker that you have **high blood pressure** or **hypertension**?	Yes No, my blood pressure was always normal (skip the next question and go to question M3) No, I have never had my blood pressure taken (skip the next question and go to question M3)	☐1 ☐2 ☐3
M2	***If yes,*** about **how long ago** were you first told by a doctor that you had high blood pressure?	(insert number of years)	_____

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Study Number _____M / F

No.	Question		
M3	Have you ever been told by a doctor or other health worker that you have **diabetes** (high blood sugar)?	Yes No, my blood sugar was always normal (skip the next question and go to question M5) No, I have never had my blood sugar taken (skip the next question and go to question M5)	☐1 ☐2 ☐3
M4	***If yes,*** about **how long ago** were you first told by a doctor that you had diabetes (a high blood sugar)?	(insert number of years)	_____
M5	Have you had a medical diagnosis of a **heart attack/ myocardial infarction**?	Yes No	☐1 ☐2
M6	Have you had a medical diagnosis of a **Stroke/ transient ischemic attack**	Yes No	☐1 ☐2
M7	Have you had a medical diagnosis of **blood vessel disease in your legs/ peripheral vascular disease**	Yes No	☐1 ☐2
M8	Have you had a medical diagnosis of a **weak heart/ heart failure**	Yes No	☐1 ☐2
M9	Have you had a medical diagnosis of **kidney disease/ renal failure**	Yes No	☐1 ☐2
M10	Have you had a medical diagnosis of **lung/chest problems? e.g. bronchitis/emphysema/COPD/Asthma**	Yes No	☐1 ☐2
M11	Do you have or have you ever been given a diagnosis of **cancer**? *If yes* what type:_____	Yes No	☐1 ☐2
M12	Do you have **rheumatoid arthritis**? (inflammation of joints)	Yes No	☐1 ☐2
M13	Do you have **osteoarthritis** ?(wear and tear arthritis)	Yes No	☐1 ☐2

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Study Number _____M / F

No.	Question	
M14	Do you have any other long standing medical conditions that are not already listed?	Yes ☐1 No ☐2
	If yes what are these conditions? *(you may leave blank if you prefer not to answer)*	

The next 2 questions are for women only

No.	Question	
W1	Have you gone through the **menopause**? i.e. have your periods stopped	Yes ☐1 No ☐2
W2	Have you ever taken the oral contraceptive pill (OCP) **or** hormone replacement therapy (HRT)?	Yes currently ☐1 Yes previously but now stopped ☐2 (Number of years stopped ____) No never ☐3

The next 3 questions are for patients with diabetes only.

No.	Question	
CD1	Have you ever been told you have **damage to your eyes** (retinopathy) from having diabetes?	Yes ☐1 No ☐2
CD2	Do you have any **foot problems** due to diabetes (neuropathy)? e.g. ulcers, numbness, have missing /lost toes due to diabetes	Yes ☐1 No ☐2
CD3	Have you ever been told that your **kidneys** have been damaged from having diabetes (nephropathy)?	Yes ☐1 No ☐2

VASCAB Version MQ.2.0 – 22 February 2007

Study Number _____M / F

Please write the name of your current medications as they are labelled from the medicine box, or your script.

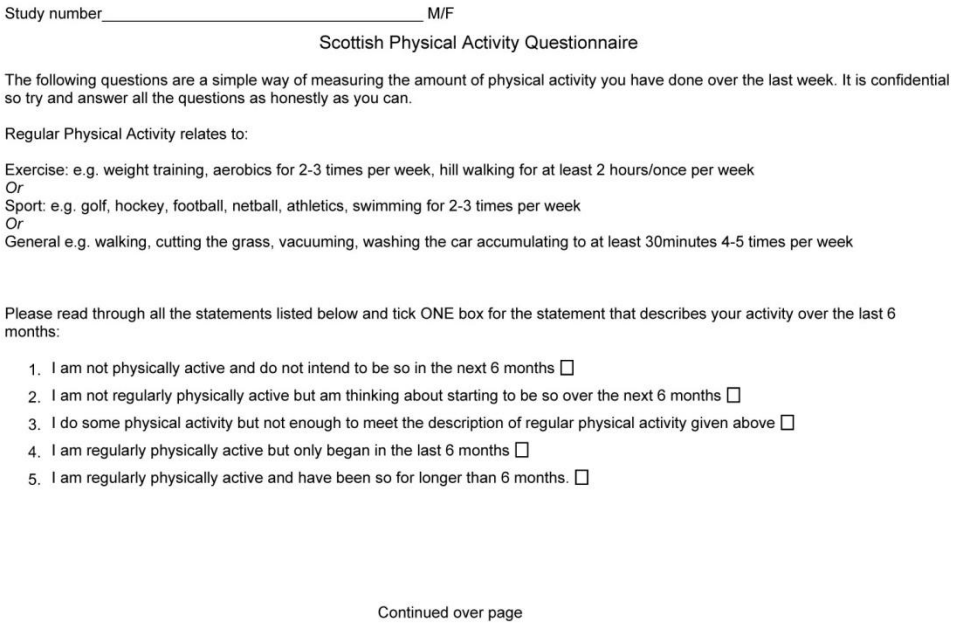
It may be easier for you just to bring a current medication list issued by your doctor or by your chemist with you. If you have such a list please leave the following box blank.

	Name of medication	**How long have you been taking this medication?**
T1	_____	(please insert years/months) _____
T2	_____	(please insert years/months) _____
T3	_____	(please insert years/months) _____
T4	_____	(please insert years/months) _____
T5	_____	(please insert years/months) _____
T6	_____	(please insert years/months) _____
T7	_____	(please insert years/months) _____
T8	_____	(please insert years/months) _____
T9	_____	(please insert years/months) _____
T10	_____	(please insert years/months) _____
T11	_____	(please insert years/months) _____

10.2 Scottish physical activity questionnaire

Participants recruited to the exercise study were asked to complete the Scottish physical activity questionnaire at visits 1 and 2.

Figure 10.2 Scottish physical activity questionnaire.



Study number_____ M/F

Scottish Physical Activity Questionnaire

The following questions are a simple way of measuring the amount of physical activity you have done over the last week. It is confidential so try and answer all the questions as honestly as you can.

Regular Physical Activity relates to:

Exercise: e.g. weight training, aerobics for 2-3 times per week, hill walking for at least 2 hours/once per week
Or
Sport: e.g. golf, hockey, football, netball, athletics, swimming for 2-3 times per week
Or
General e.g. walking, cutting the grass, vacuuming, washing the car accumulating to at least 30minutes 4-5 times per week

Please read through all the statements listed below and tick ONE box for the statement that describes your activity over the last 6 months:

1. I am not physically active and do not intend to be so in the next 6 months ☐
2. I am not regularly physically active but am thinking about starting to be so over the next 6 months ☐
3. I do some physical activity but not enough to meet the description of regular physical activity given above ☐
4. I am regularly physically active but only began in the last 6 months ☐
5. I am regularly physically active and have been so for longer than 6 months. ☐

Continued over page

Study number_____ M/F

The following questions relate to your physical activity over the **previous week**. Please mark in the appropriate box the number of **minutes** spent doing a particular activity. Please try and think carefully and be as accurate as possible with your answers and only include activities of either **moderate or vigorous** intensity. Examples are given of what should and should not be included.	**Light intensity:** Your heart rate and breathing are no different from what they were when you are standing, sitting etc **Moderate intensity:** Your heart rate and breathing rate are faster than normal. You may also sweat a little. Brisk walking or sweeping and mopping are good examples of how you might feel **Vigorous intensity:** Your heart rate is much faster and you have to breathe deeper and faster than normal. You will probably sweat. Playing football or squash are good examples of how you might feel.

Leisure time physical activity (remember do not include light intensity activities)

In the past **week** how many **minutes** did you spend each day:	Mon	Tues	Wed	Thurs	Fri	Sat	Sun
Walking out with work? e.g. do include walking to shops, walking to work, walking the dog, stair walking e.g. Do not include standing , sitting, driving, walking whilst at work							
Manual labour out with work? e.g. do include cutting grass, decorating, washing car, DIY, digging e.g. Do not include weeding, planting, pruning							
Active housework? Do include vacuuming, scrubbing floors, bed making, hanging out washing Do not include sewing, dusting, washing dishes, preparing food							
Dancing? Only include time actually spent dancing e.g. disco, line, country Do not include time spent not actually dancing							
Participating in a sport, leisure activity or training? Do include exercise classes, cycling, football, swimming, golf, jogging Do not include darts, snooker/pool, fishing,							
Other physical activity if not already covered (please write in)							

Study number_____ M/F

Physical Activity at work (Only complete if currently employed and remember not to include any light intensity activities)

In the past week how many minutes did you spend each day:	Mon	Tues	Wed	Thurs	Fri	Sat	Sun
Walking whilst at work? Do include e.g. walking up or down stairs, to and from your desk, "doing the rounds" Do not include e.g. standing, sitting at desk etc i.e. time spent not actually walking							
Manual Labour whilst at work? Do include e.g. lifting, stacking shelves, climbing ladders, building work, cleaning Do not include sitting at desk, answering telephone, driving, check-out operation							

Was last week typical of the amount of physical activity you usually do?
Yes ☐
No- I usually do more ☐ normally, how much more? _____ Minutes. Of which activity?_____
No- I usually do less ☐ normally, how much more? _____ Minutes. Of which activity?_____

Figure 10.3 Letter of ethical approval for VASCAB study

North Glasgow University Hospitals
Division

NHS
Greater
Glasgow

West Glasgow Ethics Committee 1
Western Infirmary
Dumbarton Road
Glasgow G11 6NT

Telephone: 0141 211 6238
Facsimile: 0141 211 1920

04 October 2006

Prof Anna F. Dominiczak
Director BHF Glasgow Cardiovascular Research Centre
BHF GCRC
126 University Place
University of Glasgow
Glasgow
G12 8TA

Dear Prof Dominiczak

Full title of study: **VAScular function in Coronary Artery Bypass patients (VASCAB)**
REC reference number: **06/S0703/110**

The Research Ethics Committee reviewed the above application at the meeting held on 03 October 2006.

Ethical opinion

The Committee thanked Professor Dominiczak and Dr. Jane Dymott for attending the meeting to discuss this study.

The Committee has one or two questions to the investigators which were answered to their satisfaction i.e.

a) A34 visits 0-12 - the investigators indicated that this was indeed an error.
b) The Committee wondered what the role of the fitness fanatics was and this was answered.
c) The Committee are of the opinion that the initial approach shouldbe at the pre-admission clinic visit.
d) The Committee indicated to the investigators that any further tests on the samples will require a further submission to an ethics committee.
e) A58 in respect of funding should be completed.
f) The Committee are of the view that GPs should be informed and a GP letter should be drawn up and passed to the committee for review.
g) The committee strongly feel that patients who have an intolerance of Salbutamol or GTN should be added to the Exclusion criteria.

Patient Information Sheet CABG should be amended as under:

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a) The word "minor" should be deleted prior to "scratch".
b) Volume of blood taken should be stated in "tablespoonfuls".
c) A sentence should be added in respect of gifting of the samples.
d) Page 3 - 2nd top line - should read "treat patients better who" etc

Healthy Volunteer PIS:

a) Any reference to "disease" should be deleted.
b) A sentence should be added to the effect that there is a risk that something might be picked up which could have future implications for insurance/mortgage purposes.
c) A sentence should be added in respect of "gifting of the samples".
d) "current medication" should read "any medication".
e) A sentence should be added in respect of notifying your private medical insurer of your taking part in the study.
f) Page 3 - 2nd top line should read "treat patients better" etc
g) Blood vols should be expressed in tablespoonfuls.
h) Page 2 - 2nd bottom bullet point should read "allergy against glycerol trinitrate please tell us.

All Consent Forms:

a) A tick box should be added in respect of telling GPs.

The above minor amendments should come back to the Secretary for checking and filing.

The members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation.

Ethical review of research sites

The Committee agreed that all sites in this study should be exempt from site-specific assessment (SSA). There is no need to complete Part C of the application form or to inform Local Research Ethics Committees (LRECs) about the research. The favourable opinion for the study applies to all sites involved in the research.

Conditions of approval

The favourable opinion is given provided that you comply with the conditions set out in the attached document. You are advised to study the conditions carefully.

Approved documents

The documents reviewed and approved at the meeting were:

Document	*Version*	*Date*
Application	5.1	06 September 2006
Investigator CV	CI	06 September 2006
Protocol	1.0	01 September 2006
Covering Letter		06 September 2006
Summary/Synopsis	CABG 1.0	01 September 2006

Questionnaire: non-validated follow up 2 years	F4 1.0	01 September 2006
Questionnaire: non-validated follow up 1 year	F3 1.0	01 September 2006
Questionnaire: non-validated follow-up 30 days	F2 1.0	01 September 2006
Questionnaire: Non-validated follow up 7 days	F1 1.0	01 September 2006
Questionnaire: Non-validated	Main MQ 1.0	01 September 2006
Participant Information Sheet	HE 1.0	01 September 2006
Participant Information Sheet	VV 1.0	01 September 2006
Participant Information Sheet	CABG 1.O	01 September 2006
Participant Consent Form	HE 1.0	01 September 2006
Participant Consent Form	VV B 1.0	01 September 2006
Participant Consent Form	VV A 1.0	01 September 2006
Participant Consent Form	CABG 1.0	01 September 2006
Summary Synopsis	HE 1.0	01 September 2006
Summary Synopsis	VV 1.0	01 September 2006
CV for student		06 September 2006

Research governance approval

You should arrange for the R&D Department at all relevant NHS care organisations to be notified that the research will be taking place, and provide a copy of the REC application, the protocol and this letter.

