University of Leicester

Multi-factorial prevention of cardiovascular disease and novel markers of risk in early glucose disorders

The Addition-Leicester study

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by

David Robert Webb
Department of Cardiovascular Sciences
University of Leicester

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Abstract

Introduction

Screening followed by intensive multi-factorial cardiovascular risk intervention may improve outcomes in type 2 diabetes. It is unknown whether this is achievable or clinically effective within ethnically diverse United Kingdom populations. By preventing deleterious sub-clinical arteriosclerosis and inflammation, earlier identification of glucose disorders may be particularly beneficial within high-risk south Asian groups.

Aims

To describe the rationale, design and results of a major multiethnic screening programme for type 2 diabetes, including a detailed characterisation of novel and traditional cardiovascular risk markers at baseline and one year after a controlled trial of multi-factorial intervention in screen detected cases.

Results

6749 individuals were screened with a glucose-tolerance test and 632 (9%) had an Arterial Stiffness (AS) assessment. 1480 (22%) were south Asian, 885 (18%) had abnormal glucose regulation and 196 (3.3%) had undiagnosed type 2 diabetes. Untreated cardiovascular disease risk and premature AS were significantly higher in all three of these groups compared with comparable white European and normoglycaemic controls. Vitamin D status was independently associated with AS in south Asians. Modelled cardiovascular outcomes and surrogate measures of inflammation (CRP) were improved by multi-factorial intervention but AS was not.

Conclusion

Screening for diabetes is feasible in United Kingdom multiethnic populations and identifies people at high risk of cardiovascular disease. The prevalence of undiagnosed glucose abnormalities remains high. AS is manifest in screen-detected diabetes and unlike modelled vascular outcomes and inflammation is not ameliorated by multi-factorial intervention at one year. Vitamin D may be an important, treatable determinant of AS in south Asians.

Acknowledgements

I dedicate this thesis to the family, friends and colleagues who have supported me over the last four years. Firstly, I am most grateful to my supervisors, Professors Melanie Davies and Kamlesh Khunti for their fantastic support and an enthusiasm for research that knows no bounds. Through their vibrant personalities they have created a truly unique and I think unrivalled clinical research environment here at Leicester. I have certainty benefited a great deal from being part of their team and thank them both for their unwavering support. I feel extremely privileged to have been part of a highly committed and successful team, many of whom continue to work tirelessly in the pursuit of improving the lives of people with diabetes.

I would like to thank everyone in the ADDITION research group, particularly the “intensive” team; Bala Srinivasan, Emma Healy, Su Sehmi, Fiona Alblett, Janette Barnett and Julia Stockman. A special thanks to Sandra Campbell for co-ordinating the intensive arm so efficiently and to all the practices, general practitioners and participants of the ADDITION-Leicester study. Without the dedication of all these people, and many more, this project would certainly not have been the success it is.

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Research Question

Does screening for abnormal glucose tolerance in a multiethnic population identify people with modifiable risk factors for cardiovascular disease, and does subsequent intervention improve modelled heart disease outcomes, measures of vascular stiffness and inflammation?

Justification

Population screening studies are predominantly conducted within Caucasian populations and concentrate upon identifying diabetes range hyperglycaemia. Few have comprehensively assessed cardiovascular disease risk across populations or used validated measures of arterial stiffness to further characterise vascular health amongst those screened. Universal identification of “pre-diabetes” in the impaired glucose intolerance range is unprecedented on this scale and provides an important opportunity to study post glucose tolerance test hyperglycaemic states reportedly more frequent in people of south Asian descent. Standardised collection and analysis of numerous biomedical measurements have enabled identification of putative novel risk factors contributing to arterial stiffness in south Asians. There are very few studies examining vascular stiffness indices in this group yet the incidence of cardiovascular events remains two to four fold that of the background United Kingdom rate.

Whilst acknowledging that a major clinical events intervention trial is the only way to definitively judge the efficacy of screening, in the absence of this option the most practical alternative is a shorter intervention period with modelled and surrogate endpoints. There is a paucity of data examining the clinical effectiveness or otherwise of multi-factorial cardiovascular risk intervention in diabetes cases identified through screening. The chosen range of outcomes assessed at one year is deliberately wide ranging and aims to produce highly original data of interest to researchers across a range of disciplines.

Thesis aims

1) To report the screening outcomes of a major screening programme (ADDITION-Leicester), incorporating detailed descriptions of;

i) Population sampling and uptake

ii) The demographics and ethnic mix of the screened population

iii) Adjusted prevalence estimates for undiagnosed glucose disorders stratified by ethnicity

iv) Five and ten year ethnicity modified Framingham cardiovascular risk estimates

2) To compare cardiovascular risk profiles and estimated burden of cardiovascular disease in newly diagnosed type 2 diabetes cases identified through screening and conventional diagnostic approaches

3) To categorise arterial stiffness by carotid-femoral pulse wave velocity ($_{cf}$PWV) measurement within a selected sub-populations of ADDITION-Leicester

4) To determine any ethnicity dependent effect on $_{cf}$PWV variation in this population

5) To explore the “putative” role for 25-hydroxyvitamin D in age-related arteriosclerosis as measured by $_{cf}$PWV

6) To report the effects of a twelve month randomised trial of multi-factorial cardiovascular risk intervention in people with screen detected type 2 diabetes on the following outcome measures;

i) Modelled five year CHD risk (UKPDS)

ii) Carotid-femoral Pulse Wave Velocity

iii) Markers of inflammation (C-Reactive Protein, Interleukin 6, Tumour Necrosis Factor)

Contributions

ADDITION-Leicester was a complex two phase study employing a team of health care professionals. Specially trained and highly dedicated research nurses performed daily screening duties, which included consent, biomedical measurements and administration of oral glucose tolerance tests. As clinical lead for the study I was available at all screening clinics to assist with venepuncture, deal with consent issues or medical queries and provide general medical cover. My role included the interpretation and dissemination of all biomedical results, with personal communication of newly diagnosed diabetes. I was also responsible for the intervention phase, attending over 90% of community or hospital based visits in the first two years of the study. Baseline and intervention data was independently entered onto a master database and analysed by me under the supervision of a medical statistician and Professor Davies. I was responsible for all aspects of the PACE sub-study.

Abbreviations

ADA: American Diabetes Association

ADDITION: Anglo-Danish-Dutch study of Intensive Treatment In PeOple with screeNed diabetes

AGE: Advanced Glycosylated End Product

BMI: Body Mass Index (kgm^{-2})

CHD: Coronary Heart Disease

CIMT: Carotid Intima Media Thickness

CRP: C reactive Protein

CV: Coefficient of variation

DESMOND: Diabetes Education and Self-Management for Ongoing and Newly Diagnosed

DCCT: Diabetes Control and Complications Trial

DTSQ: Diabetes Treatment satisfaction questionnaire

ECG: Electrocardiogram

FPG: Fasting Plasma Glucose

HOMA: Homeostasis Model Assessment

IEC: International expert committee on the diagnosis and classification of diabetes

IFG: Impaired Fasting Glycaemia

IGR: Impaired Glucose Regulation

IGT: Impaired Glucose Tolerance

IL6: Interleukin 6

IMD: index of medical Deprivation

MAP: Mean Arterial Pressure (diastolic and one third pulse pressure (systolic – diastolic))

NHS: National Health Service

OGTT- Oral Glucose Tolerance Test (75g standard unless otherwise indicated)

PACE: Pulse wave velocity (Adipo)Cytokine Ethnicity

PWV / cfPWV: Pulse Wave Velocity / carotid-femoral Pulse Wave Velocity

QALY: Quality Adjusted Life Yera

TNF-α: Tumour Necrosis Factor –alpha

2-HPG: Two hour serum glucose (post 75g-OGTT)

UKPDS: United Kingdom Prospective Diabetes Study

WHO: World Health Organisation

Chapter 1

Introduction

1.1 Disorders of glucose metabolism

1.1.1 An emerging epidemic of metabolic disease

Type 2 diabetes is a complex disorder characterised by profound disturbances of carbohydrate and lipid metabolism, resulting in relative insulin deficiency and chronic hyperglycaemia [1]. Already one of the most common conditions in the western world, the anticipated burden of this disease is staggering and a major threat to future healthcare prosperity [2-5]. Latest global estimates suggest some 285 million people have type 2 diabetes compared with 171 million in 2000 [6]. Tightly linked to a major obesity problem, the number of cases in many “developed” countries is expected to double again over the next twenty years [7,8]. Diabetes will undoubtedly “top” the public health agenda as predictions made fifteen years ago and considered shocking at the time, underestimate true trends in a disease now expected to reach near pandemic proportions [9,10]. Table 1.1 depicts the truly global nature of the problem with predicted prevalence estimates across a diverse range of populations.

Estimates for the United Kingdom are no less disconcerting, with the total number of people with type 2 diabetes set to increase from 4.8 to 6.2 million over the next fifteen years [11]. Although accurate incidence data is scarce, two large studies utilising community datasets report significant increases in diagnosed cases for periods covering 1994-1998 [12] and 1996-2008 [13]. Overall incidence of type 2 diabetes in the latter study increased by more than 70% over the study period, from 2.6 to 4.3 per 1000 person years. A combined estimate of the total number of cases (diagnosed and undiagnosed)

for this year in England stands at 3,099,853 or 7.4% (5.3%-10.8%) of the population [11].

Table 1.1 Prevalence of diabetes and estimated number of adults aged 20-79 years with diabetes for the years 2010 and 2030 [14]

	Prevalence (%)		Cases (thousands)		Annual increment (thousands)
	2010	2030	2010	2030	
United Kingdom	3.6	4.3	2140	2549	20
France	6.7	7.8	4164	5201	52
Sweden	5.2	6.2	484	556	4
United States	10.3	12.0	26,814	35,958	457
India	7.8	9.3	50,768	87,036	1813
Kenya	2.8	3.7	519	1,231	36
China	4.5	5.8	43,157	62,553	970

Alarmingly, these disturbing statistics fail to accurately portray the true burden of Western lifestyle-related metabolic disease, as they do not report even higher frequencies of non-diabetes range glucose abnormalities, commonly termed “pre-diabetes” or Impaired Glucose Regulation (IGR) [15].

The adverse health consequences associated with diabetes are well recognised and approaches to their prevention form the main focus of this thesis. When the projected frequency of the condition and its precursors are also considered, it becomes clear that the future health needs of many millions of people will depend upon novel approaches to its prevention and management.

1.1.2 Diagnosis and classification of glucose disorders

1.1.2.1 Type 2 diabetes

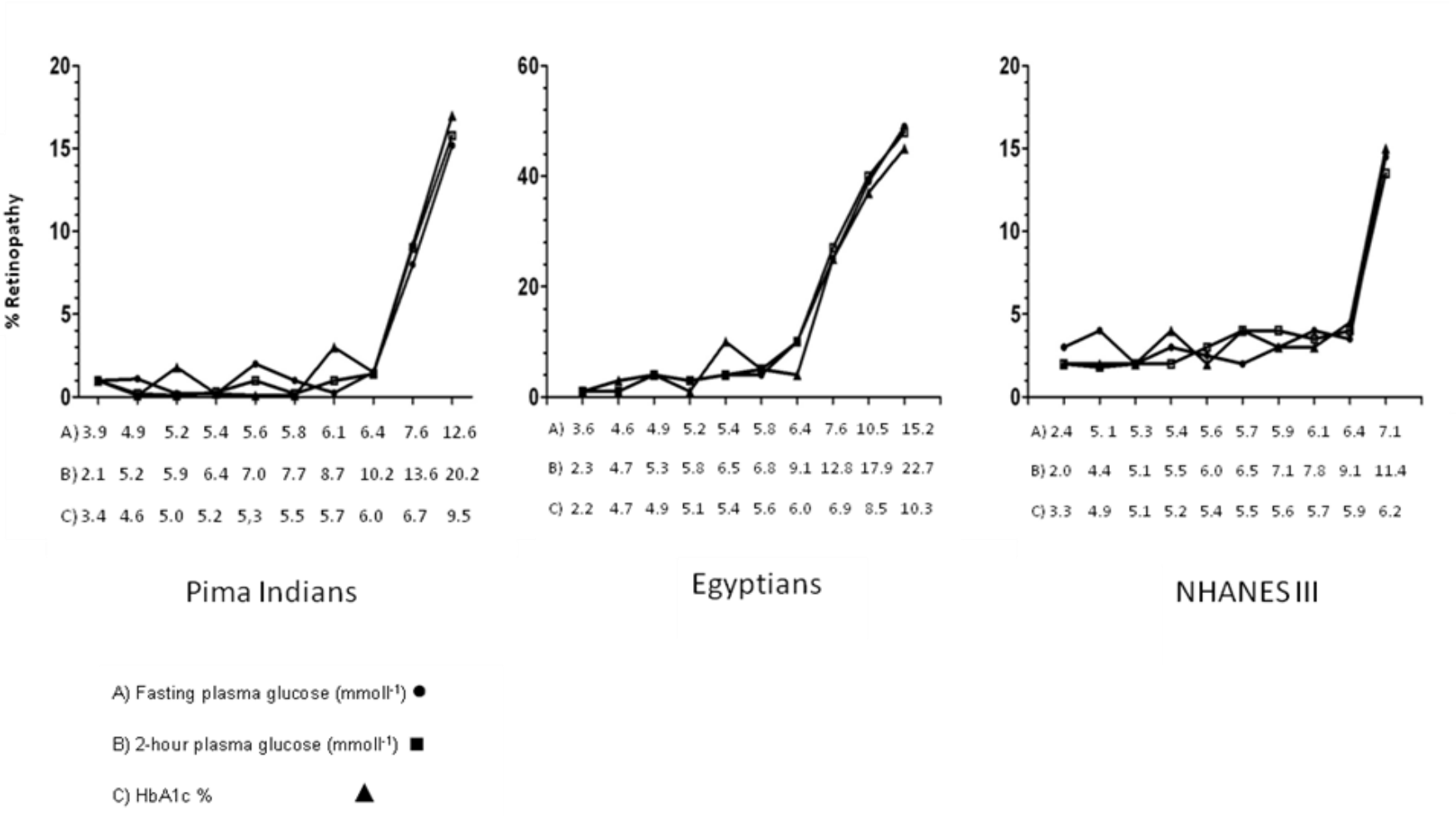
The classic signs and symptoms of diabetes, namely *polyuria*; the passing of an excessive amount of urine and *polydipsia;* excessive thirst, were first described by the ancient Egyptian Papyrus of Ebers in 1500 B.C. The term diabetes, meaning “to pass through” appears in Greek manuscripts from the first century A.D. with “Mellitus” a Latin-based derivative translated as “sweet as honey” completing its present day name in the late 1700’s [16]. The modern day diagnosis relies upon the measurement of plasma glucose concentrations in timed (fasting or after a standard metabolic stress such as an OGTT) or more recently, casual glycoslated haemoglobin ($HbA_{1c\%}$) samples. The most widely used glucose assay is the hexokinase reagent, which generates a measurable increase in hydrogenated Nicotinamide Adenine Dinucleotide (NADH) proportional to the glucose concentration of the sample [17].

Although clearly a major advance, the commercial development of reliable enzymatic glucose assays was unregulated and quickly resulted in a disorganised assortment of new biochemical criteria and definitions. By 1973 no less than six diagnostic fasting glucose thresholds were in use, each with differing time intervals, glucose loads or substrate. Unsurprisingly, the prevalence of diabetes varied markedly between and within populations and in response to increasing confusion the US National Diabetes Data Group (NDDG), quickly followed by the World Health Organisation (WHO 1980) published a standardised definition of diabetes. In doing so these organisations produced the blueprint of international criteria which would

define the disease for decades to come [18,19]. The chosen cut-points for diabetes (fasting glucose ≥7.8mmoll^{-1} (140mgdL^{-1}) or two hour post-challenge glucose of ≥11.1mmoll^{-1} (200mgdL^{-1})) were essentially arbitrary and based upon the development of "symptomatic diabetes" (i.e. polyuria, polydipsia and weight loss). In 1997, the relationship between glucose concentration and the prevalence of long-term complications was recognised by a lower fasting blood glucose cut-off, based on a consistent epidemiological pattern observed across three racially diverse populations (Figure 1.1). These studies demonstrated glucose levels below which there was little prevalent retinopathy (a common microvascular complication explained in section 1.1.3) and above which the rate increased in an almost linear fashion [20,21]. The new diagnostic threshold for fasting glucose was set at ≥7.0mmoll^{-1} (126mgdL^{-1}), whilst the two hour post-challenge level remained the same [21,22].

A series of minor modifications over the next five years attempted to consolidate the definition across organisations and remove confusing nomenclature such as "Insulin Dependent Diabetes Mellitus" (IDDM) and "Non Insulin Dependent Diabetes Mellitus" (NIDDM).

Figure 1.1 Prevalence of retinopathy within deciles of A) fasting plasma glucose ($mmoll^{-1}$) (circles), B) Two hour post-OGTT plasma glucose ($mmoll^{-1}$) (squares) and $HbA_{1c\%}$ (triangles). Because the prevalence of retinopathy differs between the three study groups, x axis scales are not identical [20,21].



Chronicled in Table 1.2 are WHO and American Diabetes Association (ADA) thresholds for fasting and post-challenge plasma glucose concentrations defining type 2 diabetes, Impaired Glucose Tolerance (IGT) and Impaired Fasting Glycaemia (IFG) since 1979 [18-27]. Disagreement remains over the practicality and clinical application of two hour post-challenge glucose, with the ADA recommending fasting measurements (fasting glucose ≥7.0mmoll^{-1} (126mgdl^{-1} on two occasions) in contrast to WHO guidance retaining both fasting and post-challenge criteria. Both organisations recognise that the diagnosis of diabetes should be based on repeated assessments unless the initial glucose estimation is associated with symptomatic hyperglycaemia.

In 2010/2011, the ADA and WHO incorporated glycosylated haemoglobin ($HbA_{1c\%}$) into their diagnostic criteria. $HbA_{1c\%}$ has a number of theoretical advantages over glucose; it can be measured spontaneously, has less biological variability and may provide an equally effective index of vascular complications. The epidemiological work presented within this thesis applies 1999 WHO diagnostic criteria and is therefore confined to serum glucose measurements [21].

1.1.2.2 Pre-diabetes: IFG and IGT

The term pre-diabetes was first introduced as a means of simplifying defined fasting and post-challenge glucose ranges conferring an increased risk of progression to frank diabetes. It encompassed recognised glucose cut-offs for IFG and IGT (Table 1.2), both of which are associated with an increased but highly variable risk of progression to diabetes. Although useful as lay terminology, the concept of “pre-diabetes” has not been universally adopted

as it incorrectly implies an inevitability of progression and its individual components (IFG/IGT) probably have differing pathophysiology. IGT is a more sensitive measure of early glucose dysregulation and reflects abnormal post-prandial glucose control secondary to skeletal muscle and liver insulin resistance [28]. IFG reflects abnormal fasting glucose control with primarily defective beta-cell function in combination with insulin resistance equivalent to IGT [29]. When reporting combined IFG and IGT populations for epidemiological purposes this thesis will refer to an Impaired Glucose Regulation (IGR) term rather than pre-diabetes. Dichotomising “at risk” populations in this manner is controversial, as unlike the curvilinear relationship observed with microvascular complications, glucose concentration and diabetes risk are continuously related. IFG and IGT are essentially arbitrary cut-offs, powerfully predicting future diabetes in some and falsely reassuring others with “normal” glucose range results. Therefore the choice of cut-off defining IFG and IGT has been largely discretionary and perhaps unsurprisingly divisive. For example, recognising the increased risk of diabetes with a fasting glucose value of ≤6.1mmoll^{-1} (110mgdl^{-1}), in 2003 the ADA revised the lower definition of IFG to ≥5.6mmoll^{-1}(100mgdl^{-1}). The WHO classification however retained the cut-off of ≥6.1mmoll^{-1} for IFG and instead chose to advocate continued use of the oral glucose tolerance test (OGTT) and the IGT two hour glucose range of 7.8 – 11.1mmoll^{-1}, an approach largely rejected by the Americans due primarily to the logistical difficulties associated with this test [24].