All researchers and research collaborators who will be participating in the research at a NHS site must obtain final research governance approval before commencing any research procedures. Where a substantive contract is not held with the care organisation, it may be necessary for an honorary contract to be issued before approval for the research can be given.

Membership of the Committee

The members of the Ethics Committee who were present at the meeting are listed on the attached sheet.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

06/S0703/110 **Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project

Yours sincerely

PP Sharon Jenner

Andrea H Torrie
Ethics Manager – West Glasgow LREC's

Email: andrea.torrie@northglasgow.scot.nhs.uk

Enclosures: *List of names and professions of members who were present at the meeting and those who submitted written comments*
Standard approval conditions SL-AC2

Copy to: R & D Department WIG

West Glasgow Ethics Committee 1

Attendance at Committee meeting on 03 October 2006

Committee Members:

Name	Profession	Present?	Notes
Dr J Hunter	***Chair***		
Dr A R Binning	***Vice-Chair***		
Mr R Donald			
Dr I Robertson			
Dr K Hanretty			
Mrs A Lees			
Ms C Cowan			
Mr R Sim			
Prof D Stewart-Tull			
Dr J Thorburn			
Dr G Robertson			
Dr D Attwood			
Mr C Rodden			
Dr K Duffy			
Mrs A H Torrie			

Figure 10.4 Letter of ethical approval for exercise study.

North Glasgow University Hospitals Division

NHS Greater Glasgow

West Glasgow Ethics Committee 2
Western Infirmary
Dumbarton Road
Glasgow
G11 6NT

Telephone: 0141 211 6238
Facsimile: 0141 211 1920

24 April 2006

Dr Christian Delles
Senior Lecturer
BHF Glasgow Cardiovascular Research Centre
University of Glasgow
126 University Place
Glasgow G12 8TA

Dear Dr Delles

Full title of study: **Mechanisms of superoxide generation in diabetes and coronary artery disease: modification by physical exercise**

REC reference number: **06/S0709/55**

The Research Ethics Committee reviewed the above application at the meeting held on 18 April 2006. The Committee wished to thank you for attending the meeting to discuss the above study

Ethical opinion

The Committee had no issues with the Study Design but a few minor amendments to the Patient Information Sheet

Study Design - Question A 71 to be signed

Patient Information Sheet

a. What will happen to me if I take part - Blood volumes to be in layman's terms i.e. teaspoon
b. Page 2 2nd sentence delete "throughout" insert "due to"
c. Page 2 separate sentence required for Salbutamol allergy
d. Page 3 amend "Inhalation of glycerol trinitrate" this is not inhaled.
e. Consent Form "a separate box stating GP to be informed of patient's participation in the study" should be added.

The above amendments to come back to the Secretary for checking and filing

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The members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation.

Ethical review of research sites

The Committee agreed that all sites in this study should be exempt from site-specific assessment (SSA). There is no need to complete Part C of the application form or to inform Local Research Ethics Committees (LRECs) about the research. The favourable opinion for the study applies to all sites involved in the research.

Conditions of approval

The favourable opinion is given provided that you comply with the conditions set out in the attached document. You are advised to study the conditions carefully.

Approved documents

The documents reviewed and approved at the meeting were:

Document	*Version*	*Date*
Application	5.1	28 March 2006
Investigator CV		28 March 2006
Protocol	1.0	28 March 2006
Covering Letter		28 March 2006
Participant Information Sheet	1.0	28 March 2006
Participant Consent Form	1.0	28 March 2006
Consent Form - Genetic	1.0	28 March 2006

Research governance approval

You should arrange for the R&D Department at all relevant NHS care organisations to be notified that the research will be taking place, and provide a copy of the REC application, the protocol and this letter.

All researchers and research collaborators who will be participating in the research at a NHS site must obtain final research governance approval before commencing any research procedures. Where a substantive contract is not held with the care organisation, it may be necessary for an honorary contract to be issued before approval for the research can be given.

Membership of the Committee

The members of the Ethics Committee who were present at the meeting are listed on the attached sheet.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

06/S0709/55	**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project

Yours sincerely

Andrea H Torrie
Ethics Manager – West Ethics Committees
e-mail: andrea.torrie@northglasgow.scot.nhs.uk

Enclosures: *List of names and professions of members who were present at the meeting and those who submitted written comments*
Standard approval conditions SL-AC2

Copy to: *R&D Department*

West Glasgow Ethics Committee 2

Attendance at Committee meeting on 18 April 2006

Committee Members:

Name	*Profession*	*Present?*	*Notes*
PRESENT:	Dr N Pace (Chairman)		
	Dr R Lindsay		
	Dr N Lucie		
	Mr J McHugh		
	Mrs J Wardlaw		
	Dr S Langridge		
	Dr S Humphreys		
	Mr K Wallace		
	Rev R Currie		
	Dr E Douglas		
	Dr M T Hosey		
	Prof M Gilhooly		
	Mrs A H Torrie		

10.3 VASCAB study participants

10.3.1 All participants

In total 126 patients with coronary artery disease (CAD) undergoing elective coronary artery bypass grafting (CABG) were recruited for the VASCAB study. Eighty control subjects were recruited in total. Table 10.1 shows demographics and clinical characteristics for patients with CAD compared to all controls. Control patients were younger than CAD patients. A higher proportion of the patients with CAD were male. There was no difference in systolic blood pressure between the two groups however patients with CAD had significantly lower diastolic blood pressure. Body mass index was significantly higher in patients with CAD compared to controls. As would be expected the use of secondary cardiovascular prevention therapies such as aspirin, statins and modifiers of rennin-angiotensin system was widespread in patients with CAD.

The control group consisted of 64 healthy controls and 16 patients attending for elective varicose vein surgery. The patients undergoing varicose vein (VV) surgery were significantly younger than the healthy controls otherwise the group were similar. Table 10.2 shows the participant characteristics for the healthy controls compared to VV controls.

Table 10.3 shows the biochemistry results for patients with CAD compared to all controls. Total cholesterol and low density lipoprotein (LDL) levels were significantly lower in the patients with CAD reflecting the widespread use of statins in this group. Triglyceride levels were significantly higher and high density lipoprotein (HDL) levels significantly lower in the CAD group. C-reactive protein (CRP), glycated haemoglobin (HbA_{1c}) and urinary albumin to creatinine ratio (ACR) were all significantly higher in the patients with CAD.

Table 10.4 shows the biochemistry results for healthy controls compared to VV controls. Healthy controls had higher LDL levels compared to the patients undergoing VV surgery there were no other significant differences.

Table 10.1. Participant characteristics for patients with CAD and all controls in the VASCAB study

	CAD (n=126)	All controls (n=80)	*P*-value
Age (years)	66.1 ±9.34	59.6±11.6	<0.0001
Male (%)	100(79.4)	43(53.8)	<0.0001
Systolic Blood pressure (mm Hg)	138.7±23.6	137.2±18.6	NS
Diastolic Blood pressure (mm Hg)	77.8±11.8	81.4±10.4	0.028
Heart rate (beats/min)	64.4±12.3	68.6±12.6	0.022
Body Mass Index (kg/m^2)	29.4±4.84	25.9±3.5	<0.0001
Current smokers (%)	11 (8.7)	5(6.3)	NS
Type 2 diabetes mellitus (%)	36(28.6)	0	n/a
Hypertension (%)	69(54.8)	21(26.3)	<0.0001
Myocardial Infarction (%)	63(50)	0	n/a
TIA/CVA (%)	9(7.1)	1(1.3)	NS
Chronic renal failure (%)	7(5.6)	0	NS
Heart failure (%)	22(17.5)	0	n/a
Aspirin (%)	105(83.3)	10(13)	<0.0001
Other antiplatelet agent (%)	34(27.0)	1(1.3)	<0.0001
Statin (%)	109(86.5)	9(11.3)	<0.0001
ACEi/ARB (%)	74(58.7)	6(7.5)	<0.0001
Beta-blocker (%)	94(74.6)	5(6.3)	<0.0001
Calcium channel blocker (%)	41(32.5)	5(6.3)	<0.0001
Nitrate (%)	68(54)	0	<0.0001
Diuretic (%)	31(24.6)	6(7.5)	0.001
Oral hypoglycaemic agent (%)	24(19)	0	n/a
Insulin (%)	8(6.3)	0	n/a

Continuous variables are mean ± standard deviation. Discrete variables are absolute numbers and percentage (%) TIA; transient ischaemic attack, CVA, cerebrovascular accident. ACEi, Angiotensin-converting enzyme inhibitor, ARB, Angiotensin receptor blocker.

Table 10.2 Participant characteristics for healthy controls and patients with VV recruited for the VASCAB study

	Healthy controls (n=64)	Varicose vein controls (n=16)
Age (years)	61.6±8.23	52.8±18.6
Male (%)	37(57.8)	7(43.8)*
Systolic Blood pressure (mm Hg)	137.2±18.4	137.3±22.2
Diastolic Blood pressure (mm Hg)	81.0±10.20	84.1±11
Heart rate (beats/min)	67.8±11.76	75.2±15.6
Body Mass Index (kg/m^2)	25.9±3.68	26.5±1.8
Current smokers (%)	3(4.7)	2(12.5)
Type 2 diabetes mellitus (%)	0	0
Hypertension (%)	18(28.1)	2(12.5)
Myocardial Infarction (%)	0	0
TIA/CVA (%)	0	1(6.3)**
Chronic renal failure (%)	0	0
Heart failure (%)	0	0
Aspirin (%)	8(12.5)	2(12.5)
Other antiplatelet agent (%)	0	1(6.3)
Statin (%)	7(10.9)	2(12.5)
ACEi/ARB (%)	5(7.8)	2(12.5)
Beta-blocker (%)	4(6.3)	2(12.5)
Calcium channel blocker (%)	4(6.3)	0
Nitrate (%)	0	0
Diuretic (%)	4(6.3)	1(6.3)

Continuous variables are mean ± standard deviation. Discrete variables are absolute numbers and percentage (%) TIA; transient ischaemic attack, CVA, cerebrovascular accident. ACEi, Angiotensin-converting enzyme inhibitor, ARB, Angiotensin receptor blocker.*P=0.038, **P=0.01.

Table 10.3 Biochemistry results for all CAD participants compared to all controls.

	CAD (n=126)	All controls (n=80)	*P*-value
Cholesterol (mmol/L)	4.00±0.94	5.70±1.14	<0.0001
Triglycerides (mmol/L)	2.04±1.21	1.51±0.78	0.001
LDL(mmol/L)	1.92±0.74	3.46±1.01	<0.0001
HDL(mmol/L)	1.17±0.31	1.55±0.41	<0.0001
CRP(mg/L)	4.09±8.08	2.00±2.33	0.003
HbA_{1c} (%)	6.16±1.12	5.52±0.31	<0.0001
Urinary ACR (mg/mmol)	4.58±17.6	1.30±0.92	0.019

All variables mean ± Standard deviation. LDL, low density lipoprotein; HDL, high density lipoprotein; CRP, C-reactive protein;HbA_{1c}, glycated haemoglobin; urinary ACR, urinary albumin:creatinine ratio.

Table 10.4 Biochemistry results for healthy controls compared to VV controls.

	Healthy controls(n=64)	VV control (n=16)
Cholesterol (mmol/L)	5.81±1.14	4.99±0.92
Triglycerides (mmol/L)	1.54±0.81	1.29±0.55
LDL(mmol/L)	3.57±0.98	2.75±1.04*
HDL(mmol/L)	1.53±0.42	1.64±0.36
CRP(mg/L)	1.78±1.96	3.42±3.87
HbA_{1c} (%)	5.53±0.33	5.41±0.15
Urinary ACR (mg/mmol)	1.31±0.97	1.22±0.44

All variables mean ± Standard deviation. LDL, low density lipoprotein; HDL, high density lipoprotein; CRP, C-reactive protein;HbA_{1c}, glycated haemoglobin; urinary ACR, urinary albumin:creatinine ratio.*P=0.034.

10.3.2 Patients with type 2 diabetes mellitus

Thirty seven (28.6%) of the participants with CAD had type 2 diabetes mellitus (DM). Participant characteristics for participants with CAD and type 2 DM compared to those with CAD alone are shown in 10.5.The patients with type 2 DM had significantly higher body mass index (BMI) compared to those with CAD alone. There was higher prevalence of hypertension in the patients with type 2 DM. Use of both diuretics and ACEi/ARB was higher in patients with type 2 DM.

Patients with type 2 DM and CAD had significantly lower HDL levels compared to patients with CAD alone (table 10.6). As would be expected HbA_{1c} levels were higher in patients with type 2 DM.