Table 1.2 Diagnostic criteria mmoll^{-1} (mgdl^{-1}) for type 2 diabetes mellitus, Impaired Glucose Tolerance (IGT) and Impaired Fasting Glycaemia (IFG)

		NDDG	WHO			ADA			IDF		IEC
		1979 [18]	1980 [19]	1999 [20,21]	2010 [22]	1997 [20,23]	2003 [24]	2010 [25]	2006 [26]	2010 [22]	2009 [27]
Diabetes Mellitus	Fasting	≥7.8 (136)	≥8.0 (140)	≥7.0 (126)	≥7.0 (126)	≥7.0 (126)	≥7.0 (126)	≥7.0 (126)	≥7.0 (126)	≥7.0 (126)	≥7.0 (126)
	2hr P-C glucose	≥11.1 (200)	≥11.1 (200)	≥11.1 (200)	≥11.1 (200)	≥11.1 (200)	≥11.1 (200)	≥11.1 (200)	≥11.1 (200)	≥11.1 (200)	≥11.1 (200)
	$HbA_{1c\%}$†	-	-	-	≥6.5	-	-	≥6.5		≥6.5	≥6.5
Impaired Glucose Tolerance (IGT)	Fasting	<7.8 (136)	<7.0* (126)	<7.0 (126)	<7.0 (126)	<7.0 (126)	<7.0 (126)	<7.0 (126)	<7.0 (126)	<7.0 (126)	NR
	2hrP-C glucose	7.8-11.0 (136-199)	8.0-11.0 (140-199)	7.8-11.0 (136-199)	7.8-11.0 (136-199)	7.8-11.0 (136-199)	7.8-11.0 (136-199)	7.8-11.0 (136-199)	7.8-11.0 (136-199)	7.8-11.0 (136-199)	NR
	$HbA_{1c\%}$†	-	-	-	-	-	-	-	-	-	6.0-6.4
Impaired Fasting Glycaemia (IFG)	Fasting	-	-	6.1-6.9 (110-125)	6.1-6.9 (110-125)	6.1-6.9 (110-125)	5.6-6.9 (100-125)	5.6-6.9 (100-125)	5.6-6.9 (100-125)	6.1-6.9 (110-125)	NR
	2hr P-C glucose	-	-	<7.8 (136)	<7.8 (136)	<7.8 (136)	<7.8 (136)	<7.8 (136)	<7.8 (136)	<7.8 (136)	NR
	$HbA_{1c\%}$†	-	-	-		-	-	5.7-6.4		5.7-6.4	6.0-6.4

*For fasting glucose concentration between 7.0-8.0 Oral Glucose Tolerance Test recommended and post load criteria followed
†The test should be performed in a laboratory using a method that is NGSP certified and standardized to the DCCT assay

2hr P-C: Two hour post-challenge glucose **NDDG**: US National Diabetes Data Group **WHO**: World Health Organisation **ADA**: American Diabetes Association **IDF**: International Diabetes Federation **IEC**: International Expert Committee

Despite continuing disagreement over cut-points defining risk of diabetes within the glucose continuum, there is definite consensus that intensive life-style and pharmacological interventions delay progression to diabetes within defined IFG and or IGT populations [30-32]. The emergence of effective preventative therapies over the last ten years is clearly attractive and this, in combination with convincing evidence of increased vascular risk in IGT has probably ensured the survival of “non-diabetes range” categories of hyperglycaemia. IGT is associated with a significant risk of cardiovascular mortality irrespective of progression to diabetes and a recent meta-analysis concluded both IFG and IGT are associated with a twenty percent increased risk of cardiovascular disease compared with normoglycaemic controls [33-35].

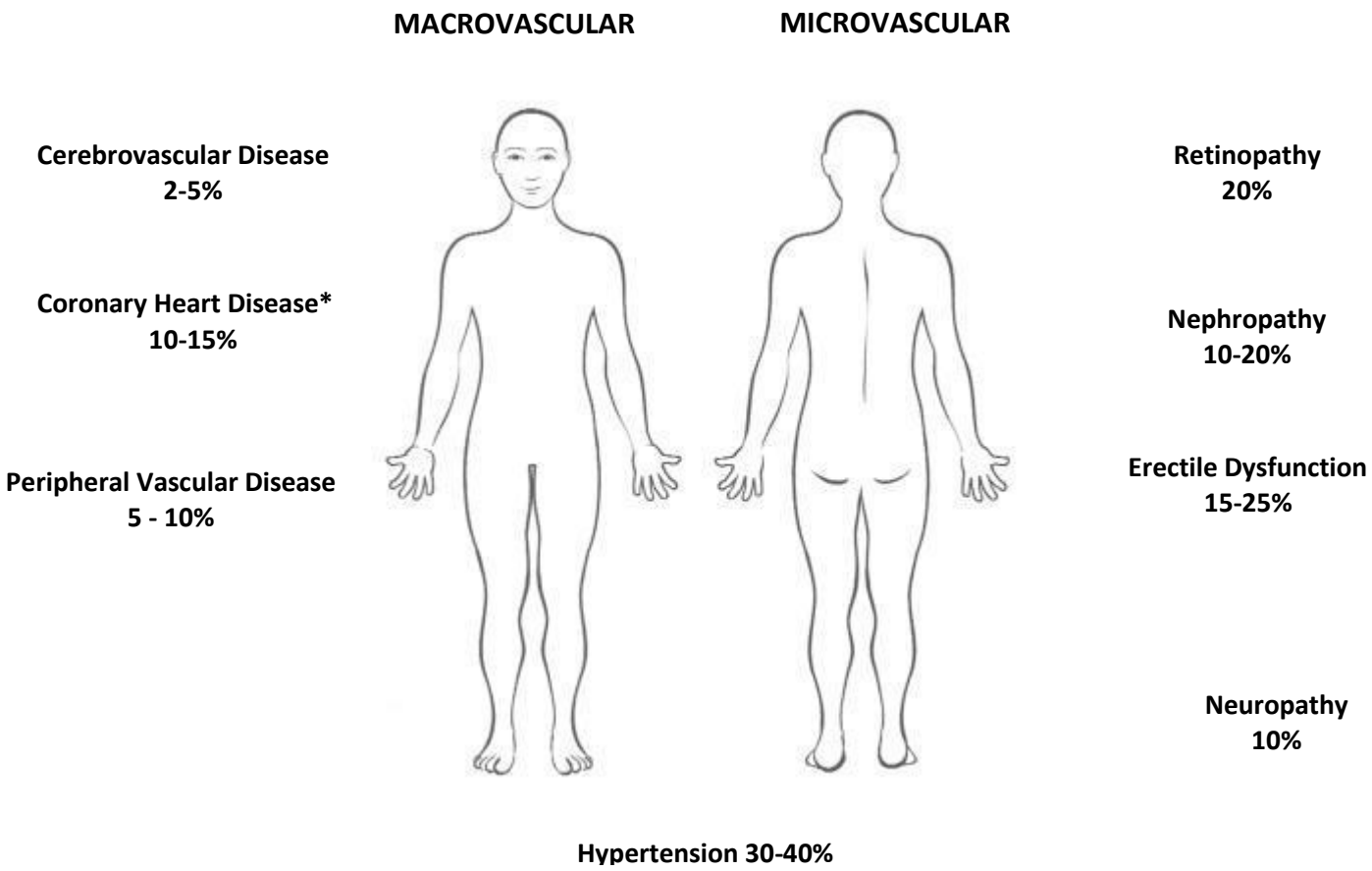
It would appear the pathogenesis of cardiovascular complications associated with the spectrum of insulin resistance begins below the diagnostic threshold for diabetes. Therefore, if the main aim of therapeutic intervention is to reduce the burden of these complications, it could be argued that the definition and or identification of glucose abnormalities should incorporate IGR range hyperglycaemia [36]. Theoretically, this would enable more rapid implementation of strategies aimed at the primary prevention of both diabetes and cardiovascular disease [37]. The most recent diagnostic criteria for diabetes remain focused on the risk of micro- rather than macrovascular complications but have at least attempted to move away from the concept of strict diagnostic cut-offs. Nevertheless, included in the International Expert Committee (IEC) criteria is a “high-risk” non-diabetes $HbA_{1c\%}$ range of 6.0 -

6.4% (Table 1.2), recommending preventative intervention and more frequent testing for people falling within this category [27].

1.1.3 The complications of type 2 diabetes: an overview

The pathophysiology of type 2 diabetes is complex, with environmental triggers such as the hypercalorific Western diet and sedentary life-style combining with genetic predisposition to drive intra-abdominal fat deposition and low grade systemic inflammation. The resultant metabolic milieu is characterised by hyperglycaemia, dyslipidemia, hypertension and obesity, and places people with diabetes at risk of specific target organ diseases (complications) as a result of small (microvascular) and large (macrovascular) vessel arterial damage (Figure 1.2). It is these vascular complications which account for the excess morbidity and mortality associated with the disease [38]. Ten year survival is just fifty percent, with the majority of deaths the result of large vessel atherosclerotic diseases such as coronary heart disease (CHD) and stroke. A diagnosis of type 2 diabetes results in a two to four fold increased risk of cardiovascular disease and a mean reduction in life expectancy of more than ten years [39].

Figure 1.2 Frequency of vascular complications in newly diagnosed type 2 diabetes mellitus [40-42]



A major reason for this is late presentation, as clinical diagnosis is typically preceded by a protracted symptom free period, during which glucose levels progressively rise and vascular disease develops. Although impossible to accurately quantify, it is estimated that the onset of type 2 diabetes precedes clinical diagnosis by an average of seven years and as a consequence, at diagnosis, forty to fifty percent will have clinically significant micro- or macrovascular complications [40-42] (Figure 1.2). Furthermore, there is evidence that intervention techniques for diabetes related macrovascular complications are often less successful, presumably due to the severity and widespread nature of the disease at presentation [43-45]. Primary prevention of complications through earlier identification of glucose disorders and IGR may therefore offer the best chance of improving long-term outcomes, particularly as the risk of vascular disease is increased below the diagnostic cut-off for diabetes [34-37].

1.1.4 The evidence base for cardiovascular risk factor treatment

The management of type 2 diabetes, on the one hand, aims to address the excess morbidity and mortality associated with its cardiovascular complications. In clinical practice, this occurs in combination with specific attempts to safely lower serum glucose levels using a combination of life-style and pharmacological treatments. Clinical trials endorse this approach in people with established diabetes, demonstrating that aggressively treating hypertension and dyslipidaemia, improves cardiovascular outcomes [46-59] (Table1.3).

Table 1.3a Primary prevention lipid lowering cardiovascular disease outcome trials in people with type 2 diabetes mellitus (1998-2008). *indicates diabetes sub-group analysis (sub-group number in parenthesis)

Study	Size (n)	Duration (Yrs)	Cohort age and diabetes duration	Intervention	Outcomes Events vs control	Comment
CARDS [46]	2,838	4.0	62 6	Atorvastatin 10mg	36% ↓ CHD 83 vs 127 (p<0.001)	>1 risk factor for CVD. 48% ↓ stroke
HPS* [47]	14,573 (5963) 2912 (PP)	4.8	62.1 9	Simvastatin 40 mg	33% ↓ CVD 135vs 196(p<0.001)	CVD event rate in placebo arm 13%
ASPEN* [48]	2,410 (1,905)	4.0	60 8	Atorvastatin 10 mg	10% ↓ CHD 100 vs 102 (p=0.1)	Non-significant results reflect "control contamination" +changing lipid lowering practice during study
ALL-HAT* [49]	10,355 (3638)	4.8	66 unknown	Pravastatin 40 mg	11% ↓ CHD 81 vs 88 (p=0.23)	Non-significant results reflect "control contamination" +changing lipid lowering practice during study
ASCOT-LLA* [50]	10,305 (2532)	3.3	63.6 8	Atorvastatin 10mg	25% ↓ CVD 116 vs 151 (p=0.03)	>2 risk factors for CVD Study terminated at 3.3 years
HHS* [51]	4081 (135)	5.0	47	Gemfibrozil 1200mg	68% ↓ CHD not significant	Insufficient power in sub-group analysis.
FIELD [52]	9795 7664 (PP)	5.0	62.2 5	Fenofibrate 200mg	19%↓CVD (p=0.01)	Statin "pollution" 30% of control Main study Secondary prevention non significant

Table 1.3b Primary prevention blood pressure lowering cardiovascular outcome trials in people with type 2 diabetes mellitus (1998-2008). *indicates diabetes sub-group analysis (sub-group number in parenthesis)

Study	Size (n)	Duration (Yrs)	Cohort age (Yrs)	Intervention	Outcomes Events vs control	Comment
HOT* [53]	18,790 (1501)	3.8	61.5	Felodipine stepped care approach	51% ↓ CVD 25vs12 (p<0.01)	<80mmHg vs <90mmHg sub-groups
UKPDS [54]	1148	8.4	55	Tight vs less tight control	32% ↓ CVD 62 vs 82 (P<0.01)	150/85 vs 180/105 Av 144/82 achieved
ABCD [55]	470	5.0	60	Nisolipine vs enalapril	51% ↓ deaths in enalapril arm	Nisolipine arm stopped due to ↑ myocardial infarction
HOPE* [56]	9267 (3577)	5.0	55	Ramipril vs placebo	37% ↓ CVD death 651 vs 826 (p<0.01)	Diabetes and > 1vascular risk factor
ALLHAT* [57]	33,357 (12,063)	4.9	>55	Amlodipine vs Lisinopril vs chlorthalidine	No difference in outcomes	BP lowering 4/2 with Lisinopril
ASCOT-BPLA* [58]	19,257 (5199)	5.5	63	Amlodipne /Perinopril Vs Atenolol /Thiazide	No difference in outcomes	BP lowering 5.9/2.5 with amlodipine
ACCORD-BP [59]	4733	4.7	62.2	Stepped care <120 vs <140 control	No difference in outcomes	

CARDS: Collaborative Atorvastatin Diabetes Study
ASPEN: Atorvastatin Study for Prevention of CHD Endpoints
ASCOT-LLA**:** Anglo-Scandinavian Cardiac Outcome Trial, Lipid lowering
FIELD: Fenofibrate Intervention and Event Lowering in Diabetes
HPS **:** Heart Protection Study
ALL-HAT: Antihypertensive and Lipid Lowering treatment to prevent Heart Attack
HHS: Helskini Heart Study
HOT**:** Hypertension Optimal Treatment study

The convincing results of these individual risk factor intervention trials form the basis of this combined approach to the treatment of type 2 diabetes.

The two largest statin therapy intervention trials to date, the Collaborative Atorvastatin Diabetes Study (CARDS) [46] and the Heart Protection Study (HPS) [47]; demonstrated highly significant relative risk reductions of 24 and 37% respectively for a first major event or death in subjects with type 2 diabetes. These trials provide definitive evidence that Simvastatin and Atorvastatin are highly effective at reducing the risk of major coronary and cerebrovascular events in patients with diabetes, even in the absence of cardiovascular disease. Event rates in the placebo arms of both HPS and CARDS (13% and 9% respectively) were high and support the concept of diabetes as a "cardiovascular disease equivalent", to be considered separately in population level vascular risk calculations. In a meta-analysis of 12 trials (not including CARDS) lipid lowering drug therapy was associated with a 21% reduction in coronary events in both primary and secondary prevention categories [60].

The combination of hypertension and diabetes appears to double the risk of cardiovascular disease and a number of landmark studies (most notably UKPDS) and sub-group analyses highlight the importance of tight blood pressure control as well as lipid lowering in people with diabetes [53-59] (summarised in Table 1.3b).

Whilst the evidence supporting the Joint British Societies (JBS-2) [61] guideline defining hypertension as a systolic blood pressure of >140/90mmHg is strong, recent outcome trials have challenged established lower thresholds in selected high risk categories, including diabetes (JBS-2 for example

recommends defining hypertension as >130/80mmHg in diabetes). Although a number of observational studies indicate greater risk at lower blood pressure levels, aggressive intervention in the ACCORD-BP study failed to demonstrate improved outcomes in individuals randomised to a blood pressure of less 120mmHg [59]. The rationale for the lower treatment targets in diabetes therefore remains based upon epidemiological extrapolation of risk suggesting there is no threshold below which risk no longer declines [55].

Intervention trials concentrating upon intensive glucose control have not been so convincing and paradoxically, in one case has even shown an increase in cardiovascular events [62-64]. Glucose lowering probably takes ten years or more to impact favourably upon macrovascular complications, suggesting that either glucose *per se* is not the major determinant of atherogenesis in diabetes or aggressive intervention is required earlier in the disease process [65].

Although a multi-factorial approach to cardiovascular risk management is considered standard practice in type 2 diabetes, outcome trials assessing the effects of combined glucose, blood pressure and lipid lowering interventions in are surprisingly scarce and confined to relatively advanced cases. In the Steno study, 160 patients with type 2 diabetes and microalbuminuria were randomised to either conventional treatment in accordance with national guidelines or an intensive stepwise behaviour modification and pharmacological intervention addressing hyperglycaemia, hypertension and dyslipidaemia. After seven years of follow-up, there was a highly significant fifty-three percent reduction in the risk of a cardiovascular event in patients

receiving intensive multi-factorial intervention. Twenty-four percent of participants treated in this way developed cardiovascular disease compared with forty-four percent in the conventional arm of the study [66]. In an extended observational follow-up, absolute risk reduction remained significant despite convergence of blood pressure, glucose and lipid differences achieved during the study. During the entire thirteen year study, the rate of death among patients in the conventional therapy group was a staggering fifty percent, a finding emphatically dispelling any notion of diabetes as a benign disease not requiring aggressive cardiovascular risk management from diagnosis [67].

A recently published *ad hoc* analysis of the ADVANCE study (Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation) showed that blood pressure in combination with intensive glucose lowering reduced the incidence of cardiovascular death by 18% (p=0.027) [68].

Although not a cardiovascular outcome trial, UKADS (United Kingdom Asian Diabetes Study) assessed whether a culturally sensitive community intervention, facilitating intensive cardiovascular risk factor management improved blood pressure, cholesterol and glycaemic control in patients of south Asian origin with pre-existing type 2 diabetes (forty percent of participants had a recorded duration of diabetes of between zero and four years) [69]. Over the duration of the trial, significant but modest reductions in blood pressure (-4.9/3.8mmHg) and cholesterol (-0.45mmoll^{-1}) but not $HbA_{1c\%}$ (+0.04%) were reported. These improvements were almost certainly the result

of increased anti-hypertensive (from 55 to 75%) and statin (from 48 to 65%) pharmacotherapy in the intervention arm.

Data from the Swedish National Diabetes Register provides further “real world” evidence that multi-factorial cardiovascular risk intervention improves outcomes in people with type 2 diabetes. This observational study prospectively categorised patients by blood pressure and glucose control into “tight” and sub-optimal” groups, and used general practice registry data to report incident cardiovascular outcomes. A median difference in $HbA_{1c\%}$ of 1.6% and blood pressure of 25/5mmHg between tight and sub-optimal control reduced the risk of non-fatal myocardial infarction and stroke by 28% and 38% respectively [70].

In summary, there is strong evidence that target driven lipid lowering and blood pressure interventions improve cardiovascular outcomes in patients with type 2 diabetes. There is also evidence, especially in established and advanced diabetes that combinations of cardiovascular risk factor interventions have additive benefits.

2.2 Screening for type 2 diabetes

1.2.1 Rationale for earlier identification and intervention

Diabetes is recognised as a major contributor to health inequalities and premature vascular morbidity, much of which could potentially be reduced with existing treatments [71]. An estimated one in seven of all deaths in the United Kingdom are directly attributable to diabetes, whilst potentially reversible cardiovascular diseases account for seventy-five percent of all deaths in people with diabetes [72]. The increasing evidence base for effective primary prevention of cardiovascular disease and type 2 diabetes, discussed in Section 1.1.4, together with a greater general awareness of both conditions has led to great interest in screening, early detection and risk reduction intervention activities.

1.2.2 Criteria for screening in asymptomatic populations

"Screening is the process of identifying those individuals who are at sufficiently high risk of a specific disorder to warrant further investigation or direct action" – World Health Organisation 1997

This core principle of screening, described by Wilson and Junger appears particularly attractive when applied to the potentially devastating consequences of type 2 diabetes [73]. Screening programmes are becoming increasingly integrated into standard medical practice, and appear particularly successful in diseases exhibiting truly latent behaviour. In the United Kingdom, candidate diseases are systematically evaluated against an established set of criteria, (based on Wilson and Jungers original work) prior to the implementation of any national programme (Table 1.4).

Table 1.4 WHO screening criteria and summary of UK NSC (2008), DUK (2009), ADA (2010), USPSTF (2008) and CTFPHE (2009) screening recommendations for non-symptomatic type 2 diabetes in adults

Wilson & Jungers criteria	**Strategy**	**UK NSC** [74]	**DUK** [75]	**ADA** [76]	**USPSTF** [77]	**CTFPHE** [78]
The condition represents an important health concern that imposes a significant burden on quality and or quantity of life.	**Population screening**	**Not recommended** **(NHS risk assessment)**	**Not Recommended**	**>45 years**	**Not Recommended**	**Not Recommended**
The natural history of the disease must be well understood The condition must have an asymptomatic period during which detection and treatment significantly reduce mortality and morbidity Tests are available that can detect the pre-clinical stage of disease and these tests are acceptable, reliable and affordable The benefits of screening outweigh the physical and psychological harm caused by tests, diagnostic procedures and treatment	**Risk factor based:** *-BMI (Kgm^{-2})* *-BP (mmHg)* *-Dyslipidaemia* *-CVD* *-Pre-diabetes* *-1^{o} relative diabetes* *-Physical inactivity* *-PCOS* **Screening frequency recommendation** **Age range (years)**	≥ 30 (≥27.5*) >140/90 - Yes Yes Yes Yes Yes **None** 40 -74	≥ 30 (≥27.5*) >140/90 Yes Yes Yes Yes Yes Yes **Three yearly** Adults	>25† >140/90 HDL-C<0.9 Yes Yes Yes Yes Yes **Three yearly** Adults	No recommendation >135/80 - Yes Yes Yes Yes Yes **None** Adults	No recommendation >135/80 Trigs>2.8 Yes Yes Yes Yes Yes **None** Adults

UKNSC: United Kingdom National Screening Committee **DUK**: Diabetes UK Guidance **ADA**: American Diabetes Association **USPSTF**: United States Preventative Services Task Force **CTFPHE**: Canadian Task Force on the Periodic Health Examination * South Asians † certain groups

A number of reputable organisations, including the United Kingdom National Screening Committee (UK NSC), the American Diabetes Association (ADA) and United States Preventative Services Task Force (USPSTF) produce updated, evidence based criteria on screening for diabetes. The latest guidance from all three bodies is detailed in Table 1.4. The UK NSC last appraised the evidence and appropriateness of screening for type 2 diabetes in 2007, as an NHS Health Technology Assessment [79]. In the following section we conduct a similar exercise in order to update the current evidence and identify deficiencies in the literature relevant to this thesis.

1.2.3 Summary of current evidence and guidance on screening

"The condition represents an important health concern that imposes a significant burden on quality and or quantity of life."

This critical requirement readily applies to diabetes in the both the community and hospital setting. In 2005, it was estimated there were 20,765 excess deaths amongst people with diabetes between the ages of 20 and 79 years [80]. Estimated global excess mortality attributable to diabetes was 2.9 million in 2000. This equates to 5.2% of world all cause mortality, making diabetes the fifth most common cause of death. Unless adequately addressed, it is estimated mortality relating to diabetes will increase by fifteen to twenty percent over the next decade and existing health inequalities will be exaggerated by a disease closely linked to socio-economic status and deprivation [81].

It is estimated ten to fifteen percent of hospital beds in the United Kingdom are occupied by people with diabetes, representing over a million bed days

per year [82]. Hyperglycaemia on admission to hospital remains a major predictor of adverse outcome and pre-existing diabetes is associated with higher rates of many common acute medical and surgical diseases [83].

Indeed, the management of diabetes and its complications already has important socio-economic implications. It is currently estimated that ten percent of the NHS budget is spent on diabetes, approximately nine billion pounds per year or one million per hour based on the 2007/8 NHS budget. In 2006, 28.4 million items to treat diabetes were prescribed at a cost of £561.4 million [84]. Between 2000 and 2008 expenditure on diabetes drugs rose by 50%, accounting for 7% of all prescription costs in the UK [85]. Often debilitating physical, emotional and personal complications of diabetes are much more difficult to quantify and this list simply serves to illustrate the magnitude of the problem facing health care providers.

“The condition must have an asymptomatic period during which detection and treatment significantly reduce mortality and morbidity”

Establishing the duration and potential adverse consequences of unchecked asymptomatic hyperglycaemia is crucial when deciding whether or not earlier intervention is appropriate. Existing estimates extrapolate plots of the frequency of microvascular complications with fasting plasma glucose concentration to arrive at a figure of approximately seven years between onset and diagnosis of disease [86]. With such a protracted latent period, the number of undiagnosed cases is likely to be significant and the opportunity to prevent progressive end-organ disease diminished.

It is estimated one in four people with diabetes in the United Kingdom, a total population of 1.1 million are undiagnosed [11] and approximately 5% of the U.S. adult population has diabetes but is unaware of the diagnosis [87]. Twenty-five percent of such cases are likely to have established retinopathy or microalbuminuria at diagnosis, and fifty percent have demonstrable large vessel atherosclerosis (Figure1.2) [40-42]. Recent UKPDS data is suggestive of a “legacy effect” with a period of untreated hyperglycaemia resulting in long-term adverse cardiovascular consequences even if blood glucose levels are appropriately treated later in the disease [65].

Thus available epidemiological evidence indicates that the latent period between the onset and clinical diagnosis of type 2 diabetes is clinically important and in many cases extremely detrimental. The diagnostic criteria for diabetes remain the same for symptomatic and asymptomatic individuals, the distinction remaining a function of plasma glucose concentration reflecting disease duration rather than severity. Retaining historically recognised and understood diagnostic categories should theoretically assist time-pressured physicians interested in identifying type 2 diabetes earlier. A more timely diagnosis may reduce complications by creating an opportunity for earlier intervention involving optimisation of glucose control and other cardiovascular risk factors (Section 1.1.4).

Despite these theoretical benefits, there is no confirmatory evidence from randomised trials that aggressive multi-factorial intervention improves cardiovascular outcomes if commenced earlier in the disease trajectory i.e. during the asymptomatic phase of diabetes. In the absence of evidence of

direct benefits of routine screening for type 2 diabetes, the decision to screen individual patients remains pragmatic and a matter for clinical judgment.

“The natural history of the disease must be well understood”

As discussed earlier, the natural history of type 2 diabetes is characterised by the emergence of post-prandial and subsequently fasting hyperglycaemia (Section 1.1.2.2). In the vast majority of cases hyperglycaemia results from a failure of beta-cell insulin secretory capacity to adequately compensate for insulin resistance in peripheral tissues [88]. The resultant inexorable decline of beta-cell function precipitates IGR and eventually diabetes range hyperglycaemia. The association of progressive dysglycaemia with the occurrence of microvascular complications in particular is well established and forms the basis of the recognised diagnostic categories discussed earlier (Section 1.1.2). By affecting intrinsic properties of the vascular wall, chronic hyperglycaemia appears to be one of a number of factors responsible for the resultant small and large vessel angiopathy characterising diabetes related complications. The current theory underpinning this highly complex relationship, together with the postulated role of chronic inflammation in the pathogenic process of vascular dysfunction in metabolic disease are described in Section 1.3

“Tests are available that can detect the pre-clinical stage of a disease and these tests are acceptable, reliable and affordable”

Analogous to the diagnostic classification discussed in Section 1.1.2, optimal screening strategies for type 2 diabetes have been the subject of intense

debate. A board range of imperfect screening tools have hindered attempts to achieve a consensus on fundamental operational issues such as screening methodology, frequency of sampling and target population selection. This in combination with concerns over significant population variation in the accuracy of available tests has resulted in the ADA and UK NSC historically adopting nationally accepted diagnostic criteria without recommending specific approaches to identifying new asymptomatic cases.

Testing restricted to individuals at increased risk will reduce the "number needed to screen" and theoretically improve the overall cost-effectiveness of the screening programme. The NSC criteria stipulate screening confined to those at increased risk, defined by advancing age (>40 years), BMI (>30 Kgm^{-2}), co-morbidities (hypertension, cardiovascular disease), family history, socio-economic status and ethnicity (Table 1.4). In 2008, the UK NSC attempted to provide primary care physicians with a clearer strategy to identify new cases of diabetes likely to be at high risk of cardiovascular complications [74]. This involves a multi-stage process reliant upon general practice registries to firstly select high risk patients (in this case those with a cardiovascular risk >20% or pre-existing disease). The second stage is a fasting glucose assessment, with a cut-off of >6.0$mmoll^{-1}$ triggering the third stage, a definitive OGTT. It is estimated this approach, conducted in a typical white European population with hypertension and an average BMI of ≥25 Kgm^{-2}, would have a sensitivity of 80% and specificity of 65% with around 8% of the population requiring an OGTT.

Random, fasting and post-challenge glucose concentration, $HbA_{1c\%}$ and glycosuria testing have all been considered for screening. A selection of

studies reporting sensitivity and specificity data for each of these modalities is illustrated in Table 1.5. Urine testing is highly specific but has poor sensitivity, whereas random capillary or plasma glucose estimations are more sensitive but generally lack specificity. Venous fasting plasma glucose has sensitivity between 40% and 65% with specificity in excess of 90% for fasting glucose values ranging from 6.1–7.8mmoll^{-1} Random and post-prandial sampling (venous or capillary) perform better than fasting tests, presumably because subjects in the initial stages of disease are more likely to meet the two hour post-challenge rather than fasting diagnostic criteria. However, the poor reproducibility and cost of the OGTT is prohibitive and its inconvenience maybe a deterrent to mass screening. Despite recommendations to screen for diabetes with standard diagnostic fasting or post-challenge glucose levels, random plasma assessments probably remain the most commonly used screening method in clinical practice [89]. The convenience of a random test, often added to a battery of other health checks is appealing and is likely to off-set concerns over lack of standardisation and repeatability with random sampling. This is also a major advantage of glycosylated haemoglobin ($HbA_{1c\%}$), which is emerging as the preferred diagnostic tool for both diagnosed and undiagnosed type 2 diabetes.

Table 1.5 Studies summarising the performance of screening tests for newly diagnosed type 2 diabetes [90-98].

Screening Test	Population	Cut-point	Sensitivity (%)	Specificity (%)	Comment
Urinalysis	WE UK	Detectable glycosuria	18 43	99 98	Inexpensive Convenient
FPG	Various	4.8 5.3 6.0 7.0	86 77 90 74	45 77 66 89	Inexpensive Inconvenient Patient reliant
RCG	WE US US SA	6.2 6.7 7.7 7.8	63 75 62 75	92 88 95 88	Inexpensive Convenient ? standardised
RPG	ADA 2000	8.9	No data reported in guidance		
2 hr PC	Pima Indians WE/SA	8.6 11.1	90 70	93 97	Inconvenient Patient reliant Difficult Expensive
$HbA_{1c\%}$	US / WE Chinese SA UK WE UK SA	5.6 6.5 7.0 6.5 6.1 6.5 6.5	89 / 86 44 / 24 25 / 12 54 88 62 79	80 / 74 99 / 99 100 / 100 100 88 98 93	Convenient Standardised ?discrimination for IFG/IGT
Questionnaire FINDRISC Cambridge Leicester	 WE WE UK WE + SA	 - - -	 77 77 81	 66 72 45	Convenient Inexpensive Can't diagnose

FPG: Fasting Plasma Glucose ($mmoll^{-1}$)
RCG: Random Capillary Glucose ($mmoll^{-1}$)
RPG: Random Plasma Glucose ($mmoll^{-1}$)
$HbA_{1c\%}$: Glycoslated Haemoglobin
2 hr PC: Two hour post-challenge glucose (post 75g-OGTT)
SA: South Asian
WE: White European
US: United states

The prevalence and socio-economic costs of diabetes are undoubtedly major threats to the future financial security of the National Health Service. Advocates of screening, argue major cost savings accrued by delaying the progression and complications of the disease will inevitably justify the additional investment required to implement national screening programmes for diabetes or even IGR. In the absence of direct evidence that treatments effective in clinically diagnosed cases are effective if introduced earlier, inevitably complex economic models have been used to estimate costs and outcomes associated with diabetes screening. The cost of screening, including diagnostic tests, inaccuracies of the chosen screening procedure and the subsequent prolonged treatment for positive cases must ultimately be less than the socio-economic burden posed by delayed intervention.

The majority of published studies in this area use either direct costs or calculated cost per Quality Adjusted Life Year (QALY) outcomes within cohorts charting the progression of diabetes and its complications. Briefly, QALYs provide a crude metric estimate of the benefits gained from a medical procedure or intervention in terms of both quality and quantity of life. The lower the ratio of cost to QALY the more cost-effective the intervention, with a value of £50,000 typically used as a benchmark indicating value for money.

Hoerger *et al.* [99] was the first to incorporate cardiovascular outcomes into economic modelling and concluded that targeted screening (of people with co-existent hypertension) was more cost-effective than universal screening ($48,146 versus $143,839). Two United Kingdom based studies used an approach incorporating potential benefits and harms of screening. Goyder *et*

al [100] found that for every 10,000 individuals screened a net of ten QALYs would be gained from a combination of avoided micro- and macrovascular complications. Gilles *et al.* [101] used similar methodology in a United Kingdom multiethnic population. Estimated costs per QALY were £14,150 for screen detected type 2 diabetes, and £6,242 for diabetes and IGT followed by life-style interventions.

Direct evidence of cost saving has been recently demonstrated by another population based screening study. Estimated medi-care testing and treatment costs were calculated over three years for a variety of screening approaches. Screening with random capillary glucose ($186,090), $HbA_{1c\%}$ ($192,261) and plasma glucose one hour after a 50g-glucose challenge ($180,635) were all lower than costs for no screening ($205,966) [102].

A recently published analysis of sequential screening strategies within a simulated North American population indicates ADA guidelines for screening are likely to be cost-effective. Complex modelling showed that screening adults aged 45 years and older every three years results in a lead time in diagnosis gained of 5.3 years and a cost per QALY of between $8461 and $11,155. Compared with no screening, all simulated screening strategies increased the number of QALYS, suggesting screening for diabetes is a reasonable and not prohibitively expensive exercise [103]

"*The benefits of screening outweigh the physical and psychological harm caused by tests, diagnostic procedures and treatment."*

Existing diagnostic tests for diabetes are straightforward, inexpensive, and rarely problematic. Although repeated venesection is inconvenient and occasionally stressful, clinical investigations are relatively non-invasive in comparison with other screening programmes. The OGTT is occasionally associated with nausea and symptomatic reactive hypoglycaemia but probably more important is the potential to cause harm through iatrogenic hypoglycaemia once a diagnosis is made and glucose-lowering treatments initiated. Hypoglycaemia has been implicated as a cause of excess mortality in trials of intensive glucose lowering in people with established diabetes [63]. Patients identified with screen detected diabetes will undoubtedly be exposed to known and unknown adverse effects of pharmacotherapy for a substantially longer period of time than those diagnosed when symptoms develop. In order to control often multiple co-existent cardiovascular risk factors patients with type 2 diabetes are typically prescribed at least four or five medications. The long-term consequences, potential interactions and costs of poly-pharmacy associated with the modern management of type 2 diabetes is an important and under researched consideration, especially pertinent to screen detected cases.

Screening will inevitably be associated with a collection of individual favourable and unfavourable experiences, some of which will be disease specific and some generic to screening. Favourable experiences include heightened awareness that a condition has been detected earlier, enhanced

motivation towards treatment, and relief attached to a negative result. Knowledge and volition are required before behaviour change and if supported by well designed self-management education is theoretically more likely to maximise the positive impact of screening. One example is the DESMOND life-style education intervention which is specifically designed to empower self-management health beliefs and has been shown to improve patient related outcomes in newly diagnosed type 2 diabetes [104].

Conversely, in those screened and found to have disease, the experience of being identified may lead to a decline in perceived health status and increased anxiety, negative effects consistently reported in other screening programmes [105]. In addition, a true negative result may be falsely reassuring, minimizing perceived risk whilst simultaneously reinforcing high risk behaviour. In respect to diabetes there is emerging evidence that screening actually has little impact on patients' psychological health. The results of a recent randomised controlled trial agree with a series of prospective comparative studies in concluding little adverse affect, increased anxiety or change (positive or negative) in perceived health status following a diagnosis of diabetes through screening [106-108].

In summary, the last ten years has seen major advances in the evidence base resolve many of the issues surrounding screening for type 2 diabetes. Establishing whether earlier identification improves cardiovascular outcomes remains critical as intensive glucose lowering in established cases appears to have limited initial effect.

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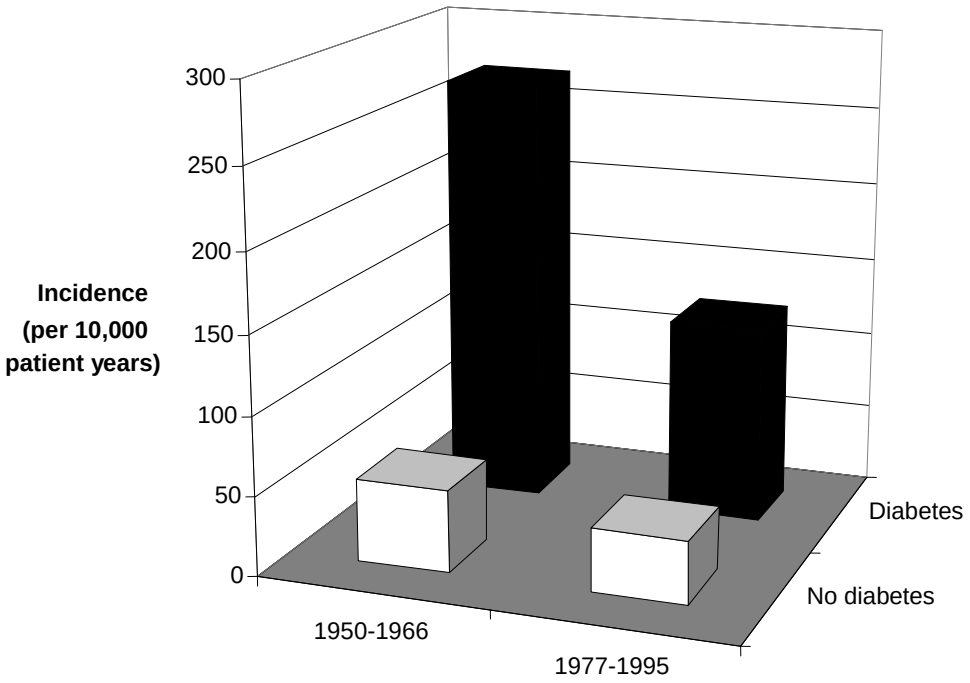
1.3 Pathogenesis of vascular complications

People with diabetes have a two to four fold increased risk of cardiovascular diseases, including CHD, arrhythmia, peripheral vascular disease, congestive cardiac failure and stroke [109-111] (Figure 1.3). An estimated ten to fifteen percent of population CHD risk is directly attributable to diabetes and myocardial diseases account for over fifty percent of all-cause mortality in diabetes [72]. Some authorities believe the risk of a vascular event may be the same whether CHD is present or not and suggest the disease should be regarded as a cardiovascular disease equivalent associated with major vascular pathology from diagnosis [112].