Table 10.5 Participant characteristics for patients with CAD and type 2 DM and patients with CAD alone

	CAD alone (n=90)	CAD and type 2 DM (n=36)	*P*-value
Age (years)	65.7±9.2	66.9±10.0	NS
Male (%)	67(77)	31 (83.8)	NS
Systolic BP (mm Hg)	139.0±24.8	140.3±19.7	NS
Diastolic BP (mm Hg)	78.8±11.3	74.7±12.7	NS
Heart rate (beats/min)	64.0±12.6	64.6±11.6	NS
Body Mass Index, kg/m^2	28.7±4.8	31.3±4.6	0.014
Current smokers (%)	8(9.2)	3(8.1)	NS
Hypertension (%)	36(41.4)	33(89.2)	0.0001
Myocardial Infarction (%)	41(47.1)	22(59.5)	NS
TIA/CVA (%)	5(5.7)	4(10.8)	NS
Chronic renal failure (%)	5(5.7)	2(5.4)	NS
Heart failure (%)	11(12.6)	11(29.7)	NS
Aspirin (%)	72(82.8)	33(89.2)	NS
Other antiplatelet agent (%)	26(29.9)	9(24.3)	NS
Statin (%)	76(87.4)	35(94.6)	NS
ACEi/ARB (%)	45(51.7)	30(81.1)	0.007
Beta-blocker (%)	64(73.6)	31(83.8)	NS
Calcium channel blocker (%)	25(28.7)	17(45.9)	NS
Nitrate (%)	51(58.6)	19(51.4)	NS
Diuretic (%)	14(16.1)	18(48.6)	0.001
Oral hypoglycaemic agent (%)	0	23(62.2)	n/a
Metformin		17 (47.2)	
Sulphonylurea		13 (36)	
Thiazolidinediones		4 (11)	
Insulin (%)	0	8(21.6)	n/a

Continuous variables are mean ± standard deviation. Discrete variables are absolute numbers and percentage (%) TIA; transient ischaemic attack, CVA, cerebrovascular accident. ACEi, Angiotensin-converting enzyme inhibitor, ARB, Angiotensin receptor blocker.

Table 10.6 Biochemistry results in patients with CAD alone compared to patients with CAD and type 2 DM

	CAD alone (n=90)	**CAD and type 2 DM (n=36)**	***P*-value**
Cholesterol (mmol/L)	4.09±0.91	3.82±0.99	NS
Triglycerides (mmol/L)	1.92±0.89	2.38±1.71	NS
LDL (mmol/L)	2.00±0.73	1.74±0.74	NS
HDL (mmol/L)	1.21±0.33	1.07±0.24	*P*=0.030
CRP(mg/L)	4.29±8.75	3.78±6.60	NS
HbA_{1c} (%)	5.62±0.37	7.39±1.29	<0.0001
Urinary ACR(mg/mmol)	2.31±6.24	3.59±4.17	0.021

All variables mean ± Standard deviation. LDL, low density lipoprotein; HDL, high density lipoprotein; CRP, C-reactive protein; HbA_{1c}, glycated haemoglobin; urinary ACR, urinary albumin:creatinine ratio.

11 References

1. British Heart Foundation (2009) Coronary Heart Disease Statistics . http//www.heartstats.org. Accessed 1/09/2010

2. The INTERHEART study investigators. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. *Lancet* 2004;**364**:937-52.

3. WHO guidelines for the definition and diagnosis of diabetes. http://www.who.int/diabetes/publications/Definition%20and%20diagnosis%20of%20diabetes_new.pdf . Accessed 01/05/2011.

4. Stumvoll M, Goldstein BJ, van Haeften TW. Type 2 diabetes: principles of pathogenesis and therapy. *The Lancet* 2005;**365**:1333-46.

5. International Diabetes Federation (2006). The Diabetes Atlas. Third Edition. Brussels

6. Wild S, Roglic G, Green A, Sicree R, King H. Global Prevalence of Diabetes. *Diabetes Care* 2004;**27**:1047-53.

7. Scottish diabetes survey (2009). http://www.diabetesinscotland.org.uk/Publications/ScottishDiabetesSurvey2009 . Accessed 20/01/2010.

8. Morrish, N. J., Wang, S. L., Stevens, L. K., Fuller, J. H., Keen, H., and . Mortality and causes of death in the WHO multinational study of vascular disease in diabetes. Diabetologia 44(0), S14-S21. 1-9-2001.

9. Haffner SM, Lehto S, Ronnemaa T, Pyorala K, Laakso M. Mortality from Coronary Heart Disease in Subjects with Type 2 Diabetes and in Nondiabetic Subjects with and without Prior Myocardial Infarction. *N Engl J Med* 1998;**339**:229-34.

10. Booth GL, Kapral MK, Fung K, Tu JV. Recent Trends in Cardiovascular Complications Among Men and Women With and Without Diabetes. *Diabetes Care* 2006;**29**:32-7.

11. Berry C, Tardif JC, Bourassa MG. Coronary Heart Disease in Patients With Diabetes: Part I: Recent Advances in Prevention and Noninvasive Management. *J Am Coll Cardiol* 2007;**49**:631-42.

12. Natali, A., Vichi, S., Landi, P., Severi, S., L'Abbate, A., and Ferrannini, E. Coronary atherosclerosis in Type II diabetes: angiographic findings and clinical outcome. Diabetologia 43(5), 632-641. 18-5-2000.

13. Selvin E, Marinopoulos S, Berkenblit G, Rami T, Brancati FL, Powe NR *et al*. Meta-Analysis: Glycosylated Hemoglobin and Cardiovascular Disease in Diabetes Mellitus. *Annals of Internal Medicine* 2004;**141**:421-31.

14. Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38. UK Prospective Diabetes Study Group. *BMJ (Clinical Research Ed.)* 1998;**317**:703-13.

15. Howard G, O'Leary DH., Zaccaro D, Haffner S et al. Insulin Sensitivity and Atherosclerosis. Circulation 93(10), 1809-1817. 15-5-1996.

16. Laaksonen DE, Niskanen L, Lakka HM, Lakka TA, Uusitupa M. Epidemiology and treatment of the metabolic syndrome. *Ann Med* 2004;**36**:332-46.

17. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. *The Lancet* 2005;**365**:1415-28.

18. Ginsberg HN. Insulin resistance and cardiovascular disease. *J Clin Invest* 2000;**106**:453-8.

19. Fuller JH, McCartney P, Jarrett RJ, Keen H, Rose G, Shipley MJ *et al*. Hyperglycaemia and coronary heart disease: the Whitehall study. *J Chronic.Dis.* 1979;**32**:721-8.

20. Stratton IM, Adler AI, Neil HA, Matthews DR, Manley SE, Cull CA *et al*. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. *BMJ (Clinical Research Ed.)* 2000;**321**:405-12.

21. The Emerging Risk Factors Collaboration. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. *The Lancet* 2010;**375**:2215-22.

22. Prepared by: British Cardiac Society, British hypertension society, Diabetes UK, Heart UK,Primary care cardiovascular society, the Stroke association:Joint British Societies' guidelines on prevention of cardiovascular disease in clinical practice. *Heart* 2005;**91**:v1-52.

23. Smith SC, Jr., Allen J, Blair SN, Bonow RO, Brass LM, Fonarow GC *et al*. AHA/ACC Guidelines for Secondary Prevention for Patients With Coronary and Other Atherosclerotic Vascular Disease: 2006 Update: Endorsed by the National Heart, Lung, and Blood Institute. *Circulation* 2006;**113**:2363-72.

24. European guidelines on cardiovascular disease prevention in clinical practice: executive summary: Fourth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (Constituted by representatives of nine societies and by invited experts). [Miscellaneous]. *European Journal of Cardiovascular Prevention & Rehabilitation* 2007;**14 Supplement 2**:E1-E40.

25. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *The Lancet* 1998;**352**:837-53.

26. The ADVANCE Collaborative Group. Intensive Blood Glucose Control and Vascular Outcomes in Patients with Type 2 Diabetes. .*New England Journal of Medicine* 2008;**358**:2560-72.

27. The Action to Control Cardiovascular Risk in Diabetes Study Group. Effects of Intensive Glucose Lowering in Type 2 Diabetes. . *New England Journal of Medicine* 2008;**358**:2545-59.

28. Duckworth W, Abraira C, Moritz T, Reda D, Emanuele N, Reaven PD *et al*. Glucose control and vascular complications in veterans with type 2 diabetes. *N Engl J Med* 2009;**360**:129-39.

29. Ray KK, Seshasai SRK, Wijesuriya S, Sivakumaran R, Nethercott S, Preiss D *et al*. Effect of intensive control of glucose on cardiovascular outcomes and death in patients with diabetes mellitus: a meta-analysis of randomised controlled trials. *The Lancet* 2009;**373**:1765-72.

30. Gill G, Woodward A, Casson I, Weston P. Cardiac arrhythmia and nocturnal hypoglycaemia in type 1 diabetes The dead in bed syndrome revisited. *Diabetologia* 2009;**52**:42-5.

31. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). *The Lancet* 1998;**352**:854-65.

32. Nissen SE,.Wolski K. Effect of Rosiglitazone on the Risk of Myocardial Infarction and Death from Cardiovascular Causes. *New England Journal of Medicine* 2009;**356**:2457-71.

33. Blood Pressure Lowering Treatment Trialists' Collaboration. Effects of Different Blood Pressure-Lowering Regimens on Major Cardiovascular Events in Individuals With and Without Diabetes Mellitus: Results of Prospectively Designed Overviews of Randomized Trials. *Arch Intern Med* 2005;**165**:1410-9.

34. Adler AI, Stratton IM, Neil HA, Yudkin JS, Matthews DR, Cull CA *et al*. Association of systolic blood pressure with macrovascular and microvascular complications of type 2 diabetes (UKPDS 36): prospective observational study. *BMJ (Clinical Research Ed.)* 2000;**321**:412-9.

35. Cushman WC, Evans GW, Byington RP, Goff DC, Jr., Grimm RH, Jr., Cutler JA *et al*. Effects of intensive blood-pressure control in type 2 diabetes mellitus. *N Engl J Med* 2010;**362**:1575-85.

36. Collins R, Peto R. Antihypertensive drug therapy:effects on stroke and coronary heart disease. In Swales JD, ed. *Textbook of hypertension*, pp 1156-64. London: Blackwell Scientific Publications, 1994.

37. Dahlof B, Sever PS, Poulter NR, Wedel H, Beevers DG, Caulfield M *et al*. Prevention of cardiovascular events with an antihypertensive regimen of amlodipine adding perindopril as required versus atenolol adding bendroflumethiazide as required, in the Anglo-Scandinavian Cardiac Outcomes Trial-Blood Pressure Lowering Arm (ASCOT-BPLA): a multicentre randomised controlled trial. *The Lancet* 2005;**366**:895-906.

38. Williams B,Lacy PS, Thom SM et al. Differential Impact of Blood Pressure-Lowering Drugs on Central Aortic Pressure and Clinical Outcomes: Principal Results of the Conduit Artery Function Evaluation (CAFE) Study. *Circulation* 2006;**113**:1213-25.

39. Sarafidis PA, Stafylas PC, Kanaki AI, Lasaridis AN. Effects of Renin-Angiotensin System Blockers on Renal Outcomes and All-cause Mortality in Patients With Diabetic Nephropathy: An Updated Meta-analysis. *Am J Hypertens* 2008;**21**:922-9.

40. Kannel WB. Lipids, diabetes, and coronary heart disease: insights from the Framingham Study. *Am Heart J* 1985;**110**:1100-7.

41. Smith G.D, Shipley MJ, Marmot M.G, Rose G. Plasma cholesterol concentration and mortality. The Whitehall Study. *JAMA* 1992;**267**:2740-1.

42. Law MR, Wald NJ, Rudnicka AR. Quantifying effect of statins on low density lipoprotein cholesterol, ischaemic heart disease, and stroke: systematic review and meta-analysis. *BMJ (Clinical Research Ed.)* 2003;**326**:1423.

43. Buchwald H, Varco RL, Boen JR, Williams SE, Hansen BJ, Campos CT *et al*. Effective Lipid Modification by Partial Ileal Bypass Reduced Long-term Coronary Heart Disease Mortality and Morbidity: Five-Year Post trial Follow-up Report From the POSCH. *Arch Intern Med* 1998;**158**:1253-61.

44. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90 056 participants in 14 randomised trials of statins. *The Lancet* 2005;**366**:1267-78.

45. British Cardiac Society, British Hyperlipidaemia Association, British Hypertension Society, British Diabetic Association. Joint British recommendations on prevention of coronary heart disease in clinical practice: summary . *BMJ (Clinical Research Ed.)* 2000;**320**:705-8.

46. De Backer G, Ambrosioni E, Borch-Johnsen K, Brotons C, Cifkova R, Dallongeville J *et al*. European guidelines on cardiovascular disease prevention in clinical practice: Third Joint Task Force of European and other Societies on Cardiovascular Disease Prevention in Clinical Practice. *Eur Heart J* 2003;**24**:1601-10.

47. Smith SC, Jr., Blair SN, Bonow RO, Brass LM, Cerqueira MD, Dracup K *et al*. AHA/ACC Guidelines for Preventing Heart Attack and Death in Patients With Atherosclerotic Cardiovascular Disease: 2001 Update: A Statement for Healthcare Professionals From the American Heart Association and the American College of Cardiology. *Circulation* 2001;**104**:1577-9.