The reasons why diabetes is such a potent risk factor for premature arterial disease remain poorly understood despite an extensive amount of published research. Inflammatory, haemodynamic, autonomic, and direct gluco-toxic effects are all implicated but a clear single mechanism remains elusive. This lack of clarity suggests either the pathogenesis is extremely complex and likely multi-factorial, or a common aetiology links the conditions and observed associations are predominantly casual [113]. Diabetes is therefore commonly regarded as an independent risk factor for a number of clinical and sub-clinical vascular abnormalities as well as a specific disease with its own hyperglycaemia related morbidities (Figures 1.2 and 1.4). Many of these features appear to overlap and therefore may respond to early aggressive cardiovascular risk intervention.

Figure 1.3 Cardiovascular disease incidence in subjects with and without diabetes from the Framingham Heart study [110,111]



1.3.1 Inflammation, diabetes and cardiovascular disease

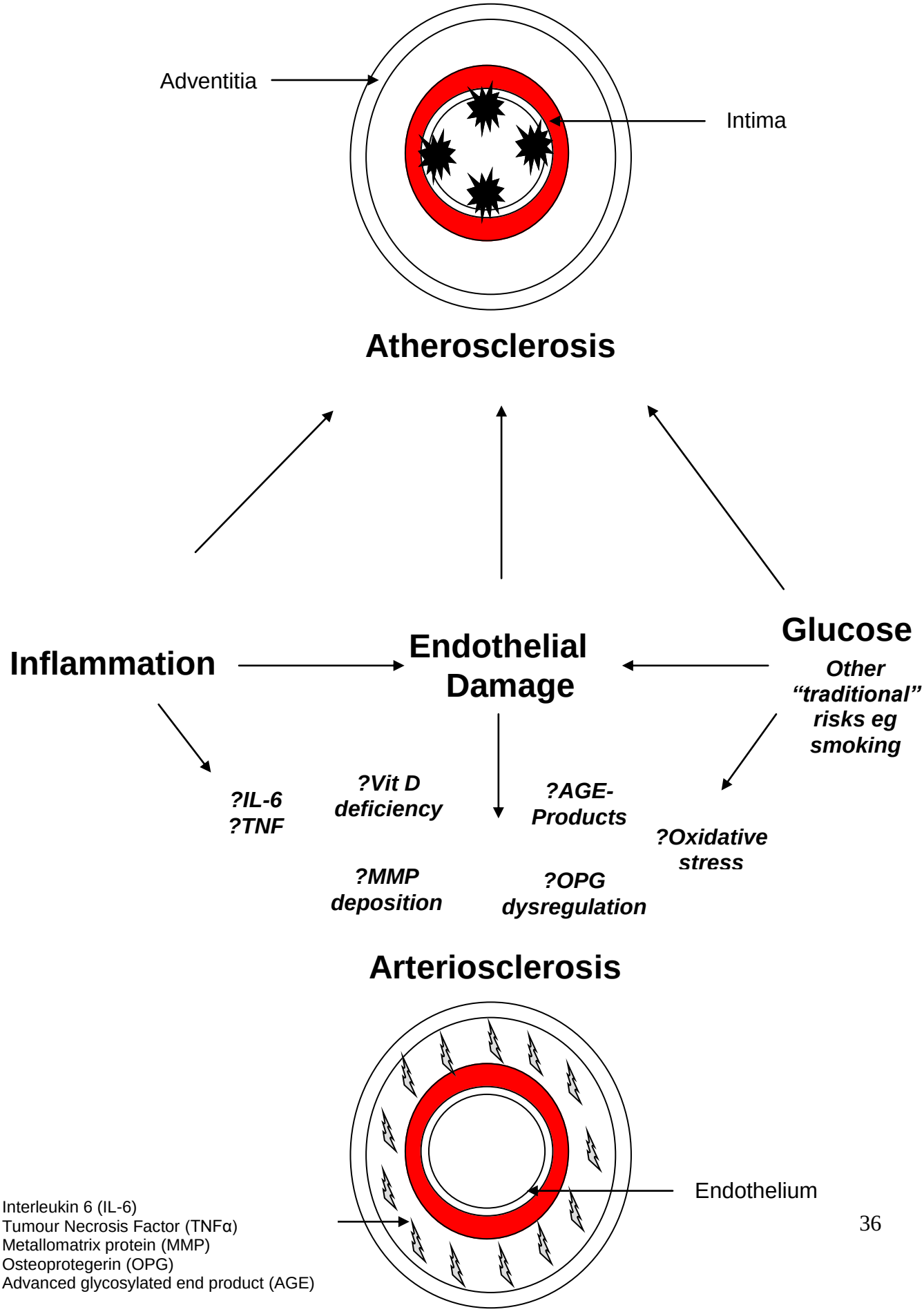
A chronic low grade inflammatory response plays a key role in the development of diabetes related vascular complications. Acute phase proteins such as C-reactive protein (CRP) and fibrinogen are persistently elevated and predict the development of symptomatic cardiovascular disease and type 2 diabetes [114,115]. Adipose tissue derived circulating pro-inflammatory cytokines such as Interleukin-6 (IL-6) and Tumour Necrosis Factor-α (TNF-α) are also significantly elevated, suggesting complex interplay with obesity, a common driver of cardio-metabolic diseases [116, 117].

1.3.2 Arterial stiffness in diabetes

Diabetes related macrovascular disease affects the inner (intimal) artery layer, resulting in thrombo-occlusive atheroma (atherosclerosis) and the elastic (medial) layer resulting in vascular stiffening (arteriosclerosis) [118,119] (Figure 1.4). Although distinct in their clinical presentation both pathologies probably share common antecedents and are major causes of mortality in diabetes. This thesis concentrates on the proposed mechanisms for vascular remodelling resulting in premature arteriosclerosis in diabetes. Diabetes is associated with conduit vessel changes which profoundly affect vascular tone and compliance [120]. Premature sclerosis appears to stiffen the elastic layer of major arteries, contributing to cardiovascular disease by increasing cardiac after-load, thereby reducing myocardial perfusion [121,122].

A number of technologies for determining indirect estimates of vascular wall stiffness (discussed in detail in Section 1.3.4) independently predict cardiovascular mortality within high risk populations including hypertension, type 2 diabetes and advanced renal disease [123-125]. Elevated pulse pressure, the only clinical sign of arterial stiffness, is evident in individuals with type 1 diabetes in early to mid-adulthood, is accentuated by microvascular complications and predicts incident cardiovascular death. Numerous cross-sectional studies associate both type 1 and type 2 diabetes with premature arterial stiffness, largely irrespective of measurement modality [124,130-139]. A number of non-invasive techniques, described in detail in Section 1.3.4, demonstrate increased central artery stiffness when subjects with diabetes are compared with age-gender-matched normoglycaemic controls (Table 1.6).

Figure 1.4 Proposed mechanisms of atherosclerosis and arteriosclerosis in diabetes



Interleukin 6 (IL-6)
Tumour Necrosis Factor (TNFα)
Metallomatrix protein (MMP)
Osteoprotegerin (OPG)
Advanced glycosylated end product (AGE)

The concept of accelerated vascular “ageing” in diabetes is widely accepted, and could explain excess cardiovascular risk unaccounted for by traditional risk factors. Future development of agents specifically targeting this process would represent a novel therapeutic approach to the management of vascular complications and if commenced early enough may prevent the cardiovascular consequences of arterial stiffness. In this respect it is less clear whether fasting and post-challenge glucose concentrations below the diabetes diagnostic thresholds are also associated with degrees of premature central artery stiffness (Table 1.6). This could be an important consideration as strategies aimed at improving outcomes in diabetes increasingly focus upon earlier identification of cardiovascular risk followed by aggressive cardio-protection.

1.3.3 Putative mechanisms of vascular stiffening

Endothelial cell dysfunction (an early manifestation of microvascular dysfunction in diabetes) may mediate vascular stiffening through inflammatory cytokine induced vascular smooth muscle cell apoptosis and medial layer calcification [126]. Apoptosis of endothelial cells as a result of prolonged hyperglycaemia and cytokine action appear to compromise the continuity of the endothelial barrier and expose smooth muscle cells to glucotoxicity and inflammatory stress. It is proposed such exposure may activate an osteoblast-like function within these cells and trigger key pathways involved in hydroxyapatite and calcium deposition [127,128] (Figure 1.4).

Diabetes is also associated with the production of Advanced Glycated End products (AGEs) formed by the non-enzymatic reaction of glucose with amine residues on ubiquitous proteins, lipids or nucleic acids. This product cross-

links collagen, is extremely resistant to degradation and may selectively activate receptors triggering inflammation and growth (Receptors for Advanced Glycation End Products – RAGE). AGE-proteins have been implicated in the pathogenesis of many diabetes related complications and may expedite vascular stiffness by reducing internal elasticity. This may occur by direct infiltration of the vessel wall or activation of pro-inflammatory cascades through RAGE (Figure 1.4).

Advanced renal disease is associated with similar structural changes which predict cardiovascular mortality and are not entirely explained by age or hypertension [123]. Like diabetes, pre-senile medial calcification occurs, which in the case of chronic kidney disease may be accelerated by disordered mineral metabolism. Although the mechanism remains unclear, vitamin D deficiency (25- or 1,25-hydroxyvitamin D), increased calcium-phosphate product, hyperphosphataemia and hyperparathyroidism have all been implicated in medial arterial calcification and cardiovascular disease in end stage renal disease [129]. It is plausible vitamin D status relates to medial calcification and vascular stiffness in other populations susceptible to vitamin D deficiency.

Table 1.6 Recent cross sectional and prospective* studies of arterial stiffness in type 1 and 2 diabetes, IGT and the Metabolic syndrome

Study		Year	Size (n)	Population	Age(Yrs)	Measurement	Result	Comment
Schram M.T.*	[130]	2003	3250	T1DM (Eurodiab)	34	Sphygmomanometer	↑PP assoc. with incident CHD	Microvascular comp. ↑P.P
Ronnback M.	[131]	2004	8474	T1DM	50	Sphygmomanometer	T1DM:↑PP& ↑Systolic BP	
Ahlgren A.R.	[132]	2005	121	T1DM	44	Echo Tracking / distensibility measure	T1DM: ↑AS Unchanged after 7 years	↑Stiffness women only
Brooks B.A.	[133]	2001	173	T2DM	56	Augmentation Index	T2DM: ↑AS	↑Stiffness Men only
Ravikumar R.	[134]	2002	100	T2DM	58	Augmentation Index	T2DM: ↑AS	Indian
Cruickshank K.*	[124]	2002	433	T2DM / IGT	60	Carotid-fem. PWV	T2DM: ↑AS (PWV:12.0 vs 10.1ms^{-1})	Predicts mortality
Kimoto E.	[135]	2003	290	T2DM	60	Ankle-brachial PWV	T2DM: ↑AS	
Rahman S.	[136]	2008	90	T2DM / IGT	56	Carotid-fem. PWV Augmentation Index	T2DM: ↑AS (PWV: 10.4 vs 8.7 ms$^{-1)}$	Newly diagnosed
Henry R.M.	[137]	2003	7447	T2DM / IGT	69	Distensibility measure	T2DM: ↑AS	Not central in IGT
Ohnishi H.	[138]	2003	232	IFG	49	Ankle-brachial PWV	IFG: ↑AS	
Schram M.T.	[139]	2004	619	T2DM / IGT	62	Carotid-fem. PWV Distensibility measure	↑AS T2DM > IGT > NGT	Central +peripheral in T2DM and IGT
V.People N.M.	[140]	2006	2960	IFG	60+	Distensibility measure	↑ AS	Only in >75 group
Safar M.E.	[141]	2006	476	MeS	51	Carotid-fem. PWV	↑ AS	
Ferreira I.	[142]	2007	313	MeS	24	Distensibility measure	↑ AS	↑Stiffness women only
Henry R.M.	[143]	2009	619	MeS	62	Distensibility measure	↑ AS central / peripheral	Not associated with inflammatory markers

T1DM: Type 1 diabetes, **T2DM**: Type 2 diabetes, **MeS**: Metabolic Syndrome, **AS**: Aortic stiffness, **PP**: Pulse Pressure

1.3.4 Estimates of arterial stiffness

Because vascular stiffness is difficult to quantify directly, non-invasive assessment of arterial mechanical properties is confined to a variety of inferred measures recorded from commercially available platforms. Several methods of generating these “stiffness indices” exist, and can be broadly categorised into techniques which apply propagative and non-propagative models to the circulation (Table 1.7). Measurement involves three main methodologies; pulse transit time or pulse wave velocity, analysis of the arterial pressure pulse or its wave contour and direct stiffness estimation using measures of diameter and distending pressure.

1.3.4.1 The propagative model: Pulse Wave Velocity

Regional estimates of arterial stiffness can be obtained by measuring the speed of pulse pressure propagation ie. pulse wave velocity (PWV) along any arterial segment. The elastic and geometric properties of the vessel wall together with the density of fluid contained within it determine the pressure wave velocity according to the *Bramwell-Hill* and *Moens-Korteweg* equations (overleaf). PWV therefore provides indirect functional information relating to the mechanical properties of the elastic arterial wall, its thickness and lumen diameter. Surface measurement of PWV is now recognised as the most simple, robust and reproducible method of estimating central arterial stiffness [144].

Table 1.7 Examples of commercially available devices, techniques and outcome measures used to determine regional, local and generalised arterial stiffness and wave reflections

	Device	Method	Outcome Measure	
Regional	Sphgomocor®	Tonometer	AoPWV,CPP	[145]
	PT4000®	Doppler	AoPWV	[146]
	Complior®	Mechanotransducer	AoPWV,CPP	[147]
	Walltrack®	Echotracking	AoPWV	[148]
Local	Walltrack®	Echotracking	QCS	[149]
	Artlab®	Echotracking	DI / YM	[150]
Generalised	PW CR2000®	Piezoelectric sensor	C1 / C2	[151]
Wave Reflections	Sphgomocor®	Tonometer	AIx	[152]
	Pulse Trace PCA ®	Photoplethysmograhy	SI_{DVP}	[153]

AoPWV: Aortic Pulse Wave Velocity, **CPP**: Central Pulse Pressure, **YM**: Young modulus, **C1/C2**: Compliance factors, **AIx**: Augmentation Index, **SI_{DVP}:** Stiffness Index, **DI**: Distensibility Index, **QCS**: Quality carotid Stiffness

Bramwell-Hill equation:

$PWV = \sqrt{(1/p \times \Delta P/\Delta A)} \times A$

p= density of blood
P= Pressure
A= Area of lumen

Moens-Korteweg equation:

$PWV = \sqrt{(E \times h / 2p \times r)}$

E= Elastic modulus
h= vessel wall thickness
r = internal radius

Transcutaneous pressure, doppler or distension sensors are applied simultaneously over two arterial sites and the time delay between emergent waveforms measured. The distance covered by the waves is assimilated to a surface assessment of the inter-recording site distance and PWV simply calculated as:

$PWV = D / PPT\ (ms^{-1})$

D= distance between sites
PPT = Pulse Pressure Time

Pressure waves can also be recorded sequentially from different sites and PPT calculated using the R-wave of a simultaneously recorded electrocardiogram as a reference time frame. Reflecting recent interest in this area, a range of devices are now available using validated tonometric or sonographic methods of waveform acquisition as novel non-invasive investigative tools (Table 1.7). Two of the most widely used devices incorporate either a single high-fidelity applanation tonometer (SphygmoCor System, ArtCor, Sydney, Australia) or a 5Hz Doppler probe (PT4000, MicroMedical, UK) to sequentially obtain proximal and distal pulses.

1.3.4.2 The non-propagative model: local distensibility measures

Local estimates are most commonly described in terms of compliance and distensibility co-efficients and are obtained through measurement of arterial changes in diameter or area and local distending pressure by means of ultrasound imaging. Absolute (compliance) or relative (distensibility) changes in lumen area for a given increase in pressure, when taken with estimates of wall thickness, allow local estimation of an elastic modulus according to the

Moens-Korteweg equation. This is probably the most robust measurement method but is technically extremely demanding and time consuming.

1.3.4.3 Pulse wave augmentation index

Analysis of the arterial pulse waveform using applanation tonometry is used to measure the augmentation in pulse pressure as a result of pulse wave reflection and amplification. Good quality registration of the arterial pressure waveform is obtained by applanating a peripheral artery using a tonometer. The peripheral pressure waveform is transformed via a mathematical function into a central artery shape. The difference between the systolic peak of the forward propagating wave and augmented wave relative to the pulse pressure represents the augmentation index due to wave reflection. Augmentation index therefore provides indirect information on arterial stiffness but is convenient and operator friendly.

1.3.5 Novel targets for intervention? Trials with stiffness outcomes

Although management of traditional risk factors is a key element of primary and secondary prevention, it is suggested that up to 20% of cardiovascular events are not predicted by classic Framingham based risk scores [154]. In the diabetic state this discrepancy maybe even greater, as particular pro-inflammatory / pro-atherosclerotic aspects of the disease are not factored into existing Framingham models. Interventions that may specifically minimize any increase, or potentially even decrease aortic stiffness may therefore provide novel therapeutic approaches to cardiovascular disease of particular benefit to patients with diabetes.

Standard blood pressure lowering, possibly in addition to novel pleiotropic actions of ACE inhibitors and Angiotensin Receptor Blockers (ARBs) have been shown to reduce central artery stiffness in populations with and without diabetes [155,156] (Table 1.8). This appears to be a class effect as other anti-hypertensives have a less predictable impact upon measures of central blood pressure and stiffness. It is postulated statins (HMG-CoA reductase inhibitors) exhibit anti-atherosclerotic properties independent of lipid lowering, although available evidence for a significant role in ameliorating age related central stiffening is controversial [157]. However, a number of small trials using high doses of Atorvastatin or Simvastatin demonstrate convincing effects on markers of arterial stiffness in populations with diabetes [158,159].

Weight loss and oral hypoglycaemic agents other than Thiazolidinediones appear to have little or no beneficial effect on these vascular markers [160-166] (Table 1.8). This is surprising, as the epidemiological evidence suggests hyperglycaemia is definitely involved in the pathogenesis of premature vascular stiffness. It is possible glucose or inflammatory mediators exert an initially reversible deleterious effect on vascular wall structure which, with prolonged exposure becomes irreversible and permanently sclerotic (the so-called “metabolic memory” theory) [167]. There are no published studies examining the effects of multi-factorial intervention on arteriosclerotic change in earlier stages of type 2 diabetes.

Table 1.8 Comparator trials assessing the effects of various treatments on arterial stiffness in patients with diabetes or Insulin Resistance Syndromes

	Size (n)	Duration (mths)	Study Population	Age (Y)	Outcome measure	Description Intervention (I) vs comparator (C)	Result
ACEI / ARB							
Tropeano A.I. [155]	57	6	T2DM ↑BP	63	Distensibility	Perindopril 8mg vs 4mg	↑carotid distensibility
Manolis A.J. [156]	42	6	T2DM	57	$_{cf}$PWV	Perindopril 4mg vs placebo	↓ PWV (I) 2.3 vs (C)0.3 ms^{-1}
Statins							
Orr J.S. [158]	26	12	BMI >30	54	$_{cf}$PWV	Atorvastatin 80mg vs placebo	↓PWV (I) 1.6 vs (C) 0.4 ms^{-1}
Effrati S. [159]	40	3	MeS	52	AIx	Simvastatin 40mg vs Ezetimibe 10mg	↓ AIx (I) 30.2 vs (C) 21.6
Biguanide							
Meyer C [160]	100	3	PCOS	31	$_{cf}$PWV	Metformin 500mg vs OCP vs placebo	↑PWV with OCP
Agarwal N.[161]	30	3	PCOS	29	AIx + $_{cf}$PWV	Metformin 500mg vs placebo	↓ PWV MF mean 0.76 ms^{-1}
Sulphonylurea							
Stakos D.A. [162]	181	24	Insulin Resistant	43	Distensibility	Glipizide 5mg vs Metformin 500 mg vs placebo	↑ PWV with Glipizide No change with Metformin
Koshiba K. [163]	34	6	T2DM	61	AIx + $_{cf}$PWV	Glibencamide 2mg vs Insulin	No PWV changes
Thiazolidinedione							
Rahman S.[164]	33	12	T2DM	47	$_{cf}$PWV	Rosiglitazone vs placeco	↓ PWV with Rosiglitazone
Yu L. [165]	46	3	T2DM	63	$_{cf}$PWV	Rosiglitazone vs placebo	
Weight Loss							
Schneider R.[166]	24	3	MeS	49	PWA	Orlistat 120mg vs placebo	No change in large artery compliance

PCOS: Polycystic Ovarian Syndrome **MeS**: Metabolic Syndrome **cfPWV**: carotid femoral Pulse Wave Velocity, **AIx**: Augmentation Index, **PWA**: Pulse Wave Analysis, **MF**: Metformin, **OCP**: Oral Contraceptive Pill

The Thiazolidinediones (PPAR-γ agonists) have been the subject of much controversy over recent years, yet of all the glucose lowering therapies these agents have been studied the most in respect to their proposed additional beneficial effects on vascular haemodynamics. A recent meta-analysis of available trial data indicates that Thiazolidinediones ameliorate aortic stiffness and carotid intima media thickness (CIMT) independently of glucose lowering capacity in people with diabetes [168].

1.4 Vitamin D deficiency as a novel cardiovascular risk factor implicated in premature arteriosclerosis

1.4.1 Extra-skeletal biological functions of vitamin D

There is currently much interest in both the recognition and treatment of vitamin D deficiency beyond its established roles in metabolic bone disease. Epidemiological studies implicate vitamin D and calcium homeostasis in a plethora of non-skeletal immune-based chronic diseases [169]. Data from large observational cohorts and trials designed for bone-related outcomes consistently demonstrate inverse relationships between serum 25-hydroxyvitamin D (cholecalciferol) and prevalent diabetes or vascular disease [170-172], whilst supplementation appears to improve incident all cause mortality [173]. Serum levels of 25-hydroxyvitamin D predict future glycaemic status in diabetes and deficiency associates with markers of insulin resistance, coronary calcification, left ventricular mass, endothelial dysfunction, and hypertension [174-178]. Diabetes related micro- and

macrovascular complications may be exacerbated by vitamin D deficiency [179].

Proposed mechanisms accounting for these pleiotropic actions focus upon immuno-modulatory effects, intracellular calcium signalling and the recent finding that the vitamin D receptor is ubiquitous [180]. It is proposed vitamin D plays an important role in homeostatic responses to chronic inflammatory stimuli and the molecule is increasingly implicated in a number of innate immune pathways. Vitamin D mediated effects relevant to arteriosclerosis include osteoprotegerin production, a “protective” factor known to inhibit medial calcification and matrix metalloproteinase-9 (MMP9), a stable zinc endopeptidase implicated in vascular remodelling [181-183] (Figure 1.4) .

1.4.2 Vitamin D and pulse wave velocity

Two cross-sectional studies have independently associated vitamin D with carotid-femoral PWV. London *et al.* [184] demonstrated an independent relationship between 25-hydroxyvitamin D status, aortic stiffness and endothelial dysfunction in patients with advanced renal disease. Andrade *et al.* [185] reported similar results after measuring 1,25-hydroxyvitamin D and augmentation index in 131 patients recruited from cardiac clinics in Canada. Conversely, population studies have so far failed to consistently demonstrate a relationship between 25-hydroxyvitmain D concentration and CIMT [186, 187]. CIMT is a recognised robust surrogate of atherosclerosis and implies alternative structural disease, such as stiffness relating to mainly medial calcification of musculo-elastic conduit arteries may be responsible for the observed inverse relationship between vitamin D concentration and cardiovascular disease. Other studies in populations with diabetes and

profound vitamin D deficiency have shown independent associations between 25-hydroxyvitamin D and CIMT, indicating the there may be a threshold concentration below which deleterious effects are triggered [188,189]. Pilot intervention studies are now emerging suggesting replacement ameliorates vascular stiffening in vitamin D deficient populations without renal failure. A landmark study, using a supplement of just 2000 units daily (approximately twice the nationally recommended adult daily intake) improved carotid femoral PWV significantly within a deplete ethnic minority North American population, suggesting vitamin D replacement may improve vascular stiffness [190].

1.5 British South Asians: a model of cardio-metabolic disease susceptibility

Greater prosperity, internal migration and transition to a non-subsistence based urban economy are regarded as the hallmarks of a “developing nation” [191]. Over the last four decades, profound changes in the quality, quantity and source of food consumed in many developing countries, combined with increasingly sedentary behaviour have led to a dramatic rise in the prevalence of diabetes and other risk factors for cardiovascular disease [2]. In India for example, the reported prevalence of cardiovascular disease has increased four-fold over the last forty years and cardiovascular disease is now the leading cause of death [192-193].

1.5.1 Cardiovascular disease

These trends appear to mimic alarming rates of cardiovascular disease already observed within many Indo-Asians migrating to Europe and North America. South Asians form the largest ethnic minority group in the United Kingdom, comprising approximately four percent of the total population. Despite universal acceptance of increased cardiovascular risk there are surprisingly few robust longitudinal studies within this group and very little intervention data [194].

Available studies indicate that incident cardiovascular disease within this diaspora have consistently failed to decline in parallel with comparable indigenous populations, implying cardiovascular treatments are either less available or less effective within these groups [195-197]. Despite a significant overall decline in mortality from vascular causes, the United Kingdom continues to report higher death rates within south Asian ethnic minority groups [198]. Reasons for this continued and indeed in some populations widening disparity are not well understood and represent a major obstacle to equitable healthcare provision.

Explanations for these observations range from simply a higher frequency of established (and presumably untreated) risk factors to complex theories of genetic susceptibility and environmental mal-adaptation, to social issues including cultural barriers impeding adequate healthcare delivery, poor nutrition and diet [194]. Significant variation in disease between ethnic groups with otherwise comparable environmental exposure implies differential

interaction of risk factors across populations or the presence of additional known and unknown factors contributing to increased risk.

A number of studies commenced before the era of modern cardiovascular prevention suggest conventional risk factors do not entirely explain the propensity for Coronary Heart Disease (CHD) within migrant south Asian populations. It is plausible Indians manifest high CHD risk when, as yet unidentified risk factors contributing to ethnic susceptibility are triggered by environmental exposures exacerbating the effects of conventional risk factors [199]. Current concepts linking sustained inflammation with the pathogenesis of atherosclerosis and insulin resistance are consistent with this hypothesis. The thrifty genotype hypothesis suggests south Asians are genetically predisposed to intra-abdominal fat accumulation and are rendered particularly susceptible to long term pro-inflammatory consequences of central obesity when exposed to the hyper-calorific and nutritionally poor Western environment [200-202].

The following section provides an epidemiological overview of cardiovascular disease and type 2 Diabetes in British south Asians. This term encompasses a number of diverse United Kingdom migrant populations tracing first or second generation ancestry to India, Pakistan, Bangladesh, Sri Lanka, or Nepal. Although referring to south Asians in this way implies a potentially meaningless heterogeneous racial mix, diasporic populations are generally geographically focused and readily trace ancestry to localised areas of the Indo-Asian subcontinent.

1.5.2 Metabolic disease and diabetes

A consistent finding from the published literature is a significantly higher prevalence of certain cardiovascular risk factors, premature CHD and stroke. The prevalence of glucose disorders and type 2 diabetes, in particular, in British south Asians is estimated at 10-20%, some five times higher than the indigenous white European population [203]. Estimates derived from a large primary care dataset suggest that compared with a Caucasian reference group, age and gender-adjusted hazard ratios for type 2 diabetes range from 4.07 for Bangladeshi women to 1.93 for Indian men [204]. Somewhat dated population based estimates of diabetes prevalence within four urban centres demonstrate a four to six fold higher prevalence in south Asians compared with white Europeans [205-207]. Complex modelling of some of these datasets predicts higher prevalence estimates in south Asians to continue and probably increase over the next twenty years [208]. Incidence data for type 2 diabetes is lacking in British south Asians with the only source data on population trends coming from point prevalence estimates from Health Surveys for England in 1999 and 2004. Perhaps unsurprisingly the prevalence of doctor-diagnosed diabetes in 2004 was significantly higher than in 1999 in most populations, including south Asians [203].

In addition to diabetes, a number of cross-sectional studies summarised in Table 1.9 demonstrate a higher prevalence of risk factors for CHD amongst British south Asians compared with both White Europeans and matched indigenous Asian populations. Although prospective data is scarce, the age-adjusted CHD mortality after sixteen years of follow up in the Southall and Brent study was 60% higher in the south Asian group [209,210]. Registry

studies report similar mortality ratios of 46% and 51% for CHD in south Asians compared to the background population [195,196,211]. The 2004 Health Survey for England reported self-reported CHD in British south Asian men over 55 years of age at 24% compared with a national average of 17% [203].

A similar pattern of increased risk characterised predominantly by insulin resistance and IGR is observed within other migrant populations [212]. In the largest global case-control study of risk factors, diabetes, waist-hip-ratio and dyslipidaemia were identified as the major contributors to myocardial infarction in south Asians of less than sixty years of age [72]. Ethnic predisposition to central obesity and its metabolic consequences is clearly important in determining cardiovascular disease risk, but even when combined with other traditional factors (e.g. social-economic deprivation, smoking, blood pressure) fails to entirely account for observed variation in vascular events between groups [199]. Assumptions that cultural incompatibility and medication compliance influence outcomes are largely unsubstantiated, implying unidentified factors contribute to increased risk in south Asians.

Table 1.9 United Kingdom based cross sectional studies comparing cardiovascular risk factors in south Asians and white Europeans

Study	Population studied	Location	Age	Size	Results
Mckeigue [209] (1991)	UK Bangladeshi vs white European	Brent	40-69	3754	SA: ↑↑ T2DM (19 vs 4%) ↑BP, ↑ smoking
Bhatnager [213] (1995)	UK Indian vs Punjabi Indian	Manchester	40-55	364	UK:↑CHD, BP, TC, ↓ Insulin sensitivity
Chambers [214] (1999)	UK Indians vs White Europeans	London	35-60	1025	SA: ↑CRP, ↑BP, ↑T2DM, ↑ obesity
Bhopal [215] (1999)	UK Bangladeshi, Indian, Pakistani, vs White European	Newcastle	25-74	1223	CHD: 6%SA vs 2%WE ↑T2DM Bangladeshi ↑ BP Indians

SA: south Asian, **WE**: white European, **BP**: Blood Pressure, **TC**: Total cholesterol, **CRP**: C-reactive protein, **T2DM**: Type 2 Diabetes Mellitus.

It is plausible cardiovascular disease prevalence within south Asian ethnic groups migrating to northern latitudes relates in part to nutritional deficiencies such as sub-optimal vitamin D status. Vitamin D deficiency is extremely common within this population due to a combination of factors including skin complexion, lack of sunlight exposure, vegetarianism, and adipose tissue vitamin sequestration. It therefore represents an ideal platform for studies exploring potential interplay between vitamin D and vascular structure / function.

1.6 Introduction summary

Diabetes is an increasingly common debilitating disease characterised by specific vascular complications which are often present at diagnosis. Common cardiovascular diseases caused by a combination of arterio- and atherosclerosis are the main cause of premature death. Arteriosclerosis causes age related central artery stiffening, predicts mortality in established diabetes and may be related to a number of novel risk factors.

Implementing existing therapies with proven cardio-protective efficacy earlier in the natural history of the disease may improve vascular outcomes. Screening for diabetes may offer that opportunity but to date there have been no vascular risk intervention studies in newly diagnosed populations identified through screening. This is especially pertinent now that many other concerns over screening have been resolved. It is important to establish whether screening identifies premature arteriosclerosis in glucose intolerant groups and subsequent intensive multi-factorial approaches improve measures of

vascular stiffness and inflammation. It is established that south Asians have high rates of diabetes and cardiovascular atherosclerosis. The mechanisms driving this susceptibility remain unknown and require investigation. Readily treatable nutritional deficiencies may contribute to premature conduit vessel sclerosis and vascular disease in this group.

Chapter 2

Methodology of the ADDITION-Leicester and PACE studies

2.1 Introduction

This chapter describes the sequential phases of the ADDITION (Anglo-Danish-Dutch study of Intensive Treatment In PeOple with screeN detected diabetes in primary care)-Leicester and PACE (Pulse wave velocity (Adipo)Cytokine Ethnicity) sub-study research programmes.

ADDITION-Leicester is one of two United Kingdom centres participating in an international study of cardiovascular risk intervention in screen detected type 2 diabetes. This pan-European collaboration will report five year cardiovascular outcomes in two thousand newly diagnosed diabetes cases with the aim of establishing the role of early detection and intensive intervention through screening [216]. Unlike other centres in the United Kingdom (Cambridge), Denmark (Copenhagen) and Netherlands (Utrecht) the screening protocol of ADDITION-Leicester adopts universal or “non-risk factor” based glucose profiling, resulting in robust characterisation of the entire study population by both fasting and post-challenge glucose indices. This is vital to research within south Asian populations in particular as the prevalence of IGT and post OGTT diabetes-range hyperglycaemia is believed to be much higher in this group.

As well as contributing to this multi-centre study, ADDITION-Leicester is a stand-alone trial (Clinical trial registry number: NCT00318032) evaluating the feasibility of population screening within a United Kingdom multiethnic group, and quantifying the impact of optimised treatments on modelled CHD risk. The study is an National Health Service (Department of Health) support for

sciences sponsored initiative specifically designed to explore health inequalities within a United Kingdom south Asian community.

The initial screening stage of ADDITION-Leicester therefore allow prevalence estimates for undiagnosed glucose disorders and untreated cardiovascular disease risk to be calculated within a unique and racially diverse population. The resultant cohort is well characterised and represents an ideal platform for novel epidemiological inter-racial comparisons in advance of exploratory mechanistic sub-studies. ADDITION-Leicester was the main source of recruitment for additional arterial measurements (carotid-femoral PWV) and serum biomarker analysis as part of the PACE study (Figure 2.1).

Volunteers identified with screen-detected type 2 diabetes enter the intervention phase of ADDITION-Leicester, a randomised controlled trial comparing intensive multi-factorial treatment with routine care in general practice according to national guidelines.

2.2 The ADDITION-Leicester study: Screening phase

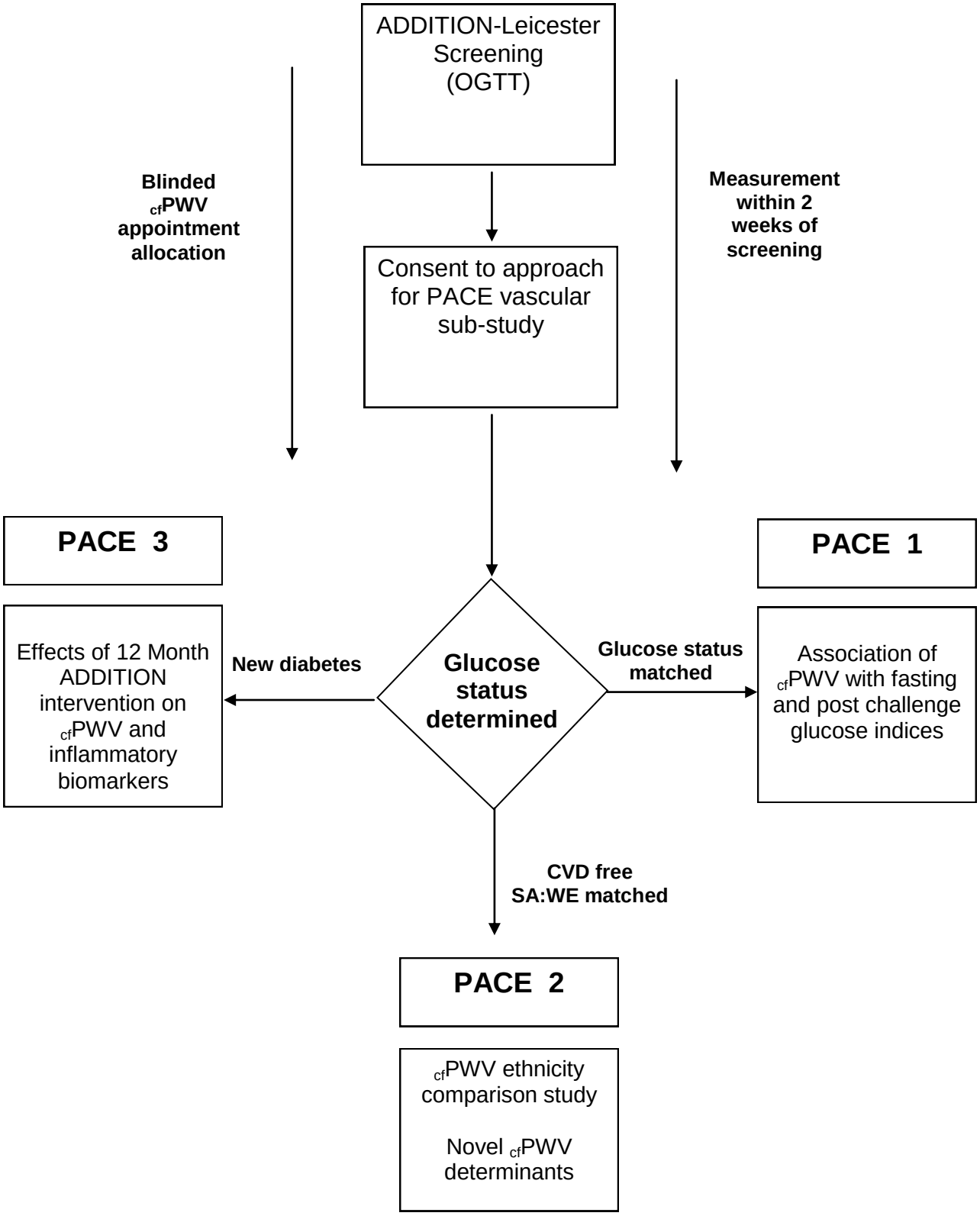
Leicester is one of three relatively deprived urban centres in the East Midlands region of the United Kingdom. The city has a population of 270,000 and is a truly plural conurbation with an estimated 65,000 (>75%) people of mostly northern Indian (Gujarati) descent. Leicester is predicted to become the first majority non-Caucasian European city by 2015 and is increasingly focused upon improving social and healthcare inequalities typical of deprived ethnic minority populations.

The ADDITION-Leicester study adopts a community based non-selective screening approach within a representative cluster of general practices. The study is coordinated from a regional academic centre hosted by the University of Leicester and University Hospitals of Leicester NHS Trust but delivered in primary care through an established diabetes research network. Local research ethics committee and Primary Care Trust approval was obtained for the study with indemnity guaranteed at trust level.