48. Gordon T, Castelli WP, Hjortland MC, Kannel WB, Dawber TR. Diabetes, blood lipids, and the role of obesity in coronary heart disease risk for women. The Framingham study. *Ann Intern Med* 1977;**87**:393-7.

49. Sharrett AR, Ballantyne CM, Coady SA, Heiss G, Sorlie PD, Catellier D *et al*. Coronary Heart Disease Prediction From Lipoprotein Cholesterol Levels, Triglycerides, Lipoprotein(a), Apolipoproteins A-I and B, and HDL Density Subfractions: The Atherosclerosis Risk in Communities (ARIC) Study. *Circulation* 2001;**104**:1108-13.

50. Gordon DJ, Probstfield JL, Garrison RJ, Neaton JD, Castelli WP, Knoke JD *et al*. High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. *Circulation* 1989;**79**:8-15.

51. Leon AS, Rice T, Mandel S, Despres JP, Bergeron J, Gagnon J *et al*. Blood lipid response to 20 weeks of supervised exercise in a large biracial population: The HERITAGE family study. *Metabolism* 2000;**49**:513-20.

52. Dattilo AM,.Kris-Etherton PM. Effects of weight reduction on blood lipids and lipoproteins: a meta- analysis. *Am J Clin Nutr* 1992;**56**:320-8.

53. Petersen M, Pedersen H, Major-Pedersen A, Jensen T, Marckmann P. Effect of Fish Oil Versus Corn Oil Supplementation on LDL and HDL Subclasses in Type 2 Diabetic Patients. *Diabetes Care* 2002;**25**:1704-8.

54. Barter PJ, Caulfield M, Eriksson M, Grundy SM, Kastelein JJP, Komajda M *et al*. Effects of Torcetrapib in Patients at High Risk for Coronary Events. *New England Journal of Medicine* 2009;**357**:2109-22.

55. Johansson J,.Carlson LA. The effects of nicotinic acid treatment on high density lipoprotein particle size subclass levels in hyperlipidaemic subjects. *Atherosclerosis* 1990;**83**:207-16.

56. Hausenloy DJ,.Yellon DM. Targeting residual cardiovascular risk: raising high-density lipoprotein cholesterol levels. *Heart* 2008;**94**:706-14.

57. Chiquette E, Ramirez G, DeFronzo R. A Meta-analysis Comparing the Effect of Thiazolidinediones on Cardiovascular Risk Factors. *Arch Intern Med* 2004;**164**:2097-104.

58. Morris JN, Heady JA, Raffle PAB, Roberts CG, Parks JW. Coronary heart disease and physical activity of work. *The Lancet* 1953;**262**:1053-7.

59. Han TS, Sattar N, Lean M. Assessment of obesity and its clinical implications. *BMJ (Clinical Research Ed.)* 2006;**333**:695-8.

60. Haslam DW,.James WP. Obesity. *Lancet* 2005;**366**:1197-209.

61. Stevens J, Couper D, Pankow J, Folsom AR, Duncan BB, Nieto FJ *et al*. Sensitivity and specificity of anthropometrics for the prediction of diabetes in a biracial cohort. *Obes.Res* 2001;**9**:696-705.

62. Ebrahim S, Kinra S, Bowen L, Andersen E, Ben Shlomo Y, Lyngdoh T *et al*. The Effect of Rural-to-Urban Migration on Obesity and Diabetes in India: A Cross-Sectional Study. *PLoS Med* 2010;**7**:e1000268.

63. Ning F, Pang ZC, Dong YH, Gao WG, Nan HR, Wang SJ *et al*. Risk factors associated with the dramatic increase in the prevalence of diabetes in the adult Chinese population in Qingdao, China. *Diabetic Medicine* 2009;**26**:855-63.

64. Avenell A, Broom J, Brown TJ, Poobalan A, Aucott L, Stearns SC *et al*. Systematic review of the long-term effects and economic consequences of treatments for obesity and implications for health improvement. *Health Technol.Assess.* 2004;**8**:iii-iv.1-182.

65. Lean M, Gruer L, Alberti G, Sattar N. Obesity Can we turn the tide? *BMJ (Clinical Research Ed.)* 2006;**333**:1261-4.

66. European Medicines Agency Press release. EMA recommends suspension of the marketing authorisation of Acomplia. http://www.ema.europa.eu/docs/en_GB/document_library/Press_release/2009/11/WC500014774.pdf (2008) Accessed 2/9/2010.

67. European Medicines Agency. European Medicines Agency recommends suspension of marketing authorisation for sibutramine. http://www.ema.europa.eu/docs/en_GB/document_library/Press_release/2010/01/WC500069995.pdf .(2010).Accessed 2/09/2010

68. Padwal RS,.Majumdar SR. Drug treatments for obesity: orlistat, sibutramine, and rimonabant. *The Lancet* 2007;**369**:71-7.

69. Rosengren A,.Wilhelmsen L. Physical activity protects against coronary death and deaths from all causes in middle-aged men. Evidence from a 20-year follow-up of the primary prevention study in Goteborg. *Ann Epidemiol* 1997;**7**:69-75.

70. Thompson PD, Buchner D, Pina IL, Balady GJ, Williams MA, Marcus BH *et al*. Exercise and Physical Activity in the Prevention and Treatment of Atherosclerotic Cardiovascular Disease: A Statement From the Council on Clinical Cardiology (Subcommittee on Exercise, Rehabilitation, and Prevention) and the Council on Nutrition, Physical Activity, and Metabolism (Subcommittee on Physical Activity). *Circulation* 2003;**107**:3109-16.

71. Wannamethee SG, Shaper AG, Walker M. Changes in physical activity, mortality, and incidence of coronary heart disease in older men. *The Lancet* 1998;**351**:1603-8.

72. The Scottish Government. The Scottish Health Survey 2008. http://www.scotland.gov.uk/Publications/2009/09/28102003/66 . Accessed 2-9-2010.

73. Dzau V, Braunwald E. Resolved and unresolved issues in the prevention and treatment of coronary artery disease: A workshop consensus statement. *Am Heart J* 1991;**121**:1244-63.

74. Ross R. Atherosclerosis--an inflammatory disease. *N Engl J Med* 1999;**340**:115-26.

75. Woollard KJ,.Geissmann F. Monocytes in atherosclerosis: subsets and functions. *Nat Rev.Cardiol* 2010;**7**:77-86.

76. Hansson GK. Mechanisms of Disease: Inflammation, Atherosclerosis, and Coronary Artery Disease. *New England Journal of Medicine* 2005;**352**:1685-95.

77. Davi G,.Patrono C. Mechanisms of Disease: Platelet Activation and Atherothrombosis. *New England Journal of Medicine* 2007;**357**:2482-94.

78. Vallance P,.Chan N. Endothelial function and nitric oxide: clinical relevance. *Heart* 2001;**85**:342-50.

79. Hamilton CA, Miller WH, Al Benna S, Brosnan MJ, Drummond RD, McBride MW *et al*. Strategies to reduce oxidative stress in cardiovascular disease. *Clin Sci.(Lond)* 2004;**106**:219-34.

80. Zieman SJ, Melenovsky V, Kass DA. Mechanisms, Pathophysiology, and Therapy of Arterial Stiffness. *Arterioscler Thromb Vasc Biol* 2005;**25**:932-43.

81. Brunner H, Cockcroft JR, Deanfield J, Donald A, Ferrannini E, Halcox J *et al*. Endothelial function and dysfunction. Part II: Association with cardiovascular risk factors and diseases. A statement by the Working Group on Endothelins and Endothelial Factors of the European Society of Hypertension. *J Hypertens* 2005;**23**:233-46.

82. Drexler H. Endothelial dysfunction: Clinical implications. *Progress in Cardiovascular Diseases* 2001;**39**:287-324.

83. Landmesser U, Hornig B, Drexler H. Endothelial Function: A Critical Determinant in Atherosclerosis? *Circulation* 2004;**109**:II-27.

84. Cai H,.Harrison DG. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. *Circ Res* 2000;**87**:840-4.

85.Deanfield JE, Halcox JP, Rabelink TJ. Endothelial Function and Dysfunction: Testing and Clinical Relevance. *Circulation* 2007;**115**:1285-95.

86. Taniyama Y,.Griendling KK. Reactive Oxygen Species in the Vasculature: Molecular and Cellular Mechanisms. *Hypertension* 2003;**42**:1075-81.

87. Guzik TJ, West NEJ, Black E, McDonald D, Ratnatunga C, Pillai R *et al*. Vascular Superoxide Production by NAD(P)H Oxidase : Association With Endothelial Dysfunction and Clinical Risk Factors. *Circ Res* 2000;**86**:e85-e90.

88. Droge W. Free Radicals in the Physiological Control of Cell Function. *Physiol.Rev.* 2002;**82**:47-95.

89. Hink U, Li H, Mollnau H, Oelze M, Matheis E, Hartmann M *et al*. Mechanisms underlying endothelial dysfunction in diabetes mellitus. *Circ Res* 2001;**88**:E14-E22.

90. Griendling KK,.FitzGerald GA. Oxidative Stress and Cardiovascular Injury: Part II: Animal and Human Studies. *Circulation* 2003;**108**:2034-40.

91. Jay D, Hitomi H, Griendling KK. Oxidative stress and diabetic cardiovascular complications. *Free Radical Biology and Medicine* 2006;**40**:183-92.

92. Libby P, Ridker PM, Maseri A. Inflammation and Atherosclerosis. *Circulation* 2002;**105**:1135-43.

93. Greenwald S. Ageing of the conduit arteries. *J.Pathol.* 2007;**211**:157-72.

94. Safar ME, Levy BI, Struijker-Boudier H. Current Perspectives on Arterial Stiffness and Pulse Pressure in Hypertension and Cardiovascular Diseases. *Circulation* 2003;**107**:2864-9.

95. Oliver JJ,.Webb DJ. Noninvasive Assessment of Arterial Stiffness and Risk of Atherosclerotic Events. *Arterioscler Thromb Vasc Biol* 2003;**23**:554-66.

96. Hamilton PK, Lockhart CJ, Quinn CE, McVeigh GE. Arterial stiffness: clinical relevance, measurement and treatment. *Clin.Sci.* 2007;**113**:157-70.

97. O'Rourke MF,.Adji A. An updated clinical primer on large artery mechanics: implications of pulse waveform analysis and arterial tonometry. *Curr.Opin.Cardiol* 2005;**20**:275-81.

98. Scottish Intercollegiate Guidelines Network. Management of stable angina. http://www.sign.ac.uk/pdf/sign96.pdf .(2007). Accessed 2-9-2010.

99. Pell JP, Slack R. The Scottish Coronary Revaascularisation Register. http://www.scs-online.org.uk/pdf/AR_0809.pdf .(2009).Accessed 3-9-2010.

100. King SB, Kosinski AS, Guyton RA, Lembo NJ, Weintraub WS. Eight-year mortality in the Emory Angioplasty versus Surgery Trial (EAST). *J Am Coll Cardiol* 2000;**35**:1116-21.

101. Investigators TB. Influence of Diabetes on 5-Year Mortality and Morbidity in a Randomized Trial Comparing CABG and PTCA in Patients With Multivessel Disease : The Bypass Angioplasty Revascularization Investigation (BARI). *Circulation* 1997;**96**:1761-9.

102. Ahmed JM, Hong MK, Mehran R, Dangas G, Mintz GS, Pichard AD *et al*. Influence of diabetes mellitus on early and late clinical outcomes in saphenous vein graft stenting. *J Am Coll Cardiol* 2000;**36**:1186-93.

103. Pell JP, Pell AC, Jeffrey RR, Jennings K, Oldroyd K, Eteiba H *et al*. Comparison of survival following coronary artery bypass grafting vs. percutaneous coronary intervention in diabetic and non-diabetic patients: retrospective cohort study of 6320 procedures. *Diabet.Med* 2004;**21**:790-2.

104. Scottish Intercollegiate Guidelines Network. Management of diabetes. www.sign.ac.uk (SIGN publication no.116). 2010. Accessed 5-9-2010.

105. Arnott AS. Assessment of functional capacity in cardiac rehabilitation: Reproducibility of a 10-m shuttle walk test. *Coronary Health Care* 1997;**1**:30-6.

106. Buckley JP, Sim J, Eston RG, Hession R, Fox R. Reliability and validity of measures taken during the Chester step test to predict aerobic power and to prescribe aerobic exercise. *British Journal of Sports Medicine* 2004;**38**:197-205.

107. Lowther M, Mutrie N, Loughlan C, McFarlane C. Development of a Scottish physical activity questionnaire: a tool for use in physical activity interventions. *British Journal of Sports Medicine* 1999;**33**:244-9.

108. Wilkinson IB, Hall IR, MacCallum H, Mackenzie IS, McEniery CM, van der Arend BJ *et al*. Pulse-Wave Analysis: Clinical Evaluation of a Noninvasive, Widely Applicable Method for Assessing Endothelial Function. *Arterioscler Thromb Vasc Biol* 2002;**22**:147-52.

109. Hayward CS, Kraidly M, Webb CM, Collins P. Assessment of endothelial function using peripheral waveform analysis: A clinical application. *J Am Coll Cardiol* 2002;**40**:521-8.

110. Chowienczyk PJ, Kelly RP, MacCallum H, Millasseau SC, Andersson TLG, Gosling RG *et al*. Photoplethysmographic assessment of pulse wave reflection : Blunted response to endothelium-dependent beta2-adrenergic vasodilation in type II diabetes mellitus. *J Am Coll Cardiol* 1999;**34**:2007-14.