2.2.1 Identification of an eligible population

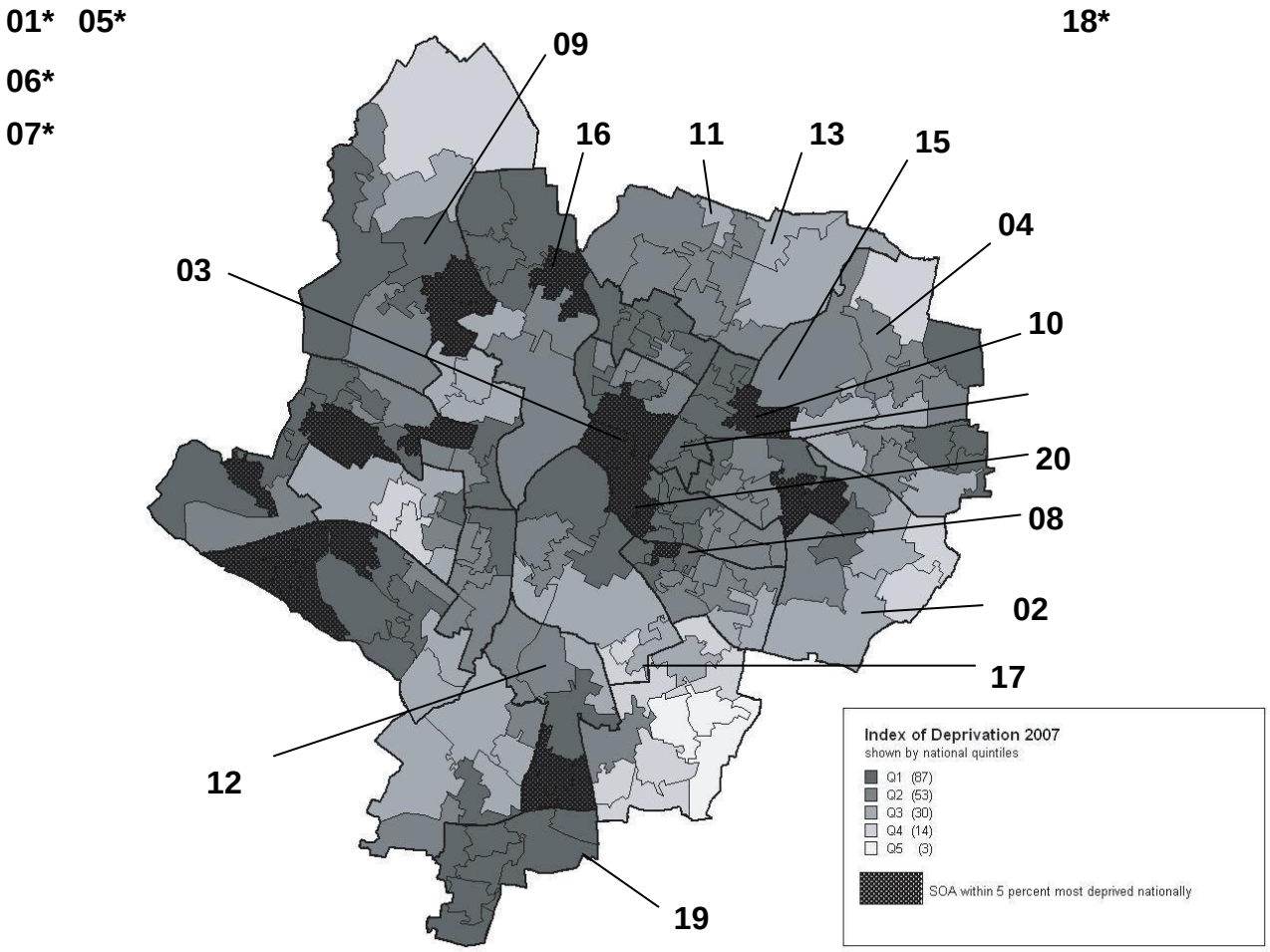
Clinical leads from forty-six general practices forming the Leicestershire and Rutland Strategic Health Authority were approached to participate in ADDITION-Leicester. Twenty-eight practices consented to an initial database search using an extraction programme compatible with the widely used clinical EMIS (Egton Medical Information Systems Ltd, York, UK) system. This specialised software generates an anonymised master practice list that matches individuals to a random unique identifier (a six-digit and single letter ADDITION-Leicester number). A list representative of the practice population was considered essential to further participation in the study and eight practices were excluded at this stage due to data extraction failure or search software incompatibility. The master practice list captures practice population demographics (age, gender, postal address, occupation, medical history, active prescriptions) and known type 2 diabetes frequency, enabling future comparison of responder / non-responder characteristics.

Figure 2.1 Pulse Wave Velocity, (**A**dipo) **C**ytokine **E**thicity PACE sub study recruitment flow



Applying the study criteria to the master practice list produces an eligible population which is reunited with the practice dataset to provide personal details necessary to post an invitation for screening (first mailer). Those meeting the inclusion criteria were sent details of the study along with a returnable request for culturally appropriate information written in five major south Asian languages (Hindi, Gujarati, Bengali, Urdu and Punjabi). Having expressed an interest in the study, potential participants were sent individual screening appointments at either a hospital site or community-based mobile screening unit located. Non-responders were sent a second invitation (second mailer) within six months. To ensure confidentiality was maintained practice staff handled initial database searches and mailing tasks. South Asian ethnicity was defined at this stage by forename and surname mapping using specialised software developed from census data (Nam Pehchan) [217]. Practice deprivation scores were calculated as the mean master practice list deprivation score using the Index of Medical Deprivation (IMD) 2004 [218]. The size, geographical location and deprivation status of the twenty practices participating in ADDITION-Leicester is shown in Figure 2.2. Our calculated mean practice IMD scores match national survey deprivation quintiles and depicted practices within the Leicester City boundary are typical of an urban United Kingdom deprivation distribution [219]. To ensure all twenty sites were covered within the study timeframe, in six practices the entire eligible population was sent information regarding the study whilst a random sample of the population were included in the remaining practices.

Figure 2.2 Leicester city boundary indicating district deprivation quintiles and location of participating practices in the ADDITION-Leicester study



Practice No.	List size (n)	IMD (sd)	T2DM (%)	R / I
01	1857	17.3 (7.3)	7.8	R
02	9527	26.3 (12.1)	7.4	R
03	4444	48.3 (22.3)	4.6	I
04	8651	22.9 (13.5)	4.5	I
05	5018	14.5 (5.3)	4.2	R
06	4110	11.3 (7.8)	2.9	R
07	9000	16.7 (8.1)	3.0	R
08	7276	29.9 (14.0)	8.3	R
09	3935	16.1 (6.6)	4.4	I
10	3105	30.3 (13.6)	2.9	R
11	4689	18.7 (9.8)	4.9	I
12	2870	30.8 (11.8)	5.2	I
13	4200	20.6 (10.0)	3.8	R
14	4111	29.5(15.8)	6.7	I
15	13,444	26.0 (13.9)	4.7	I
16	3673	28.4 (11.6)	7.5	R
17	3155	12.2 (7.6)	4.0	R
18	34,375	10.1 (6.4)	3.5	R
19	4221	11.5 (7.7)	4.6	I
20	17,650	28.9 (10.7)	8.2	I

IMD: Index of multiple deprivation mean (standard deviation) for practice

T2DM (%): Prevalence of diagnosed type 2 diabetes mellitus

R / I : randomisation allocation:
R: Routine care arm I: Intensive care arm

*** Practices 01, 05, 06, 07, 18 situated outside city boundary (Coalville, Loughborough and Melton Mowbray)**

2.2.2 Inclusion and Exclusion criteria

Inclusion and exclusion criteria for the study were similar to the multicentre ADDITION-Europe study [216]. White Europeans between the ages of 40-75 years and south Asians, Afro-Caribbean's and other races between the ages of 25-75 years were included (Table 2.1). A lower age cut-off was chosen due to the reported higher risk of type 2 diabetes at a younger age within these groups.

2.2.3 Screening visit measurements

Individuals were asked to fast for eight hours prior to attending a screening appointment and to bring a list of prescribed medications with them. At baseline (V0) and annual pre-diabetes cohort screening visits a standard OGTT was undertaken following informed consent. This test was postponed if in the preceding three days instructions to follow a normal unrestricted diet were not followed or participants reported fever or unusual physical activity. All OGTTs were performed according to WHO standardised methodology (Appendix A). Plasma samples were obtained immediately before (Fasting Plasma Glucose FPG) and two hours post-challenge (2-HPG), along with fasting samples for serum urea and electrolytes, liver function tests (LFT), lipids (total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides), $HbA_{1c\%}$, and renal function (creatinine and MDRD eGFR) (Table 2.2). A spot urine sample for urinalysis and albumin excretion rate was also collected. All samples were analysed within three hours of collection.

Table 2.1 Inclusion and exclusion criteria for ADDITION-Leicester

Inclusion Criteria

1. White European subjects aged between 40-75 years
2. Asian, black or Chinese subjects aged between 25-75 years

Exclusion Criteria

1. Housebound
2. Known to have a terminal Illness
3. Known to have Diabetes Mellitus
4. Known active psychotic illness which deems the person unable to give informed consent
5. Pregnant or lactating
6. Patients taking part in any other clinical trials

Anthropometric measurements were performed by trained staff following standard operating procedures, with height being measured to the nearest 0.1 cm using a rigid stadiometer and weight in light indoor clothing measured to the nearest 0.1 Kg with a Seca scale (Tanita, Europe). Body fat percentage was measured via calibrated bioimpedence (Tanita Europe). Body mass index (kgm^{-2}) was defined as weight in kilograms divided by height in metres squared. Waist circumference was measured at the mid-point between the lower costal margin and the level of the anterior superior iliac crest to the nearest 0.1 cm.

Blood pressure was measured three times using an Omron M4 blood pressure machine (Omron Healthcare, Milton Keynes, United Kingdom) with the participant in a seated position. An average of the second and third readings was documented in line with British Hypertension Society Guidelines [61] with written instructions for abnormal readings. A twelve lead Electrocardiogram (ECG) was performed using a Nihon Kohden CardioFax Gem machine (Nihon Kohden Europe GmbH, Rosbach vor der Höhe, Germany). An in-house physician interpreted ECGs on the day of the visit, coded for ischaemia and left ventricular hypertrophy and reported back to the general practitioner [220].

Self-completed questionnaires were used to assess baseline smoking status, alcohol consumption, occupation, and ethnicity. Validated questionnaires measuring physical activity, diabetes risk, treatment satisfaction (DTSQ) and psychological domains of well being and anxiety were also included (Table 3.2) [97,98,221-225]. All measurements were performed by a dedicated team of nurses trained to document relevant medical information and family history on a standardised case report form (CRF) during a 1:1 interview. The clinical team were unaware of participants study group allocation or glucose diagnosis.

Table 2.2 Summary of assessments performed at Baseline (v0), annual Pre-diabetes and Randomised Controlled Trial (RCT) visits (v1-5) of the ADDITION-Leicester study

Visit	**Baseline Screening (v0)**	**Pre-diabetes annual**	**T2DM RCT *Year 1 (v1)* *intensive /routine***	**T2DM RCT *Year 2 (v2)* intensive only**	**T2DM RCT *Year 3 (v3)* Intensive*/routine**	**T2DM RCT *Year 4(v4)* intensive only**	**T2DM RCT *Year 5 (v5)* *intensive /routine***
Medical Procedures:							
Blood Pressure	✓	✓	✓	✓	✓	✓	✓
Electrocardiogram (ECG)	✓	✓	✓	✓	✓*	✓	✓
Foot Check	-	-	✓	✓	✓*	✓	✓
Biochemical measurements:							
75g-OGTT: Fasting & 120 min glucose	✓	✓	-	-	-	-	-
UE, LFT, Lipid profile, $HbA_{1c\%}$	✓	✓	✓	✓	✓	✓	✓
Renal function (eGFR) & urine ACR	✓	✓	✓	✓	✓	✓	✓
TFT	-	-	✓	✓	✓*	✓	✓
Anthropometric measurements:							
Height / Weight / BMI	✓	✓	✓	✓	✓	✓	✓
Hip circumference	✓	✓	✓	✓	✓	✓	✓
Waist circumference	✓	✓	✓	✓	✓*	✓	✓
Bioimpedence (%body fat)	✓	✓	✓	✓	✓*	✓	✓
Screening Questionnaires:							
Medical / family history	✓	✓	✓	✓	✓*	✓	✓
Self-reported Questionnaires:							
FINDRISC, Cambridge risk scores [97,98]	✓	✓	✓	✓	✓*	✓	✓
EuroQol, EQ-5D [225],	✓	✓	✓	✓	✓*	✓	✓
WHO-5, BFI 44, SF36 & 12 [222,224]	✓	✓	✓	✓	✓*	✓	✓
IPAQ [221]	✓	✓	✓	✓	✓*	✓	✓
DTSQ [223]	✓	-	✓	✓	✓*	✓	✓
Arterial measurements sub study:							
$_{cf}$PWV	✓	✓	✓	-	-	-	✓
Biobank storage aliquots:							
8 x 2ml Plasma(4), serum (4),	✓	✓	✓	✓	✓*	✓	✓
Genetic Sample:							
Whole blood (EDTA)	✓	-	-	-	-	-	-

At major visits (V0-V4, and pre-diabetes assessments) further venesection was performed for future biomarker research (Table 2.2). Consent was obtained for the -80^{o}C storage of multiple anonymised serum, plasma and whole blood aliquots. These samples contribute to a biobank facilitating translational research exploring the pathogenesis of insulin resistance, vascular complications and genetics of type 2 diabetes.

An option for further physiological measurements was incorporated as a sub-study amendment at visits V0, V1 and V4 (PACE study). Volunteers consented to return on a separate occasion for non-invasive arterial assessments and blood tests. Trans-cutaneous ultrasonic Pulse Wave Velocity ($_{cf}$PWV) was performed under controlled conditions according to a strict protocol within two weeks of their screening appointment.

2.2.4 Diagnosis and reporting

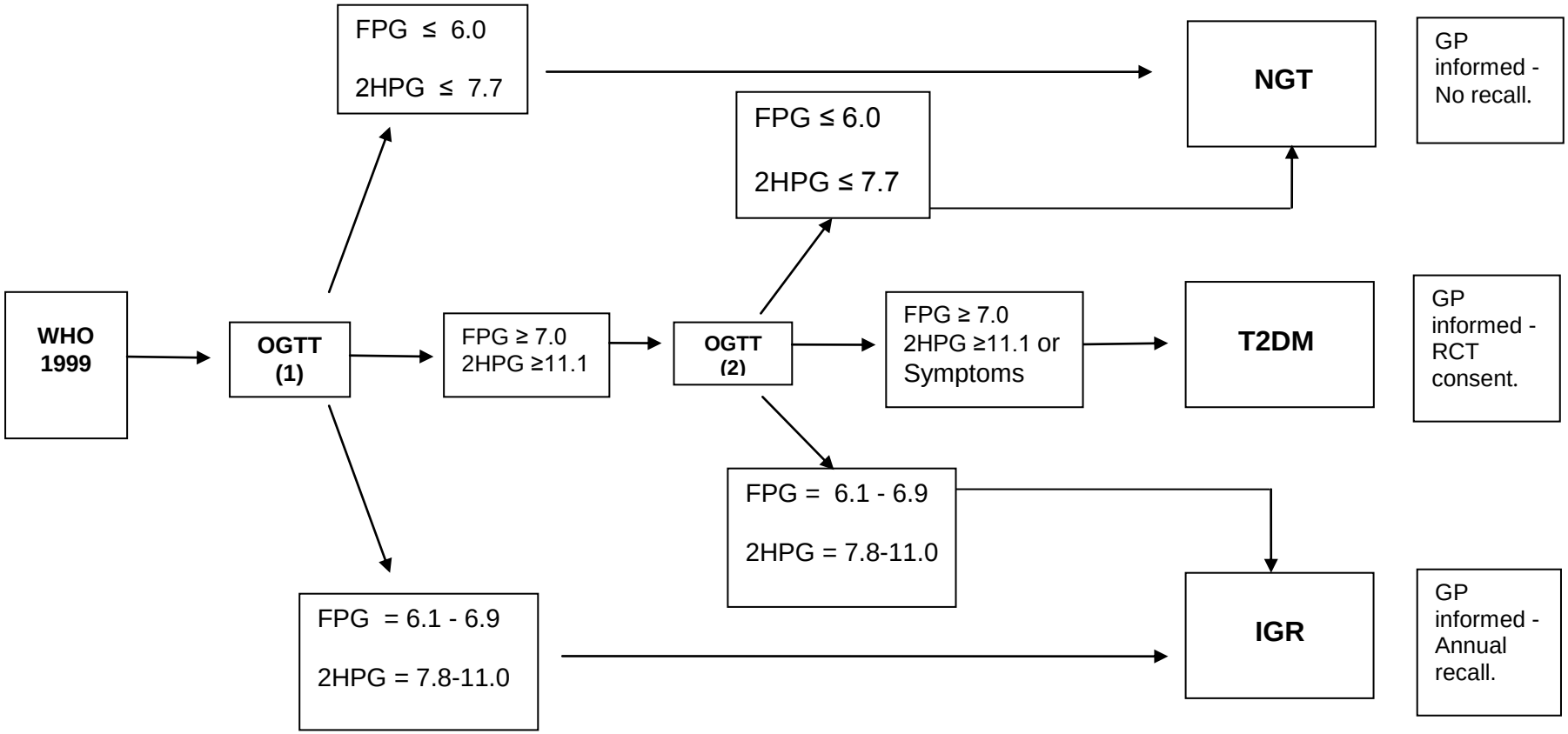
Results were relayed via written correspondence and copied to participant and general practitioner. All biochemical measurements were performed in house at the University Hospitals of Leicester NHS Trust. $HbA_{1c\%}$ was analysed by a DCCT aligned Biorad Variant II system (Bio-Rad Laboratories, Hemel Hempstead, UK). Participants were categorised according to WHO criteria [21,226]. Impaired Glucose Regulation (IGR) was defined as any combination of IFG and or IGT. The diagnosis of diabetes was confirmed by an in-house physician on the basis of two abnormal glucose results obtained on separate visits, unless hyperosmolar symptoms suggestive of hyperglycaemia were reported at the screening visit. Asymptomatic individuals with a diabetes range OGTT were re-called for a second test (rescreen) within

two weeks. In the event of discordant OGTT results (e.g. baseline diabetes followed by rescreen IGR) participants were categorised as having IGR (Figure 1.3 and Section 1.1.2.2). Volunteers diagnosed with diabetes were entered into a cluster randomised controlled trial of multi-factorial cardiovascular risk intervention whilst those identified with IGR were given lifestyle advice and invited to join the ADDITION-Leicester pre-diabetes Cohort Study (Section 2.2.5).

2.2.5 ADDITION-Leicester pre-diabetes cohort study

Volunteers found to be within IGR fasting or post-challenge glucose categories at baseline were provided with written life-style advice and invited for annual rescreen. The ADDITION-Leicester pre-diabetes cohort study annual screening protocol is identical to the baseline visit (Table 2.2), all results are relayed to participant and general practitioner and those with diabetes range results are recalled for a second OGTT. The process for continued follow-up differs however, as newly diagnosed type 2 diabetes is considered an endpoint and is returned to the care of the primary care specialist rather than entering the trial phase. Continued pre-diabetes or normal glucose tolerance range results are invited for further annual assessments.

Figure 2.3 ADDITION-Leicester algorithm for the diagnosis of screen-detected IGR (pre diabetes) and type 2 diabetes



Fpg: fasting plasma glucose pre OGTT (mmol/l)
2hpg: 2-hour plasma glucose post OGTT (mmol/l)
NGT: Normal Glucose Tolerance
IGR: Impaired Glucose Regulation (pre-diabetes) (composite of IFG and/or IGT glucose ranges)
T2DM: Type 2 diabetes mellitus

2.3 The ADDITION-Leicester study treatment phase

2.3.1 Randomisation

Randomisation was performed by an independent steering committee provided with practice demographics, deprivation status and approximate type 2 diabetes prevalence within individual practices. Practices and not individuals were randomised by minimisation with a ratio of 1:1 control to active treatment.

2.3.2 Treatment arms and visits

The screen detected diabetes control group (routine care arm) receive usual care within the primary care setting, according to national recommendations for management of type 2 diabetes and cardiovascular disease. These participants are reviewed one and five years post diagnosis, when anthropometric, biochemical and questionnaire data are collected. The screen detected intervention group (intensive care arm) are introduced to dedicated, specialist physicians and nurses who provide a structured, intensified, protocol-driven, multi-factorial approach again within the primary care setting (Table 2.3). On-going professional support is provided in the first year through an individualised peripatetic clinic offering two monthly visits from a diabetes specialist nurse or physician. Ultimately, participants are encouraged to self-manage their diabetes by identifying personalised goals which facilitate individualised behaviour and life-style change. At annual visits (V1-V4 Table 2.2) interim outcome measures and additional biomedical assessments are performed, including urinary Albumin Creatinine Ratio, an ECG and thyroid function tests.

Table 2.3 ADDITION-Leicester intensive arm algorithm for the management of hyperglycaemia, hypertension and dyslipidaemia

	Basic Treatment TARGET	if above TARGET add	if above TARGET+BMI>19 or creatinine>130umol/L	Supplementary treatment If still above TARGET	
Blood Glucose	***HbA1c <6.5%*** SMBG Tuition DESMOND attendance Dietary Advice	***HbA1c >6.5%*** SMBG Tuition DESMOND attendance Biguanides	***HbA1c >6.5%*** SMBG Tuition DESMOND attendance Insulin basal/bolus	***HbA1c >6.5%*** Biguanides Sulphonylureas Thiazolidinediones THEN Stop Thiazolidinedione Add basal Insulin (bedtime)	***HbA1c >6.5% and on Biguanides Sulphonylureas Insulin*** (basal/bolus) Intensify & titrate insulin
Blood Pressure	***BP < 130/80mmHg*** No treatment	***BP >130/80mmHg*** ACE inhibitor / ARB	***BP >135/80mmHg*** Calcium Channel Blocker Thiazide Diuretic	***BP >130/80*** ACE / ARB/ Thiazide Diuretic Calcium channel /alpha/beta Blocker	
Cholesterol	***<3.5mmol/L*** Diet	***>3.5mmol/L*** ***TG >6.0mmol/L*** Diet Statin Consider Ezetimibe/Fibrate			
Acetylsalicylic Acid	75mg to all patients, unless contraindications of gastrointestinal bleeding, ulcers or haemophilia. If Aspirin contraindicated, consider Clopidogrel.				
Statin Therapy	Simvastatin 40mg or Atorvastatin 20mg				

This visit includes a standardised foot examination incorporating vascular doppler assessment, ankle-brachial pressure indices and monofilament neuropathy testing, and a digital retinal examination independently verified by operators blinded to the participants study group.

2.3.3 Intensive intervention

Care for the intensive arm is based upon a paradigm of multi-factorial intervention shown to improve mortality in type 2 diabetes [66]. Structured education (Diabetes Education and Self-Management for Ongoing and Newly Diagnosed diabetes – DESMOND [104]) is initially offered to all patients in the intensive arm with attendance ideally, within the first two months of diagnosis. Sessions are delivered by two trained educators and aim to facilitate life-style changes in relation to dietary habits, physical activity levels, smoking cessation and glucose monitoring. Those participants who are unable, or decline the opportunity to attend the structured education programme are offered one-to-one advice with a dietitician. All volunteers are offered a glucometer, and encouraged to maintain a reflective diary.

Patients without specific contra-indications are advised to take Aspirin 75mg orally and prescribed lipid lowering therapy (Simvastatin 40 mg daily) if total cholesterol concentration exceeds 3.5mmoll^{-1}. An individualised, stepwise approach to management according to specified algorithms is adopted to ensure optimisation of hypertension, dyslipidaemia and hyperglycaemia according to protocol-driven targets using medication within existing licensed indications (Table 2.3). Recommended drug choices take into account treatment efficacy, side-effects and cost, the main priority being achievement

of treatment targets whilst maintaining flexibility and low rates of adverse events. The approach is deliberately pragmatic with the final decision on choice of medication determined by the health care professional and patient. Treatment targets for the intensive care arm are; $HbA_{1c\%}$ <7.0% (with initiation of treatment at 6.5%), blood pressure <135/85 mmHg, and total cholesterol <3.5 $mmoll^{-1}$ [66]. After the first year, community visits are extended to every four months but continue to be guided by protocol driven blood pressure, $HbA_{1c\%}$, and lipid targets.

2.4 The PACE sub-study

The main aim of the PACE study was to define PWV as a measure of arterial stiffness within a representative sample of the ADDITION-Leicester programme. Consent was obtained for further vascular-related measurements during ADDITION-Leicester screening and arrangements made for a single-session PWV assessment once glucose status was confirmed (Figure 2.1). PACE measurements were performed within two weeks of ADDITION-Leicester screening and operators were blinded to glucose results where possible. Independent administrators selected consenting volunteers to ensure PACE was able to answer three specific research questions. Firstly, is there evidence of premature arterial stiffness in screen-detected diabetes and IGR categories and how do fasting and post-challenge glucose indices associate with PWV measures? (PACE 1) Secondly, does south Asian ethnicity confer additional risk of vascular disease through accelerated arteriosclerosis and could this effect be associated with underlying vitamin D status? (PACE 2) Thirdly, does multi-factorial cardiovascular risk management

in screen-detected type 2 diabetes reduce PWV and inflammatory bio-markers at 12 months? (PACE 3)

2.4.1 Carotid-femoral Pulse Wave Velocity Measurement

2.4.1.1 Principles of non-invasive pulse wave velocity estimation

In the PACE study a 4MHz Doppler probe (Micro medical PT4000) is placed transcutaneously over carotid and then femoral pulsations. Differences between the frequency of transducer sound waves and those reflected from travelling objects (eg. red blood cells) form the basis of this recognised method of estimating fluid velocity. The time taken for an arterial pulsation to travel between chosen measurement sites (eg.carotid–femoral) can be calculated by either simultaneously placing probes over the areas in question (eg. Syphocor Complior) or sequentially recording waveforms gated to an electrocardiographic reference point (Micro Medical PT4000) (Figure 2.4). Assuming the distance between the two sites can be accurately obtained, carotid-femoral pulse wave velocity ($_{cf}$PWV) can be readily derived from the simple equation distance (meters) / time (seconds) (Section 1.3.4).

2.4.1.2 Pulse wave velocity ($_{cf}$PWV) preparation protocol

A single operator (DW) performed all arterial measurements within a temperature controlled research facility at the University teaching hospital. A standard protocol ensured participants were adequately prepared prior to PWV measurement (Appendix B). An overnight fast was required and volunteers were asked to refrain from smoking, consuming caffeine containing drinks or engaging in vigorous physical activity on the morning of the test. Resting supine brachial artery blood pressure and heart rate were recorded

using a semi-automated oscillometric device (Omron MS-I). After 10 minutes rest these were re-assessed every 5 minutes. PWV measurements were taken when haemodynamic stability was achieved (defined as two readings within Systolic (+/-9 mmHG), Diastolic(+/-6 mmHG), and HR(+/-8 beatsmin^{-1}). Blood Pressure and heart rate concordance was ensured prior to subsequent $_{cf}$PWV measurements. Subjects were supine whilst $_{cf}$PWV was measured using a 4MHz continuous-wave Doppler ultrasound probe (Micro Medical Pulse Trace PT4000). The cutaneous distance between the site of the femoral pulsation and the sternal notch was repeatedly measured and entered into the device according to manufactures guidelines. The origin of the pulse waveform was gated to the R-wave of an attached 3 lead electrocardiogram as a timing reference.

2.4.1.3 $_{cf}$PWV measurement

The arterial pulse waveform was recorded sequentially over the left carotid and femoral arteries (Figures 2.4 and 2.5). Once a stable waveform was obtained (defined by internal software as <5% standard deviation of calculated transit time), continuous waveforms were captured and recorded at the femoral site, previously marked during carotid–femoral surface measurement. The device calculated the time delay by sensing the foot of the waves (Figure 2.4). At least three continuous waveforms were recorded and a mean $_{cf}$PWV calculated. All measurements were recorded in a $_{cf}$PWV patient record form.

2.4.2 Quantitative analysis of serum biomarkers

In order to assess the anti-inflammatory effects of multi-factorial intervention in PACE 3, consent was obtained for venesection to measure the following battery of serum biomarkers; high sensitivity C-reactive protein (CRP), adiponectin, Tumour Necrosis Factor-alpha (TNF-α), Interleukin-6 (IL6) and 25-hydroxyvitamin D. All biomarker measurements were performed on fasting samples after spinning and storage at -80^{O}C. Biomarker analyses took place at the Unilever Discover Laboratory, Sharnbrook, Bedfordshire)

2.4.2.1 C-reactive Protein (hs-CRP)

Quantitative analysis of baseline and twelve month serum CRP concentration was performed simultaneously on the ABX Pentra clinical chemistry analyser using a latex-enhanced immunoturbidimetric assay. The Horibda ABX Pentra CRP assay has a minimum detection dose of 0.1mgl^{-1}, with intra-, inter- assay precision of 0.92–4.15 and 2.32– 2.92 mgl^{-1} respectively. Accepted coefficient of variance was <10%.

2.4.2.2 Adiponectin

Quantitative analysis of baseline and twelve month serum adiponectin concentration was performed simultaneously on the PerkinElmer AutoDELFIA 1235 automatic immunoassay system using a time resolved fluoroscent immuno assay. The R&D systems human adiponectin MAB assay has a minimum detection dose of 0.056ngmll^{-1}, with intra- inter- assay precision of 2.6–4.0 and 4.0–7.4 ngl^{-1} respectively. Accepted coefficient of variance was <10%.

Figure 2.4 Calculating the transit time of the arterial pulse wave using an electrocardiogram with the Micro medical PT4000

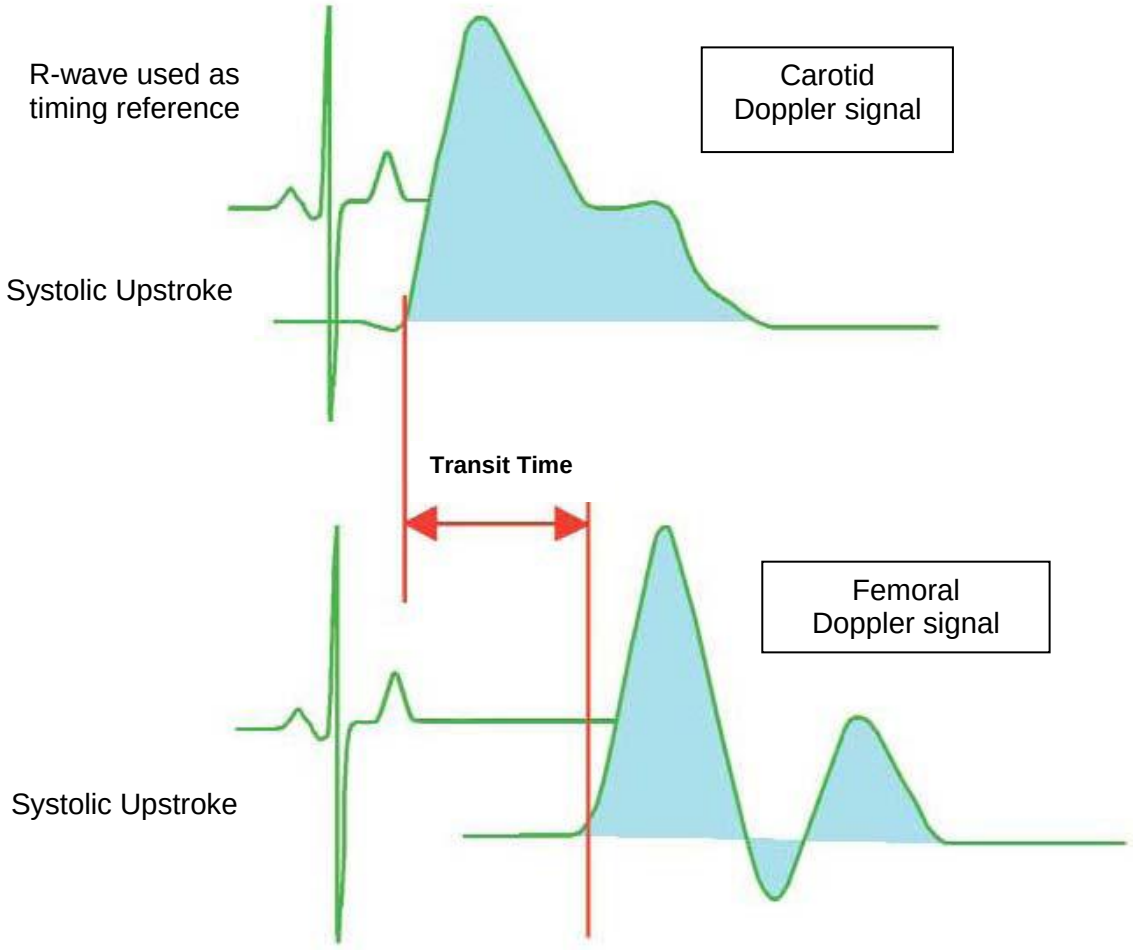
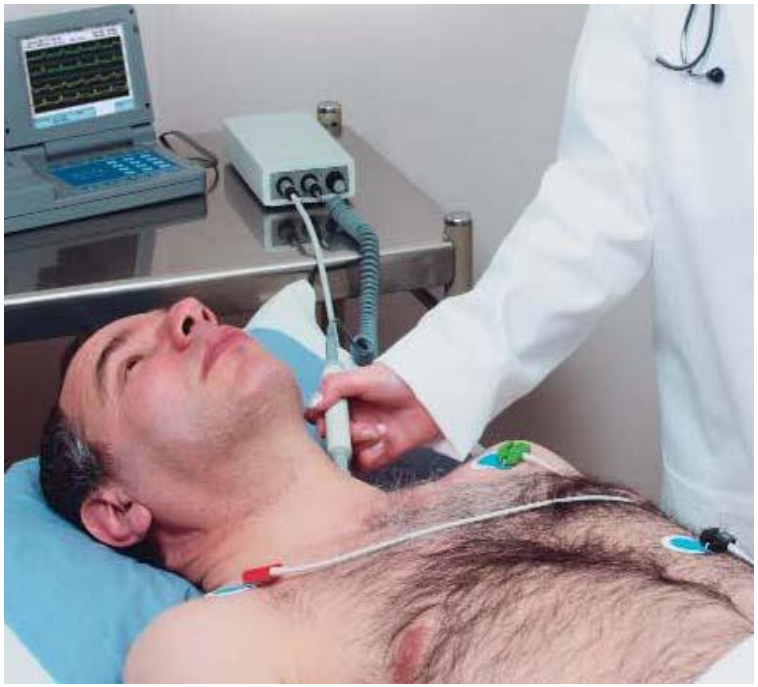


Figure 2.5 Measurement of the carotid pulse waveform using Doppler technology and the Micro medical PT4000 device (Reproduced with permission)



2.4.2.3 Tumour Necrosis Factor-alpha (TNF-α) and Interleukin-6 (IL-6)

Quantitative analyses of baseline and twelve month serum TNF-α and IL-6 concentrations were performed on the Perkin Elmer Viktor 1420 multi-label counter system using a Quantikine high sensitivity ELISA. The R&D systems human TNF-α/TNFSF1A assay has a minimum detection dose of 0.106 $pgmll^{-1}$, with intra- inter-assay precision of 3.1–8.5 and 7.4-10.6 pgl^{-1} respectively. The R&D systems human IL-6 assay has a minimum detection dose of 0.039 $pgmll^{-1}$, with intra- inter- assay precision of 6.9–7.8 and 6.5-9.6 pgl^{-1} respectively.

2.4.2.4 25-Hydroxyvitamin D

25-Hydroxyvitamin D measurements were performed by a competitive EIA (Enzyme immunoassay), range 7.3 – 384 mMl^{-1} (mid range intra- and inter assay co-efficient of variation (CV) 6% (IDS, Boldon Business Park Tyne and Wear, United Kingdom).

Chapter 3

Screening results of the ADDITION-Leicester study

Comparison of undiagnosed glucose disorders and cardiovascular risk in south Asians and white Europeans attending a population based screening programme for diabetes

3.1 Summary

Aims: To determine the frequency of undiagnosed glucose abnormalities and burden of unidentified cardiovascular disease risk among south Asians and white Europeans attending a systematic screening programme for type 2 diabetes (ADDITION-Leicester).

Methods: Random samples of individuals (n=66,320, 30% south Asian) from 20 general practices were invited for an OGTT and cardiovascular risk assessment. Age-adjusted comparisons and ten year risk among screen detected people with diabetes or IGR was computed using the Framingham ETHRISK engine.

Results: 6,041 subjects (48% Male, 22% south Asian) aged 40-75 years inclusive were included. Undiagnosed glucose disorders occurred more frequently in south Asians than white Europeans, age-gender-adjusted odds ratios were 1.74 (95% CI: 1.42 - 2.13) and 2.30 (95% CI: 1.68 - 3.16) for IGT and diabetes respectively. Prevalence of any undetected glucose disorder was 17.5%. Adjusted ten year cardiovascular disease risk was similar in screen detected IGR and diabetes (18.3% vs. 21.6%), and was higher in south Asians across the glucose spectrum. Absolute cardiovascular disease risk reductions of up to 13% in those with screen detected type 2 diabetes and 6% in IGR are achievable using existing cardio-protective therapies.

Conclusion: Population screening with an OGTT identifies a significant burden of cardiovascular disease risk and undiagnosed glucose disorders especially within south Asian groups. Cardiovascular risk is similar in screened detected IGR and diabetes categories. Strategies enticing ethnic minorities to consider screening programmes are urgently needed as significant risk reduction may be possible once a glucose abnormality is identified.

3.2 Introduction

Screening for type 2 diabetes mellitus identifies people earlier in the disease trajectory who may have more to gain from aggressive cardiovascular risk management [227]. As intensive glucose lowering appears not to rapidly improve cardiovascular mortality in individuals with established disease [62-64], it is likely that any major benefits of screening will stem from the management of multiple risk factors, in addition to hyperglycaemia [66]. Modelling studies suggest that targeted screening for type 2 diabetes is cost-effective when the likely vascular benefits of optimised blood pressure and lipid control are also considered [79].

Thus, earlier identification of other risk factors for cardiovascular disease in addition to diabetes, followed by intensive multi-factorial intervention may be required to more rapidly improve outcomes. The United Kingdom Department of Health vascular check programme, aimed at initially screening all 40-70 year olds for hypertension (>140/90 mmHg) and hypercholesterolaemia (Total Cholesterol >5.0mmoll^{-1}, LDL-cholesterol >3.0mmoll^{-1}) in advance of glucose testing reinforces a model of care centered on screening for vascular risk rather than diabetes alone [74].

The extent of achievable cardiovascular risk reduction depends not only upon the efficacy of available treatments but also the existing level of background risk. Screening policy in the United Kingdom currently recommends targeting populations known to be at high risk of diabetes, with the expectation that this will also identify individuals at the greatest risk of cardiovascular disease [74,228]. Ethnic minority south Asian groups may benefit the most from such approaches, especially if latent yet modifiable cardiovascular risk factors are more prevalent than in white Europeans [207,208]. It is unknown whether screening for asymptomatic glucose disorders to

reduce overall cardiovascular morbidity is achievable within British south Asians or yields populations at increased yet potentially modifiable risk.