111.Dawes M, Chowienczyk PJ, Ritter JM. Effects of Inhibition of the L-Arginine/Nitric Oxide Pathway on Vasodilation Caused by ¯-Adrenergic Agonists in Human Forearm. *Circulation* 1997;**95**:2293-7.

112. Celermajer DS, Sorensen KE, Gooch VM, Miller 01, Sullivan ID, Lloyd JK *et al*. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *The Lancet* 1992;**340**:1111-5.

113. Dikalov S, Griendling KK, Harrison DG. Measurement of Reactive Oxygen Species in Cardiovascular Studies. *Hypertension* 2007;**49**:717-27.

114. Berry C, Hamilton CA, Brosnan MJ, Magill FG, Berg GA, McMurray JJV *et al*. Investigation Into the Sources of Superoxide in Human Blood Vessels : Angiotensin II Increases Superoxide Production in Human Internal Mammary Arteries. *Circulation* 2000;**101**:2206-12.

115. Fink B, Dikalov S, Bassenge E. A new approach for extracellular spin trapping of nitroglycerin-induced superoxide radicals both in vitro and in vivo. *Free Radical Biology and Medicine* 2000;**28**:121-8.

116. Fortuno A, Olivan S, Beloqui O, San Jose G, Moreno MU, Diez J *et al*. Association of increased phagocytic NADPH oxidase-dependent superoxide production with diminished nitric oxide generation in essential hypertension. [Article]. *Journal of Hypertension* 2004;**22**:2169-75.

117. Friedlander Y, Kidron M, Caslake M, Lamb T, McConnell M, and Bar H. Low density lipoprotein particle size and risk factors of insulin resistance syndrome. Atherosclerosis 2000 148(1), 141-149.

118. Nichols WW. Clinical measurement of arterial stiffness obtained from noninvasive pressure waveforms. *Am J Hypertens* 2005;**18**:3S-10S.

119. Payne RA, Wilkinson IB, Webb DJ. Arterial Stiffness and Hypertension: Emerging Concepts. *Hypertension* 2010;**55**:9-14.

120. The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC).2007 Guidelines for the management of arterial hypertension. *Journal of Hypertension* 2007;**25**:1105-87.

121. Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D *et al*. Expert consensus document on arterial stiffness: methodological issues and clinical applications. *Eur Heart J* 2006;**27**:2588-605.

122. Cecelja M,.Chowienczyk P. Dissociation of Aortic Pulse Wave Velocity With Risk Factors for Cardiovascular Disease Other Than Hypertension: A Systematic Review. *Hypertension* 2009;**54**:1328-36.

123. Blacher J, Asmar R, Djane S, London GM, Safar ME. Aortic Pulse Wave Velocity as a Marker of Cardiovascular Risk in Hypertensive Patients. *Hypertension* 1999;**33**:1111-7.

124. Lehmann ED, Watts GF, Gosling RG. Aortic distensibility and hypercholesterolaemia. *The Lancet* 1992;**340**:1171-2.

125. Lehmann ED, Gosling RG, Sonksen PH. Arterial wall compliance in diabetes. *Diabetic Medicine* 1992;**9**:114-9.

126. Vaitkevicius PV, Fleg JL, Engel JH, O'Connor FC, Wright JG, Lakatta LE *et al*. Effects of age and aerobic capacity on arterial stiffness in healthy adults. *Circulation* 1993;**88**:1456-62.

127. Lacy PS, O'Brien DG, Stanley AG, Dewar MM, Swales PP, Williams B. Increased pulse wave velocity is not associated with elevated augmentation index in patients with diabetes. *J Hypertens* 2004;**22**:1937-44.

128. McEniery CM, Wallace S, Mackenzie IS, McDonnell B, Yasmin, Newby DE *et al*. Endothelial Function Is Associated With Pulse Pressure, Pulse Wave Velocity, and Augmentation Index in Healthy Humans. *Hypertension* 2006;**48**:602-8.

129. Blacher J, Guerin AP, Pannier B, Marchais SJ, Safar ME, London GM. Impact of Aortic Stiffness on Survival in End-Stage Renal Disease. *Circulation* 1999;**99**:2434-9.

130. Boutouyrie P, Tropeano AI, Asmar R, Gautier I, Benetos A, Lacolley P *et al*. Aortic Stiffness Is an Independent Predictor of Primary Coronary Events in Hypertensive Patients: A Longitudinal Study. *Hypertension* 2002;**39**:10-5.

131. Cruickshank K, Riste L, Anderson SG, Wright JS, Dunn G, Gosling RG. Aortic Pulse-Wave Velocity and Its Relationship to Mortality in Diabetes and Glucose Intolerance: An Integrated Index of Vascular Function? *Circulation* 2002;**106**:2085-90.

132. Laurent S, Boutouyrie P, Asmar R, Gautier I, Laloux B, Guize L *et al*. Aortic Stiffness Is an Independent Predictor of All-Cause and Cardiovascular Mortality in Hypertensive Patients. *Hypertension* 2001;**37**:1236-41.

133. Mackenzie IS, McEniery CM, Dhakam Z, Brown MJ, Cockcroft JR, Wilkinson IB. Comparison of the Effects of Antihypertensive Agents on Central Blood Pressure and Arterial Stiffness in Isolated Systolic Hypertension. *Hypertension* 2009;**54**:409-13.

134. Karalliedde J, Smith A, DeAngelis L, Mirenda V, Kandra A, Botha J *et al*. Valsartan Improves Arterial Stiffness in Type 2 Diabetes Independently of Blood Pressure Lowering. *Hypertension* 2008;**51**:1617-23.

135. Kass DA, Shapiro EP, Kawaguchi M, Capriotti AR, Scuteri A, deGroof RC *et al*. Improved Arterial Compliance by a Novel Advanced Glycation End-Product Crosslink Breaker. *Circulation* 2001;**104**:1464-70.

136. Van Bortel LM, Duprez D, Starmans-Kool MJ, Safar ME, Giannattasio C, Cockcroft J *et al*. Clinical applications of arterial stiffness, Task Force III: recommendations for user procedures. *Am J Hypertens* 2002;**15**:445-52.

137. Pannier B, Guerin AP, Marchais SJ, Safar ME, London GM. Stiffness of Capacitive and Conduit Arteries: Prognostic Significance for End-Stage Renal Disease Patients. *Hypertension* 2005;**45**:592-6.

138. Cameron JD, Bulpitt CJ, Pinto ES, Rajkumar C. The Aging of Elastic and Muscular Arteries: A comparison of diabetic and nondiabetic subjects. *Diabetes Care* 2003;**26**:2133-8.

139. Chen CH, Nevo E, Fetics B, Pak PH, Yin FCP, Maughan WL *et al*. Estimation of Central Aortic Pressure Waveform by Mathematical Transformation of Radial Tonometry Pressure : Validation of Generalized Transfer Function. *Circulation* 1997;**95**:1827-36.

140. Wilkinson I, MacCallum H, Flint Laura, Cockcroft JR, Newby David E, Webb DJ. The Influence of heart rate on augmentation index and central arterial pressure in humans. *J Physiol* 2000;5**25**:263-70.

141. Williams B. Pulse wave analysis and hypertension: evangelism versus scepticism. *J Hypertens* 2004;**22**:447-9.

142. Gallagher D, Adji A, 'Rourke MF. Validation of the transfer function technique for generating central from peripheral upper limb pressure waveform. *Am J Hypertens* 2004;**17**:1059-67.

143. Segers P, Rietzschel E, Heireman S, De Buyzere M, Gillebert T, Verdonck P *et al*. Carotid Tonometry Versus Synthesized Aorta Pressure Waves for the Estimation of Central Systolic Blood Pressure and Augmentation Index[ast]. *Am J Hypertens* 2005;**18**:1168-73.

144. Hope SA, Tay DB, Meredith IT, Cameron JD. Use of Arterial Transfer Functions for the Derivation of Central Aortic Waveform Characteristics in Subjects With Type 2 Diabetes and Cardiovascular Disease. *Diabetes Care* 2004;**27**:746-51.

145. McEniery CM, Yasmin, Hall IR, Qasem A, Wilkinson IB, Cockcroft JR. Normal Vascular Aging: Differential Effects on Wave Reflection and Aortic Pulse Wave Velocity: The Anglo-Cardiff Collaborative Trial (ACCT). *J Am Coll Cardiol* 2005;**46**:1753-60.

146. Gatzka CD, Kingwell BA, Cameron JD, Berry KL, Liang YL, Dewar EM *et al*. Gender differences in the timing of arterial wave reflection beyond differences in body height. *Journal of Hypertension* 2001;**19**:2197-203.

147. Hayward CS,.Kelly RP. Gender-Related Differences in the Central Arterial Pressure Waveform. *J Am Coll Cardiol* 1997;**30**:1863-71.

148.Mitchell GF, Parise H, Benjamin EJ, Larson MG, Keyes MJ, Vita JA *et al*. Changes in Arterial Stiffness and Wave Reflection With Advancing Age in Healthy Men and Women: The Framingham Heart Study. *Hypertension* 2004;**43**:1239-45.

149. Smulyan H, Marchais SJ, Pannier B, Guerin AP, Safar ME, London GM. Influence of Body Height on Pulsatile Arterial Hemodynamic Data. *J Am Coll Cardiol* 1998;**31**:1103-9.

150. Nurnberger J, Keflioglu-Scheiber A, Opazo Saez AM, Wenzel RR, Philipp T, Schafers RF. Augmentation index is associated with cardiovascular risk. *J Hypertens* 2002;**20**:2407-14.

151. Wilkinson IB, Prasad K, Hall IR, Thomas A, MacCallum H, Webb DJ *et al*. Increased central pulse pressure and augmentation index in subjects with hypercholesterolemia. *J Am Coll Cardiol* 2002;**39**:1005-11.

152. Weber T, Auer J, O'Rourke MF, Kvas E, Lassnig E, Berent R *et al*. Arterial Stiffness, Wave Reflections, and the Risk of Coronary Artery Disease. *Circulation* 2004;**109**:184-9.

153. London GM, Blacher J, Pannier B, Guerin AP, Marchais SJ, Safar ME. Arterial Wave Reflections and Survival in End-Stage Renal Failure. *Hypertension* 2001;**38**:434-8.

154. Weber T, Auer J, O'Rourke MF, Kvas E, Lassnig E, Lamm G *et al*. Increased arterial wave reflections predict severe cardiovascular events in patients undergoing percutaneous coronary interventions. *Eur Heart J* 2005;**26**:2657-63.

155. Dart AM, Gatzka CD, Kingwell BA, Willson K, Cameron JD, Liang YL *et al*. Brachial Blood Pressure But Not Carotid Arterial Waveforms Predict Cardiovascular Events in Elderly Female Hypertensives. *Hypertension* 2006;**47**:785-90.

156. Chirinos JA, Zambrano JP, Chakko S, Veerani A, Schob A, Willens HJ *et al*. Aortic Pressure Augmentation Predicts Adverse Cardiovascular Events in Patients With Established Coronary Artery Disease. *Hypertension* 2005;**45**:980-5.

157. Salomaa V, Riley W, Kark JD, Nardo C, Folsom AR. Non Insulin-Dependent Diabetes Mellitus and Fasting Glucose and Insulin Concentrations Are Associated With Arterial Stiffness Indexes : The ARIC Study. *Circulation* 1995;**91**:1432-43.

158. Megnien JL, Simon A, Valensi P, Flaud P, Merli I, Levenson J. Comparative effects of diabetes mellitus and hypertension on physical properties of human large arteries. *J Am Coll Cardiol* 1992;**20**:1562-8.

159. Woolam GL, Schnur PL, Vallbona C, Hoff HE. The pulse wave velocity as an early indicator of atherosclerosis in diabetic subjects. *Circulation* 1962;**25**:533-9.

160. Schram MT, Henry RMA, van Dijk RAJM, Kostense PJ, Dekker JM, Nijpels G *et al*. Increased Central Artery Stiffness in Impaired Glucose Metabolism and Type 2 Diabetes: The Hoorn Study. *Hypertension* 2004;**43**:176-81.

161. Brooks BA, Molyneaux LM, Yue DK. Augmentation of central arterial pressure in Type 2 diabetes. *Diabetic Medicine* 2001;**18**:374-80.

162. Maple-Brown LJ, Piers LS, O'Rourke MF, Celermajer DS, O'Dea K. Central obesity is associated with reduced peripheral wave reflection in Indigenous Australians irrespective of diabetes status. *Journal of Hypertension* 2005;**23**:1403-7.

163. O'Rourke, MF, Karamanoglu, M, Lee, L, Cutfield, G, Avolio, A, and Gallagher, DE. Derivation of central aortic from radial artery systolic pressure using a nonocclusive cuff to attenuate wave reflection in the hand. Aust NZ J Med 23, 84. 1993. 1993.

164.Yasmin,.Brown MJ. Similarities and differences between augmentation index and pulse wave velocity in the assessment of arterial stiffness. *QJM* 1999;**92**:595-600.

165. Kelly RP, Millasseau SC, Ritter JM, Chowienczyk PJ. Vasoactive Drugs Influence Aortic Augmentation Index Independently of Pulse-Wave Velocity in Healthy Men. *Hypertension* 2001;**37**:1429-33.

166. Lemogoum D, Flores G, Van den Abeele W, Ciarka A, Leeman M, Degaute JP *et al*. Validity of pulse pressure and augmentation index as surrogate measures of arterial stiffness during beta-adrenergic stimulation. [Article]. *Journal of Hypertension* 2004;**22**:511-7.

167. Chen CH, Ting CT, Lin SJ, Hsu TL, Yin FCP, Siu CO *et al*. Different Effects of Fosinopril and Atenolol on Wave Reflections in Hypertensive Patients. *Hypertension* 1995;**25**:1034-41.

168. Ting CT, Chen CH, Chang MS, Yin FCP. Short- and Long-term Effects of Antihypertensive Drugs on Arterial Reflections, Compliance, and Impedance. *Hypertension* 1995;**26**:524-30.

169. Laurent S, Tropeano AI, Boutouyrie P. Pulse pressure reduction and cardiovascular protection. *J Hypertens Suppl* 2006;**24**:S13-S18.

170. Cockcroft JR, Wilkinson IB, Evans M, McEwan P, Peters JR, Davies S *et al*. Pulse Pressure Predicts Cardiovascular Risk in Patients With Type 2 Diabetes Mellitus. *Am J Hypertens* 2005;**18**:1463-7.

171. Franklin SS, Khan SA, Wong ND, Larson MG, Levy D. Is Pulse Pressure Useful in Predicting Risk for Coronary Heart Disease? : The Framingham Heart Study. *Circulation* 1999;**100**:354-60.

172. Millar JA, Lever AF, Burke V. Pulse pressure as a risk factor for cardiovascular events in the MRC Mild Hypertension Trial. *Journal of Hypertension* 1999;**17**:1065-72.

173. Boutouyrie P, Bussy C, Lacolley P, Girerd X, Laloux B, Laurent S. Association Between Local Pulse Pressure, Mean Blood Pressure, and Large-Artery Remodeling. *Circulation* 1999;**100**:1387-93.

174. Waddell TK, Dart AM, Medley TL, Cameron JD, Kingwell BA. Carotid Pressure Is a Better Predictor of Coronary Artery Disease Severity Than Brachial Pressure. *Hypertension* 2001;**38**:927-31.

175. Safar ME, Blacher J, Pannier B, Guerin AP, Marchais SJ, Guyonvarc'h PM *et al*. Central Pulse Pressure and Mortality in End-Stage Renal Disease. *Hypertension* 2002;**39**:735-8.

176. London G, Guerin A, Pannier B, Marchais S, Benetos A, Safar M. Increased systolic pressure in chronic uremia. Role of arterial wave reflections. *Hypertension* 1992;**20**:10-9.

177. Guzik TJ, Mussa S, Gastaldi D, Sadowski J, Ratnatunga C, Pillai R *et al*. Mechanisms of Increased Vascular Superoxide Production in Human Diabetes Mellitus: Role of NAD(P)H Oxidase and Endothelial Nitric Oxide Synthase. *Circulation* 2002;**105**:1656-62.

178. Hamilton CA, Berg G, McIntyre M, Mcphaden AR, Reid JL, and Dominiczak AF. Effects of nitric oxide and superoxide on relaxation in human artery and vein. Atherosclerosis (1997) 133(1), 77-86.

179. Al Benna S, Hamilton CA, McClure JD, Rogers PN, Berg GA, Ford I *et al*. Low-Density Lipoprotein Cholesterol Determines Oxidative Stress and Endothelial Dysfunction in Saphenous Veins From Patients With Coronary Artery Disease. *Arterioscler Thromb Vasc Biol* 2006;**26**:218-23.

180.Hamilton CA, Brosnan MJ, Al Benna S, Berg G, Dominiczak AF. NAD(P)H Oxidase Inhibition Improves Endothelial Function in Rat and Human Blood Vessels. *Hypertension* 2002;**40**:755-62.

181. Antoniades C, Shirodaria C, Warrick N, Cai S, de Bono J, Lee J *et al*. 5-Methyltetrahydrofolate Rapidly Improves Endothelial Function and Decreases Superoxide Production in Human Vessels: Effects on Vascular Tetrahydrobiopterin Availability and Endothelial Nitric Oxide Synthase Coupling. *Circulation* 2006;**114**:1193-201.

182. Ludmer PL, Selwyn AP, Shook TL, Wayne RR, Mudge GH, Alexander RW *et al*. Paradoxical Vasoconstriction Induced by Acetylcholine in Atherosclerotic Coronary Arteries. *New England Journal of Medicine* 1986;**315**:1046-51.

183. Haynes WG, Strachan FE, Webb DJ. Endothelin ETA and ETB Receptors Cause Vasoconstriction of Human Resistance and Capacitance Vessels In Vivo. *Circulation* 1995;**92**:357-63.

184. Joannides R, Haefeli WE, Linder L, Richard V, Bakkali EH, Thuillez C *et al*. Nitric Oxide Is Responsible for Flow-Dependent Dilatation of Human Peripheral Conduit Arteries In Vivo. *Circulation* 1995;**91**:1314-9.

185. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA *et al*. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: A report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol* 2002;**39**:257-65.

186. Rambaran C, Jiang B, Ritter JM, Shah A, Kalra L, Chowienczyk PJ. Assessment of endothelial function: comparison of the pulse wave response to beta 2-adrenoceptor stimulation with flow mediated dilatation. *Br.J Clin Pharmacol.* 2008;**65**:238-43.

187. Fichtlscherer S, Breuer S, Zeiher AM. Prognostic Value of Systemic Endothelial Dysfunction in Patients With Acute Coronary Syndromes: Further Evidence for the Existence of the "Vulnerable" Patient. *Circulation* 2004;**110**:1926-32.

188. Suwaidi JA, Hamasaki S, Higano ST, Nishimura RA, Holmes DR, Jr., Lerman A. Long-Term Follow-Up of Patients With Mild Coronary Artery Disease and Endothelial Dysfunction. *Circulation* 2000;**101**:948-54.

189. Halcox JPJ, Schenke WH, Zalos G, Mincemoyer R, Prasad A, Waclawiw MA *et al*. Prognostic Value of Coronary Vascular Endothelial Dysfunction. *Circulation* 2002;**106**:653-8.

190. Hasdai D, Gibbons RJ, Holmes DR, Jr., Higano ST, Lerman A. Coronary Endothelial Dysfunction in Humans Is Associated With Myocardial Perfusion Defects. *Circulation* 1997;**96**:3390-5.

191. Zeiher AM, Krause T, Schachinger V, Minners J, Moser E. Impaired Endothelium-Dependent Vasodilation of Coronary Resistance Vessels Is Associated With Exercise-Induced Myocardial Ischemia. *Circulation* 1995;**91**:2345-52.

192. Pieper GM. Review of Alterations in Endothelial Nitric Oxide Production in Diabetes : Protective Role of Arginine on Endothelial Dysfunction. *Hypertension* 1998;**31**:1047-60.

193. Watts GF, O'Brien SF, Silvester W, Millar JA. Impaired endothelium-dependent and independent dilatation of forearm resistance arteries in men with diet-treated non-insulin-dependent diabetes: role of dyslipidaemia. *Clin Sci.(Lond)* 1996;**91**:567-73.

194. McVeigh GE, Brennan GM, Johnston GD, McDermott BJ, McGrath LT, Henry WR *et al*. Impaired endothelium-dependent and independent vasodilation in patients with type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 1992;**35**:771-6.

195. Hogikyan RV, Galecki AT, Pitt B, Halter JB, Greene DA, Supiano MA. Specific Impairment of Endothelium-Dependent Vasodilation in Subjects with Type 2 Diabetes Independent of Obesity. *J Clin Endocrinol Metab* 1998;**83**:1946-52.

196. Anastasiou E, Lekakis JP, Alevizaki M, Papamichael CM, Megas J, Souvatzoglou A *et al*. Impaired endothelium-dependent vasodilatation in women with previous gestational diabetes. *Diabetes Care* 1998;**21**:2111-5.

197. Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G, Baron AD. Obesity/insulin resistance is associated with endothelial dysfunction. Implications for the syndrome of insulin resistance. *J Clin Invest* 1996;**97**:2601-10.

198. Ferri C, Desideri G, Baldoncini R, Bellini C, De Angelis C, Mazzocchi C *et al*. Early activation of vascular endothelium in nonobese, nondiabetic essential hypertensive patients with multiple metabolic abnormalities. *Diabetes* 1998;**47**:660-7.

199. Kim JA, Montagnani M, Koh KK, Quon MJ. Reciprocal relationships between insulin resistance and endothelial dysfunction: molecular and pathophysiological mechanisms. *Circulation* 2006;**113**:1888-904.

200. White J, Guerin T, Swanson H, Post S, Zhu H, Gong M *et al*. Diabetic HDL-associated myristic acid inhibits acetylcholine-induced nitric oxide generation by preventing the association of endothelial nitric oxide synthase with calmodulin. *Am J Physiol Cell Physiol* 2008;**294**:C295-C305.

201. Sjoholm A,.Nystrom T. Endothelial inflammation in insulin resistance. *The Lancet* 2005;**365**:610-2.

202. Romero MJ, Platt DH, Tawfik HE, Labazi M, El Remessy AB, Bartoli M *et al*. Diabetes-induced Coronary Vascular Dysfunction Involves Increased Arginase Activity. *Circ Res* 2008;**102**:95-102.

203. Nitenberg A, Pham I, Antony I, Valensi P, Attali JR, Chemla D. Cardiovascular outcome of patients with abnormal coronary vasomotion and normal coronary arteriography is worse in type 2 diabetes mellitus than in arterial hypertension: A 10 year follow-up study. *Atherosclerosis* 2005;**183**:113-20.

204. Creager MA, Cooke JP, Mendelsohn ME, Gallagher SJ, Coleman SM, Loscalzo J *et al*. Impaired vasodilation of forearm resistance vessels in hypercholesterolemic humans. *J Clin Invest* 1990;**86**:228-34.

205. Chowienczyk PJ, Watts GF, Cockcroft JR, Ritter JM. Impaired endothelium-dependent vasodilation of forearm resistance vessels in hypercholesterolaemia. *The Lancet* 1992;**340**:1430-2.

206. John S, Delles C, Jacobi J, Schlaich MP, Schneider M, Schmitz G *et al*. Rapid improvement of nitric oxide bioavailability after lipid-lowering therapy with cerivastatin within two weeks. *J Am Coll Cardiol* 2001;**37**:1351-8.

207. John S, Schlaich M, Langenfeld M, Weihprecht H, Schmitz G, Weidinger G *et al*. Increased Bioavailability of Nitric Oxide After Lipid-Lowering Therapy in Hypercholesterolemic Patients : A Randomized, Placebo-Controlled, Double-blind Study. *Circulation* 1998;**98**:211-6.

208. John S, Schneider MP, Delles C, Jacobi J, and Schmieder RE. Lipid-independent effects of statins on endothelial function and bioavailability of nitric oxide in hypercholesterolemic patients. American heart journal 2005. 149(3), 473.

209. Lupattelli G, Marchesi S, Roscini AR, Siepi D, Gemelli F, Pirro M *et al*. Direct association between high-density lipoprotein cholesterol and endothelial function in hyperlipemia. *The American Journal of Cardiology* 2002;**90**:648-50.

210. Spieker LE, Sudano I, Hurlimann D, Lerch PG, Lang MG, Binggeli C *et al*. High-Density Lipoprotein Restores Endothelial Function in Hypercholesterolemic Men. *Circulation* 2002;**105**:1399-402.

211. Kuvin JT, R+ñmet ME, Patel AR et al. A novel mechanism for the beneficial vascular effects of high-density lipoprotein cholesterol: Enhanced vasorelaxation and increased endothelial nitric oxide synthase expression. American heart journal ,2002 144(1), 165-172. 1-7-

212. Schneider MP, Delles C, Fleischmann E, Schmidt BMW, John S, Schmieder RE. Effect of elevated triglyceride levels on endothelium-dependent vasodilation in patients with hypercholesterolemia. *The American Journal of Cardiology* 2003;**91**:482-4.

213. Lundman P, Eriksson MJ, Stnhlinger M, Cooke JP, Hamsten A, Tornvall P. Mild-to-moderate hypertriglyceridemia in young men is associated with endothelial dysfunction and increased plasma concentrations of asymmetric dimethylarginine. *J Am Coll Cardiol* 2001;**38**:111-6.

214. Gudmundsson GS, Sinkey CA, Chenard CA, Stumbo PJ, Haynes WG. Resistance vessel endothelial function in healthy humans during transient postprandial hypertriglyceridemia. *The American Journal of Cardiology* 2000;**85**:381-5.

215.Taddei S, Virdis A, Mattei P, Salvetti A. Vasodilation to acetylcholine in primary and secondary forms of human hypertension. *Hypertension* 1993;**21**:929-33.

216. Linder L, Kiowski W, Buhler FR, Luscher TF. Indirect evidence for release of endothelium-derived relaxing factor in human forearm circulation in vivo. Blunted response in essential hypertension. *Circulation* 1990;**81**:1762-7.

217. Cockcroft JR, Chowienczyk PJ, Benjamin N, Ritter JM. Preserved Endothelium-Dependent Vasodilatation in Patients with Essential Hypertension. *N Engl J Med* 1994;**330**:1036-40.

218. Waring WS, Sinclair HM, Webb DJ. Effects of salbutamol and glyceryl trinitrate on large arterial stiffness are similar between patients with hypertension and adults with normal blood pressure. *!Lost Data* 2006;**62**:621-6.

219. Chan NN, Colhoun HM, Vallance P. Cardiovascular risk factors as determinants of endothelium-dependent and endothelium-independent vascular reactivity in the general population. *J Am Coll Cardiol* 2001;**38**:1814-20.

220. Preik M, Kelm M, R+¦sen P, Tsch+¦pe D, Strauer BE. Additive Effect of Coexistent Type 2 Diabetes and Arterial Hypertension on Endothelial Dysfunction in Resistance Arteries of Human Forearm Vasculature. *Angiology* 2000;**51**:545-54.

221. Nitenberg A, Chemla D, Antony I. Epicardial coronary artery constriction to cold pressor test is predictive of cardiovascular events in hypertensive patients with angiographically normal coronary arteries and without other major coronary risk factor. *Atherosclerosis* 2004;**173**:115-23.

222. Ghiadoni L, Magagna A, Versari D, Kardasz I, Huang Y, Taddei S *et al*. Different Effect of Antihypertensive Drugs on Conduit Artery Endothelial Function. *Hypertension* 2003;**41**:1281-6.

223. Heitzer T, Herttuala SY, Wild E, Luoma J, Drexler H. Effect of vitamin E on endothelial vasodilator function in patients with hypercholesterolemia, chronic smoking or both. *J Am Coll Cardiol* 1999;**33**:499-505.

224. Taddei S, Virdis A, Ghiadoni L, Magagna A, Salvetti A. Vitamin C Improves Endothelium-Dependent Vasodilation by Restoring Nitric Oxide Activity in Essential Hypertension. *Circulation* 1998;**97**:2222-9.

225. Ceriello A,.Motz E. Is Oxidative Stress the Pathogenic Mechanism Underlying Insulin Resistance, Diabetes, and Cardiovascular Disease? The Common Soil Hypothesis Revisited. *Arterioscler Thromb Vasc Biol* 2004;**24**:816-23.

226. Goldin A, Beckman JA, Schmidt AM, Creager MA. Advanced Glycation End Products: Sparking the Development of Diabetic Vascular Injury. *Circulation* 2006;**114**:597-605.

227. Solymoss BC, Bourassa MG, Lesperance J, Levesque S, Marcil M, Varga S *et al*. Incidence and clinical characteristics of the metabolic syndrome in patients with coronary artery disease. *Coron.Artery Dis.* 2003;**14**:207-12.

228. Hamburg NM, Larson MG Vita JA,Vasan RS et al.. Metabolic Syndrome, Insulin Resistance, and Brachial Artery Vasodilator Function in Framingham Offspring Participants Without Clinical Evidence of Cardiovascular Disease. The American Journal of Cardiology 101(1), 82-88. 1-1-2008.

229. Ardigo D, Franzini L, Valtuena S, Lucilla, Monti D, Reaven GM, and Zavaroni I. Relation of Plasma Insulin Levels to Forearm Flow-Mediated Dilatation in Healthy Volunteers. The American Journal of Cardiology 97(8), 1250-1254. 15-4-2006.

230. Pistrosch F, Passauer J, Fischer S, Fuecker K, Hanefeld M, Gross P. In Type 2 Diabetes, Rosiglitazone Therapy for Insulin Resistance Ameliorates Endothelial Dysfunction Independent of Glucose Control. *Diabetes Care* 2004;**27**:484-90.

231. Mather KJ, Verma S, Anderson TJ. Improved endothelial function with metformin in type 2 diabetes mellitus. *J Am Coll Cardiol* 2001;**37**:1344-50.

232. de Aguiar LGK, Bahia LR, Villela N, Laflor C, Sicuro F, Wiernsperger N *et al*. Metformin Improves Endothelial Vascular Reactivity in First-Degree Relatives of Type 2 Diabetic Patients With Metabolic Syndrome and Normal Glucose Tolerance. *Diabetes Care* 2006;**29**:1083-9.

233. Edwards DG, Schofield RS, Lennon SL, Pierce GL, Nichols WW, Braith RW. Effect of exercise training on endothelial function in men with coronary artery disease. *The American Journal of Cardiology* 2004;**93**:617-20.

234. Noyan, Gokce, Joseph, A., V, Marie, McDonnell, Armour, R. Forse, Nawfal, Istfan, Maria, Stoeckl, Izabella, Lipinska, John, F. Keaney, and Caroline, M. Apovian. Effect of medical and surgical weight loss on endothelial vasomotor function in obese patients. The American Journal of Cardiology 95(2), 266-268. 15-1-2005.

235. Hambrecht R, Wolf A, Gielen S, Linke A, Hofer J, Erbs S *et al*. Effect of exercise on coronary endothelial function in patients with coronary artery disease. *N Engl J Med* 2000;**342**:454-60.

236. Packard, Chris, Caslake, Muriel, and Shepherd, James. The role of small, dense low density lipoprotein (LDL): a new look. International journal of cardiology 74, S17-S22. 30-6-2000.

237. Lupattelli G, Lombardini R, Schillaci G et al. Flow-mediated vasoactivity and circulating adhesion molecules in hypertriglyceridemia: Association with small, dense LDL cholesterol particles. American heart journal 200 140(3), 521-526..

238. Timimi FK, Ting HH, Haley EA, Roddy MA, Ganz P, Creager MA. Vitamin C Improves Endothelium-Dependent Vasodilation in Patients With Insulin-Dependent Diabetes Mellitus. *J Am Coll Cardiol* 1998;**31**:552-7.

239. Lonn E, Yusuf S, Hoogwerf B, Pogue J, Yi Q, Zinman B *et al*. Effects of Vitamin E on Cardiovascular and Microvascular Outcomes in High-Risk Patients With Diabetes: Results of the HOPE Study and MICRO-HOPE Substudy. *Diabetes Care* 2002;**25**:1919-27.

240. MRC/BHF Heart Protection Study of antioxidant vitamin supplementation in 20 536 high-risk individuals: a randomised placebo-controlled trial. *The Lancet* 2002;**360**:23-33.

241. Wilkinson IB, Fuchs SA, Jansen IM, Spratt JC, Murray GD, Cockcroft JR *et al*. Reproducibility of pulse wave velocity and augmentation index measured by pulse wave analysis. *J Hypertens* 1998;**16**:2079-84.

242. Lassegue B,.Clempus RE. Vascular NAD(P)H oxidases: specific features, expression, and regulation. *Am J Physiol Regul.Integr.Comp Physiol* 2003;**285**:R277-R297.

243.Heymes C, Bendall JK, Ratajczak P, Cave AC, Samuel JL, Hasenfuss G *et al*. Increased myocardial NADPH oxidase activity in human heart failure. *J Am Coll Cardiol* 2003;**41**:2164-71.

244. Bao XM, Wu CF, Lu GP. Atorvastatin inhibits homocysteine-induced oxidative stress and apoptosis in endothelial progenitor cells involving Nox4 and p38MAPK. *Atherosclerosis* 2010;**210**:114-21.

245. Griendling KK, Minieri CA, Ollerenshaw JD, Alexander RW. Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. *Circ Res* 1994;**74**:1141-8.

246. Griendling KK,.FitzGerald GA. Oxidative Stress and Cardiovascular Injury: Part I: Basic Mechanisms and In Vivo Monitoring of ROS. *Circulation* 2003;**108**:1912-6.

247. Tsimikas S. In Vivo Markers of Oxidative Stress and Therapeutic Interventions. *The American Journal of Cardiology* 2008;**101**:S34-S42.

248. Tsimikas S, Brilakis ES, Miller ER, McConnell JP, Lennon RJ, Kornman KS *et al*. Oxidized Phospholipids, Lp(a) Lipoprotein, and Coronary Artery Disease. *New England Journal of Medicine* 2009;**353**:46-57.

249. Ehara S, Ueda M, Naruko T, Haze K, Itoh A, Otsuka M *et al*. Elevated Levels of Oxidized Low Density Lipoprotein Show a Positive Relationship With the Severity of Acute Coronary Syndromes. *Circulation* 2001;**103**:1955-60.

250. Morrow JD. Quantification of Isoprostanes as Indices of Oxidant Stress and the Risk of Atherosclerosis in Humans. *Arterioscler Thromb Vasc Biol* 2005;**25**:279-86.

251. Schwedhelm E, Bartling A, Lenzen H, Tsikas D, Maas R, Brummer J *et al*. Urinary 8-iso-Prostaglandin F2{alpha} as a Risk Marker in Patients With Coronary Heart Disease: A Matched Case-Control Study. *Circulation* 2004;**109**:843-8.

252. Fortuno A, San Jose G, Moreno MU, Beloqui O, Diez J, Zalba G. Phagocytic NADPH Oxidase Overactivity Underlies Oxidative Stress in Metabolic Syndrome. *Diabetes* 2006;**55**:209-15.

253.Zalba G, Beloqui O, Jose GS, Moreno MU, Fortuno A, Diez J. NADPH Oxidase-Dependent Superoxide Production Is Associated With Carotid Intima-Media Thickness in Subjects Free of Clinical Atherosclerotic Disease. *Arterioscler Thromb Vasc Biol* 2005;**25**:1452-7.

254. Chen JW, Chen YH, Lin SJ. Long-term exposure to oxidized low-density lipoprotein enhances tumor necrosis factor-[alpha]-stimulated endothelial adhesiveness of monocytes by activating superoxide generation and redox-sensitive pathways. *Free Radical Biology and Medicine* 2006;**40**:817-26.

255.Son SM. Role of vascular reactive oxygen species in development of vascular abnormalities in diabetes. *Diabetes Research and Clinical Practice* 2007;**77**:S65-S70.

256. Devaraj S,.Jialal I. Low-Density Lipoprotein Postsecretory Modification, Monocyte Function, and Circulating Adhesion Molecules in Type 2 Diabetic Patients With and Without

Macrovascular Complications : The Effect of {alpha}-Tocopherol Supplementation. *Circulation* 2000;**102**:191-6.

257. Venugopal SK, Devaraj S, Yang T, Jialal I. +¦-Tocopherol Decreases Superoxide Anion Release in Human Monocytes Under Hyperglycemic Conditions Via Inhibition of Protein Kinase C. *Diabetes* 2002;**51**:3049-54.

258. Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y *et al*. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature* 2000;**404**:787-90.

259. Law MR,.Morris JK. By how much does fruit and vegetable consumption reduce the risk of ischaemic heart disease? *Eur J Clin Nutr*. 1998;**52**:549-56.

260. The Heart Outcomes Prevention Evaluation Study Investigators. Vitamin E Supplementation and Cardiovascular Events in High-Risk Patients. *N Engl J Med* 2000;**342**:154-60.

261. Touyz RM. Reactive Oxygen Species, Vascular Oxidative Stress, and Redox Signaling in Hypertension: What Is the Clinical Significance? *Hypertension* 2004;**44**:248-52.

262. Berry C, Anderson N, Kirk AJB, Dominiczak AF, McMurray JJV. Renin angiotensin system inhibition is associated with reduced free radical concentrations in arteries of patients with coronary heart disease. *Heart* 2001;**86:**217-20.

263. Wagner AH, Kohler T, Ruckschloss U, Just I, Hecker M. Improvement of Nitric Oxide-Dependent Vasodilatation by HMG-CoA Reductase Inhibitors Through Attenuation of Endothelial Superoxide Anion Formation. *Arterioscler Thromb Vasc Biol* 2000;**20**:61-9.

264. Adams V, Linke A, Krankel N, Erbs S, Gielen S, Mobius-Winkler S *et al*. Impact of Regular Physical Activity on the NAD(P)H Oxidase and Angiotensin Receptor System in Patients With Coronary Artery Disease. *Circulation* 2005;**111**:555-62.

265. Kukidome D, Nishikawa T, Sonoda K, Imoto K, Fujisawa K, Yano M *et al*. Activation of AMP-Activated Protein Kinase Reduces Hyperglycemia-Induced Mitochondrial Reactive Oxygen Species Production and Promotes Mitochondrial Biogenesis in Human Umbilical Vein Endothelial Cells. *Diabetes* 2006;**55**:120-7.

266. Donato AJ, Eskurza I, Silver AE, Levy AS, Pierce GL, Gates PE *et al*. Direct Evidence of Endothelial Oxidative Stress With Aging in Humans: Relation to Impaired Endothelium-Dependent Dilation and Upregulation of Nuclear Factor-{kappa}B. *Circ Res* 2007;**100**:1659-66.

267. Hamilton CA, Brosnan MJ, McIntyre M, Graham D, Dominiczak AF. Superoxide Excess in Hypertension and Aging : A Common Cause of Endothelial Dysfunction. *Hypertension* 2001;**37**:529-34.

268. Krauss RM. Lipids and Lipoproteins in Patients With Type 2 Diabetes. *Diabetes Care* 2004;**27**:1496-504.

269. Austin MA, King MC, Vranizan KM, Krauss RM. Atherogenic lipoprotein phenotype. A proposed genetic marker for coronary heart disease risk. *Circulation* 1990;**82**:495-506.

270. Austin MA, Hokanson JE, Edwards KL. Hypertriglyceridemia as a cardiovascular risk factor. *Am J Cardiol* 1998;**81**:7B-12B.

271. Ginsberg H, Zhang YL, and Antonio H. Regulation of Plasma Triglycerides in Insulin Resistance and Diabetes. Archives of medical research 2005 36(3), 232-240.

272. Krauss RM and Blanche PJ. Detection and quntification of LDL subfractions. Curr.Opin.Lipidol 3, 377-383. 1992.

273. Reaven GM, Chen YD, Jeppesen J, Maheux P, Krauss RM. Insulin resistance and hyperinsulinemia in individuals with small, dense low density lipoprotein particles. *J Clin Invest* 1993;**92**:141-6.

274. Packard CJ,.Shepherd J. Lipoprotein Heterogeneity and Apolipoprotein B Metabolism. *Arterioscler Thromb Vasc Biol* 1997;**17**:3542-56.

275. Tan CE, Forster L, Caslake MJ, Bedford D, Watson TDG, McConnell M *et al*. Relations Between Plasma Lipids and Postheparin Plasma Lipases and VLDL and LDL Subfraction Patterns in Normolipemic Men and Women. *Arterioscler Thromb Vasc Biol* 1995;**15**:1839-48.

276. Austin MA, Breslow JL, Hennekens CH, Buring JE, Willett WC, Krauss RM. Low-density lipoprotein subclass patterns and risk of myocardial infarction. *JAMA* 1988;**260**:1917-21.

277. Rizzo M,.Berneis K. Low-density lipoprotein size and cardiovascular risk assessment. *QJM* 2006;**99**:1-14.

278. Tribble DL, Rizzon M, Chait A, Lewis DM, Blanche PJ, and. Krauss RM. Enhanced oxidative susceptibility and reduced antioxidant content of metabolic precursors of small, dense low-density lipoproteins. The American journal of medicine 2001 110(2), 103-110.

279. Anber, V., Griffin, B. A., McConnell, M., Packard, C. J., and Shepherd, J. Influence of plasma lipid and LDL-subfraction profile on the interaction between low density lipoprotein with human arterial wall proteoglycans. Atherosclerosis.1996. 124(2), 261-271

280. Blanche PJ, Gong EL, Forte TM, Nichols AV. Characterization of human high-density lipoproteins by gradient gel electrophoresis. *Biochim.Biophys.Acta* 1981;**665**:408-19.

281. Li XA, Titlow WB, Jackson BA, Giltiay N, Nikolova-Karakashian M, Uittenbogaard A *et al*. High Density Lipoprotein Binding to Scavenger Receptor, Class B, Type I Activates Endothelial Nitric-oxide Synthase in a Ceramide-dependent Manner. *Journal of Biological Chemistry* 2002;**277**:11058-63.

282. Stone NJ. Nonpharmacologic Management of Mixed Dyslipidemia Associated with Diabetes Mellitus and the Metabolic Syndrome: A Review of the Evidence. *The American Journal of Cardiology* 2008;**102**:14L-8L.

283. Foster GD, Wyatt HR, Hill JO, McGuckin BG, Brill C, Mohammed BS *et al*. A randomized trial of a low-carbohydrate diet for obesity. *N Engl J Med* 2003;**348**:2082-90.

284. Kraus WE, Houmard JA, Duscha BD, Knetzger KJ, Wharton MB, McCartney JS *et al*. Effects of the amount and intensity of exercise on plasma lipoproteins. *N Engl J Med* 2002;**347**:1483-92.

285. Mooradian AD. Dyslipidemia in type 2 diabetes mellitus. *Nat Clin Pract End Met* 2009;**5**:150-9.

286. Effect of fenofibrate on progression of coronary-artery disease in type 2 diabetes: the Diabetes Atherosclerosis Intervention Study, a randomised study. *The Lancet* 2001;**357**:905-10.

287. Vakkilainen J, Steiner G, Ansquer JC, Aubin F, Rattier S, Foucher C *et al*. Relationships Between Low-Density Lipoprotein Particle Size, Plasma Lipoproteins, and Progression of Coronary Artery Disease: The Diabetes Atherosclerosis Intervention Study (DAIS). *Circulation* 2003;**107**:1733-7.

288. Rubins HB, Robins SJ, Collins D, Fye CL, Anderson JW, Elam MB *et al*. Gemfibrozil for the Secondary Prevention of Coronary Heart Disease in Men with Low Levels of High-Density Lipoprotein Cholesterol. *N Engl J Med* 1999.

289. Effects of long-term fenofibrate therapy on cardiovascular events in 9795 people with type 2 diabetes mellitus (the FIELD study): randomised controlled trial. *The Lancet* 2005;**366**:1849-61.

290. Ginsberg HN, Elam MB, Lovato LC, Crouse JR, III, Leiter LA, Linz P *et al*. Effects of combination lipid therapy in type 2 diabetes mellitus. *N Engl J Med* 2010;**362**:1563-74.

291. Taylor AJ, Villines TC, Stanek EJ, Devine PJ, Griffen L, Miller M *et al*. Extended-release niacin or ezetimibe and carotid intima-media thickness. *N Engl J Med* 2009;**361**:2113-22.

292. Brown BG, Zhao XQ, Chait A, Fisher LD, Cheung MC, Morse JS *et al*. Simvastatin and niacin, antioxidant vitamins, or the combination for the prevention of coronary disease. *N Engl J Med* 2001;**345**:1583-92.

293. Canner PL, Berge KG, Wenger Nk, Stamler J, Friedman L, Prineas RJ *et al*. Fifteen year mortality in Coronary Drug Project patients:long-term benefit with niacin. *J Am Coll Cardiol* 1986;**8**:1245-55.

294. Anderson TJ, Meredith IT, Charbonneau F, Yeung AC, Frei B, Selwyn AP *et al*. Endothelium-Dependent Coronary Vasomotion Relates to the Susceptibility of LDL to Oxidation in Humans. *Circulation* 1996;**93**:1647-50.

295. Tamai O, Matsuoka H, Itabe H, Wada Y, Kohno K, Imaizumi T. Single LDL Apheresis Improves Endothelium-Dependent Vasodilatation in Hypercholesterolemic Humans. *Circulation* 1997;**95**:76-82.

296. Treasure CB, Klein JL, Weintraub WS, Talley JD, Stillabower ME, Kosinski AS *et al*. Beneficial Effects of Cholesterol-Lowering Therapy on the Coronary Endothelium in Patients with Coronary Artery Disease. *New England Journal of Medicine* 2009;**332**:481-7.

297. Mineo C,.Shaul PW. Circulating cardiovascular disease risk factors and signaling in endothelial cell caveolae. *Cardiovascular Research* 2006;**70**:31-41.

298. Shaul PW. Regulation of Endothelial nitric oxide synthase: Location, Location, Location. *Annual Review of Physiology* 2003;**64**:749-74.

299. Rikitake Y,.Liao JK. Rho GTPases, Statins, and Nitric Oxide. *Circ Res* 2005;**97**:1232-5.

300. Wenzel P, Daiber A, Matthias, Oelze M, et al. Mechanisms underlying recoupling of eNOS by HMG-CoA reductase inhibition in a rat model of streptozotocin-induced diabetes mellitus. Atherosclerosis 198(1), 65-76. 1-5-2008.

301. Robinson JG, Smith B, Maheshwari N, Schrott H. Pleiotropic Effects of Statins: Benefit Beyond Cholesterol Reduction?: A Meta-Regression Analysis. *J Am Coll Cardiol* 2005;**46**:1855-62.

302. Sattar N, Preiss D, Murray HM, Welsh P, Buckley BM, de Craen AJ *et al*. Statins and risk of incident diabetes: a collaborative meta-analysis of randomised statin trials. *The Lancet* 2010;**375**:735-42.

303. Kastelein JJ, Akdim F, Stroes ES, Zwinderman AH, Bots ML, Stalenhoef AF *et al*. Simvastatin with or without ezetimibe in familial hypercholesterolemia. *N Engl J Med* 2008;**358**:1431-43.

304. Stepp DW, Ou J, Ackerman AW, Welak S, Klick D, Pritchard KA, Jr. Native LDL and minimally oxidized LDL differentially regulate superoxide anion in vascular endothelium in situ. *Am J Physiol Heart Circ Physiol* 2002;**283**:H750-H759.

305. Pritchard KA, Jr., Groszek L, Smalley DM, Sessa WC, Wu M, Villalon P *et al*. Native Low-Density Lipoprotein Increases Endothelial Cell Nitric Oxide Synthase Generation of Superoxide Anion. *Circ Res* 1995;**77**:510-8.

306. Cosentino F, Hurlimann D, Delli Gatti C et al. Chronic treatment with tetrahydrobiopterin reverses endothelial dysfunction and oxidative stress in hypercholesterolaemia. *Heart* 2008;**94**:487-92.

307. Gaw A, Packard CJ, Murray EF, Lindsay GM, Griffin BA, Caslake MJ *et al*. Effects of simvastatin on apoB metabolism and LDL subfraction distribution. *Arterioscler Thromb* 1993;**13**:170-89.

308. Leon AS, Sanchez OA. Response of blood lipids to exercise training alone or combined with dietary intervention. *Medicine & Science in Sports & Exercise* 2001;**33**:S502-S515.

309. Fagard RH. Exercise characteristics and the blood pressure response to dynamic physical training. *Medicine & Science in Sports & Exercise* 2001;**33**:S484-S492.

310. Gill JMR,.Malkova D. Physical activity, fitness and cardiovascular disease risk in adults: interactions with insulin resistance and obesity. *Clin.Sci.* 2006;**110**:409-25.

311. Tuomilehto J, Lindstrom J, Eriksson JG, Valle TT, Hamalainen H, Ilanne-Parikka P *et al*. Prevention of Type 2 Diabetes Mellitus by Changes in Lifestyle among Subjects with Impaired Glucose Tolerance. *New England Journal of Medicine* 2001;**344**:1343-50.

312. Reduction in the Incidence of Type 2 Diabetes with Lifestyle Intervention or Metformin. *New England Journal of Medicine* 2009;**346**:393-403.

313. Pan XR, Li GW, Hu YH, Wang JX, Yang WY, An ZX *et al*. Effects of diet and exercise in preventing NIDDM in people with impaired glucose tolerance. The Da Qing IGT and Diabetes Study. *Diabetes Care* 1997;**20**:537-44.

314. Boule NG, Haddad E, Kenny GP, Wells GA, Sigal RJ. Effects of Exercise on Glycemic Control and Body Mass in Type 2 Diabetes Mellitus: A Meta-analysis of Controlled Clinical Trials. *JAMA: The Journal of the American Medical Association* 2001;**286**:1218-27.

315. Batty GD, Shipley MJ, Marmot M, Davey Smith G. Physical activity and cause-specific mortality in men with Type 2 diabetes/impaired glucose tolerance: evidence from the Whitehall study. *Diabetic Medicine* 2002;**19**:580-8.

316. Katzmarzyk PT, Church TS, Blair SN. Cardiorespiratory Fitness Attenuates the Effects of the Metabolic Syndrome on All-Cause and Cardiovascular Disease Mortality in Men. *Arch Intern Med* 2004;**164**:1092-7.

317. Morrato EH, Hill JO, Wyatt HR, Ghushchyan V, Sullivan PW. Physical Activity in U.S. Adults With Diabetes and At Risk for Developing Diabetes, 2003. *Diabetes Care* 2007;**30**:203-9.

318. Scottish Intercollegiate Guidelines Network. Cardiac Rehabilitation. www.sign.ac.uk . 2007. Accessed 2010.

319. Jolliffe J, Rees K, Taylor R, Thompson DR, Oldridge N, and Ebrahim S. Exercise-based rehabilitation for coronary heart disease. Cochrane database of systematic Reviews (1). 2001.

320. Arak-Lukmann A, Zilmer M, Maaroos J et al. Oxidative stress before and after exercise conditioning in patients following surgical revascularization of the myocardium. *International Journal of Rehabilitation Research* 2002;**25**:305-12.

321. Byberg L, Zethelius B, McKeigue PM, Lithell HO. Changes in physical activity are associated with changes in metabolic cardiovascular risk factors. *Diabetologia* 2001;**44**:2134-9.

322. Kim J, Wei Y, Sowers JR. Role of Mitochondrial Dysfunction in Insulin Resistance. *Circ Res* 2008;**102**:401-14.

323. Davidson SM,.Duchen MR. Endothelial Mitochondria: Contributing to Vascular Function and Disease. *Circ Res* 2007;**100**:1128-41.

324. Madamanchi NR,.Runge MS. Mitochondrial Dysfunction in Atherosclerosis. *Circ Res* 2007;**100**:460-73.

325. Nisoli E, Clementi E, Carruba MO, Moncada S. Defective Mitochondrial Biogenesis: A Hallmark of the High Cardiovascular Risk in the Metabolic Syndrome? *Circ Res* 2007;**100**:795-806.

326. Graham D, Huynh NN, Hamilton CA, Beattie E, Smith RAJ, Cocheme HM *et al*. Mitochondria-Targeted Antioxidant MitoQ10 Improves Endothelial Function and Attenuates Cardiac Hypertrophy. *Hypertension* 2009;**54**:322-8.