The aim of this study is to report the uptake for ADDITION-Leicester and subsequent yield, by racial group (white Europeans versus south Asians) of undiagnosed glucose disorders defined as IGR and newly diagnosed type 2 diabetes. The burden of undiagnosed glucose disorders and cardiovascular risk within these groups is compared by presenting age-gender-adjusted frequencies and odd ratios for individual risk factors in addition to a Framingham based composite risk measure. Secondly, the biomedical characteristics, pre-existing cardio-protective treatments and UKPDS estimated CHD risk of the ADDITION-Leicester screen detected population is compared with a diabetes cohort (DESMOND) diagnosed through current clinical practice.

3.3 Methods

3.3.1 Recruitment of the screened population: ADDITION-Leicester

A detailed description of the screening process of ADDITION-Leicester is described in Chapter 2. Twenty local community practices participated in a screening programme inviting a random sample of people between 40-75 years inclusive (25-75 years for south Asian) to attend a single-session glucose and cardiovascular risk assessment between 2005 and 2009. This included an OGTT, plasma lipid profile, blood pressure and standardised anthropometric measurements. Self-completed questionnaires were used to assess medical history, smoking status and ethnicity. Deprivation level was calculated using a standard geographically determined index [219].

The diagnosis of diabetes, IFG or IGT was based on current WHO criteria [21,226]. Impaired Glucose Regulation (IGR) referred to a composite of IFG and or IGT. Those with diabetes range results were rescreened within two weeks to confirm the result according to current gold-standard practice. IGR range results were not rescreened but were offered annual follow up OGTTs through the ADDITION-Leicester pre-diabetes prospective study (Chapter 2). Participants diagnosed with diabetes were entered into the trial phase of the study (Chapter 4).

3.3.2 A conventionally diagnosed diabetes control population: DESMOND

DESMOND is a structured education programme specifically designed to facilitate lifestyle change in people with newly diagnosed type 2 diabetes [229]. In 2008, the programme was validated with a randomised controlled trial recruiting patients considered to be typical of primary care practice [104]. This was a large study conducted across thirteen cluster randomised primary care sites, with patients referred by their general practitioner or practice nurse. Importantly, the trial did not stipulate

adherence to any particular diagnostic criteria and the participating practices were instructed not to proactively identify new cases by screening. Patients were referred within four weeks of clinician diagnosis with biomedical data collected at practice level. $HbA_{1c\%,}$ blood pressure, blood lipids (total, HDL and LDL cholesterol and triglycerides), body weight and waist circumference were measured with standardised procedures ensuring valid comparison of clinical measurements and anthropometric data. Baseline data provided a newly diagnosed control population representative of current United Kingdom clinical practice.

3.3.3 Determination of cardiovascular risk

Cardiovascular risk was assessed using the ETHRISK calculator. This commercially available Framingham based epidemiological tool is a ten year risk equation incorporating gender, age, smoking status, systolic blood pressure, total and HDL cholesterol levels adjusted for south Asian ethnicity [230]. Subjects with a previous cardiovascular event, including myocardial infarction, stroke, and angina were excluded from cardiovascular disease risk calculations. The term composite cardiovascular disease includes self-reported myocardial infarction, angina, stroke, angioplasty, CABG and lower limb angioplasty or bypass procedures.

3.3.4 Statistical methods

Data were presented firstly as unadjusted mean (standard deviation (SD)) or count (%) and secondly as age and gender-adjusted means (95% confidence intervals). Non-normally distributed variables (IPAQ physical activity measure, serum triglycerides) were log transformed. The differing age inclusion criteria for white Europeans and south Asians in ADDITION-Leicester was handled in two ways. Firstly all models were reported as unadjusted means, age-gender-adjusted means and age-gender-adjusted odd ratios. Secondly, all comparative analyses were repeated using a modified

ADDITION-Leicester dataset with south Asians aged 25-40 years removed. The prevalence of diabetes, IFG and IGT in the revised group were compared by ethnicity using logistic regression, adjusted for age, gender, central obesity (using ethnicity specific cut-points of waist circumference), deprivation [219] and total energy expenditure [221]. Analyses were carried out using Stata (version 11.0) with statistical significance taken at $p<0.05$.

3.4 Results

3.4.1 Screening uptake

66,320 patients from twenty practices met the inclusion criteria for ADDITION-Leicester. The median number of eligible patient's per practice was 1,996, ranging from 707 to 14,895. A random sample of 30,950 were invited (46% of eligible population) and 6,749 (22% of invited population) subsequently screened (Figure 3.1). The median number of patients screened per practice was 299, ranging from 16 to 1,023. The eligible, invited and screened populations of individual practices are listed in Appendix C. In total 4,687 white Europeans, 1,684 south Asians and 378 other or self-reported mixed ethnicities were screened. Excluding the 331 south Asians of less than 40 years of age produced a revised population of 1,353, consisting of 1,272 Indians (94%), 30 Pakistanis (2.2%), 5 Bangladeshis (0.4%) and 46 of mixed race or non-specified Asian nationalities (3.4%). Even though predominantly Indo-Asian, the term south Asian was used to define this population.

Table 3.1 demonstrates age-gender demographics of those eligible to participate, those invited and those eventually screened. The screened population were older than both the eligible and invited, whilst the south Asian eligible, invited and screened populations were younger than the white European. Even after removal of those aged less than 40, the remaining Asians were on average 5.6 years younger than white

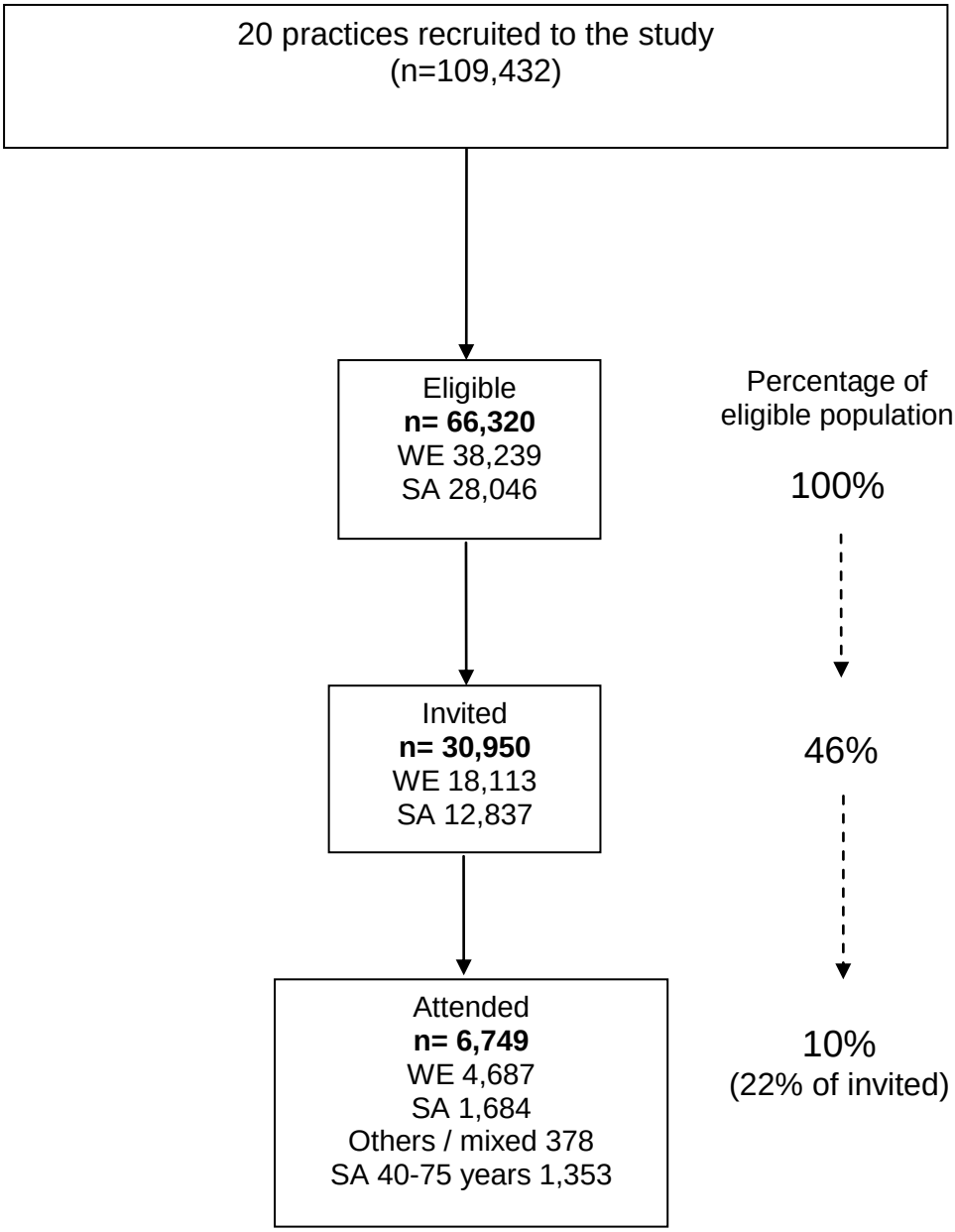
Europeans. There was a similar age discrepancy (approximately 2 years) between those eligible and invited to attend screening. More females were screened, although the numbers of males and females eligible and invited were similar. The proportion of males and females screened were similar across ethnicities.

3.4.2 Characteristics of those screened

Ethnicity stratified biomedical comparisons of the ADDITION-Leicester population are shown in Table 3.2. Data displayed is unadjusted and then adjusted for the effects of age and gender. Table 3.3 illustrates age and gender adjusted data for the sample excluding south Asians aged 25-40 years. There are marked differences between the white European and south Asian cohorts. The south Asian cohort is more deprived with lower BMI, waist circumference, total cholesterol, LDL and HDL cholesterol. There are fewer current smokers in this group but it is less physically active, with higher $HbA_{1c\%}$ levels, self-reported cardiovascular disease, medication use and ten-year estimated cardiovascular disease risk than the white European cohort. There are no differences in total body fat, blood pressure or aspirin use.

With reference to Tables 3.3 and 3.4 data is shown as age-gender adjusted mean with 95% confidence intervals (95% CIs) for continuous outcomes and age-gender adjusted percentages for categorical variables. Age-gender adjusted Odds Ratios (OR) and 95% CIs give the odds of being south Asian. Physical activity is log transformed and measured as total energy expenditure, MET-min per week, measured using I-PAQ [221].

Figure 3.1 Flow diagram illustrating the selection and recruitment process of ADDITION-Leicester



Key:

WE: White Europeans

SA: south Asians

Table 3.1: Age (SD) and gender of people included at each stage of screening

	All[a] (n= 6749*)	**White Europeans** (n= 4687*)	**South Asians** (n=1684*)
Age (Yrs)			
Eligible population	49.6 (12.6)	54.3 (10.5)	43.1 (12.4)
Invited	51.7 (12.8)	56.4 (10.2)	45.0 (13.0)
Screened	56.1 (10.8)	58.6 (9.5)	49.2 (11.1)
Screened 40-75	57.3 (9.6)	58.6 (9.5)	53.0 (8.7)
Gender (% male)			
Eligible population	50.4	50.2	50.6
Invited	50.1	51.4	48.2
Screened	47.7	47.1	49.3
Screened 40-75	47.7	47.1	49.2

Key

[a] Includes ethnicities other than White European and south Asian

*Screened population

Table 3.2 Unadjusted anthropometric and biomedical characteristics of the screened ADDITION-Leicester population

	White Europeans (n= 4687)	**South Asians (n=1684)**	**p (age-gender adjusted)**
Age, years (SD)	58.6 (9.5)	49.2 (11.1)	-
Gender, male n (%)	2209 (47.1)	830 (49.3)	0.03
Deprivation, Median [IQR]	13.5 [8.8 to 21.2]	22.6 [13.2 to 31.4]	<0.0001
Physical activity [IQR]	2319 [693 to 4518]	2578 [918 to 4746]	<0.0001
Weight, kg (SD)	80.0 (16.0)	72.1 (14.8)	<0.0001
BMI, kgm^{-2} (SD)	28.3 (5.0)	27.3 (5.0)	<0.0001
Waist, cm (SD)	94.7 (13.5)	91.9 (12.3)	<0.0001
Body Fat, % (SD)	33.7 (8.6)	31.9 (9.0)	<0.0001
Systolic blood pressure, mmHg (SD)	139.0 (19.4)	132.1 (19.2)	0.99
Diastolic blood pressure, mmHg (SD)	85.7 (10.5)	84.6 (10.8)	0.19
Current smoker (%)	750(16.1)	167 (10.0)	<0.0001
$HbA_{1c\%}$ (SD)	5.7 (0.6)	5.8 (0.6)	<0.0001
Total cholesterol, $mmoll^{-1}$ (SD)	5.7 (1.1)	5.2 (0.9)	<0.0001
LDL cholesterol. $mmoll^{-1}$ (SD)	3.6 (0.9)	3.3 (0.8)	<0.0001
HDL cholesterol. $mmoll^{-1}$ (SD)	1.4 (0.4)	1.2 (0.3)	<0.0001
Ethrisk CVD ten year risk (%) (SD)	14.0 (9.7)	14.5 (15.3)	<0.0001
Composite self-reported CVD n (%)	4.3 (8.8)	114 (6.8)	<0.0001
Anti-hypertensives n (%)	118 (25.2)	305 (18.1)	<0.0001
Statin n (%)	576 (12.3)	146 (8.7)	0.001
Aspirin n (%)	494 (10.5)	99 (5.9)	0.28

Table 3.3 Age-gender adjusted anthropometric and biomedical characteristics of the modified study population (n=6041)

*South Asians aged 40-75 years (n=1353)

	White Europeans	**South Asians***	**Adjusted**	**p value**
	Adjusted mean (95% CI)	**Adjusted mean (95% CI)**	**Odds Ratio (95% CI)**	
n	4688	1353		
Deprivation Score	17.2 (16.9 to 17.6)	23.6 (22.9 to 24.3)	1.04 (1.03 to 1.04)	<0.0001
Physical activity (IPAQ)	7.8 (7.7 to 7.9)	7.5 (7.3 to 7.5)	0.76 (0.71 to 0.82)	<0.0001
Weight, kg	80.2 (79.8 to 80.7)	71.2 (70.4 to 72.0)	0.95 (0.94 to 0.95)	<0.0001
BMI, kgm^{-2}	28.3 (28.2 to 28.4)	27.6 (27.3 to 27.8)	0.97 (0.96 to 0.98)	<0.0001
Waist, cm	94.5 (94.2 to 94.9)	92.9 (92.2 to 93.6)	0.99 (0.98 to 0.99)	<0.0001
Body fat (%)	33.6 (33.4 to 33.8)	33.1 (32.7 to 33.5)	0.99 (0.98 to 1.00)	0.05
Systolic blood pressure, mmHg	138.0 (137.5 to 138.5)	137.8 (136.8 to 138.8)	1.00 (1.00 to 1.00)	0.85
Diastolic blood pressure, mmHg	85.7 (85.4 to 86.0)	85.7 (85.1 to 86.2)	1.00 (0.99 to 1.01)	0.78
Current smoker (%)	16.6 (15.6 to 17.6)	6.0 (4.1 to 7.8)	0.37 (0.29 to 0.45)	<0.0001
$HbA_{1c\%}$	5.64 (5.62 to 5.66)	5.91 (5.87 to 5.94)	2.06 (1.82 to 2.34)	<0.0001
Total cholesterol, $mmoll^{-1}$	5.63 (5.62 to 5.68)	5.26 (5.21 to 5.32)	0.68 (0.64 to 0.73)	<0.0001
LDL cholesterol, $mmoll^{-1}$	3.62 (3.59 to 3.65)	3.35 (3.30 to 3.40)	0.70 (0.65 to 0.76)	<0.0001
HDL cholesterol, $mmoll^{-1}$	1.40 (1.39 to 1.41)	1.26 (1.25 to 1.28)	0.27 (0.21 to 0.33)	<0.0001
Ethrisk ten year risk (%)	13.2 (13.0 to 13.4)	18.7 (18.3 to 19.1)	1.11 (1.10 to 1.12)	<0.0001
Composite vascular disease (%)	8.0 (7.3 to 8.8)	10.9 (9.4 to 12.4)	1.51 (1.20 to 1.91)	<0.0001
Anti-hypertensives (%)	23.4 (22.2 to 24.6)	28.1 (25.9 to 30.3)	1.35 (1.15 to 1.58)	<0.0001
Statin (%)	11.2 (10.3 to 12.1)	14.2 (12.5 to 15.9)	1.40 (1.14 to 1.73)	0.001
Aspirin (%)	9.6 (8.8 to 10.4)	10.5 (8.9 to 12.1)	1.12 (0.88 to 1.42)	0.67

3.4.3 Prevalence of abnormal glucose tolerance

Of the 6041 patients included, 17.5% (n=1056) had abnormal glucose tolerance, comprising 3.3% (n=196) with diabetes, 2.6% (n=157) with IFG and 9.7% (n=585) with IGT and 2.0% (n=118) with both IFG and IGT. The south Asian cohort had a significantly higher prevalence of IGT (adjusted OR 1.66, 95%CI 1.33 to 2.06), IGT or IFG (adjusted OR 1.53, 95%CI 1.26 to 1.87), IGT and IFG (adjusted OR 1.78 95%CI 1.12 to 2.81) and diabetes (adjusted OR 2.18, 95%CI 1.56 to 3.06) compared to the white European cohort. The adjusted odds of having any glucose disorder were 1.8 times higher in the south Asians compared to the white Europeans (adjusted OR 1.80, 95%CI 1.52 to 2.14). High levels of ten-year cardiovascular disease risk were seen across the abnormal glucose spectrum, ranging from 15.2% in the exclusive IGT white European group to 27.7% in south Asians with screen detected type 2 diabetes. Cardiovascular disease risk was higher in south Asian across all glucose categories (Table 3.4).

3.4.4 Comparison of conventionally detected and screened detected diabetes

Demographics and biomedical data of the DESMOND control cohort (n=819) and full ADDITION-Leicester screened diabetes group (consisting of all white Europeans aged 40-75 and south Asians aged 25-75 years with diabetes) (n=214) are compared in Table 3.5. Those with conventionally detected type 2 diabetes are markedly different to screen detected cases. There are significantly fewer south Asians in the conventionally detected group (9.2% versus 37.3%). Those who are conventionally detected tend to be larger than those who are screen detected, with significantly higher weight, BMI and waist

circumference. They also have a significantly higher $HbA_{1c\%}$ (8.1 versus 7.3, $p<0.0001$). Lower medication levels in the screen detected group give rise to significantly higher levels of systolic and diastolic blood pressure and total and LDL cholesterol. Statistically higher levels of ten-year CHD and cardiovascular disease risk are seen in the screen detected group (CHD: 15.4% versus 13.3%, p=0.002; cardiovascular disease: 20.8% versus 17.2%, p=0.0001).

Conventionally diagnosed people with type 2 diabetes are prescribed significantly more blood pressure and lipid lowering treatment than screen detected cases. Blood pressure remains inadequately controlled in conventional cases already on treatment (mean systolic blood pressure 142.6mmHg) and screen detected cases irrespective of anti-hypertensive usage (mean systolic blood pressure on treatment 148.4mmHg and not on treatment 145.4mmHg) (Table 3.6a). Lipid lowering therapies are again more frequently prescribed in conventionally diagnosed cases (41% versus 30%). Unlike blood pressure, mean total cholesterol concentration is significantly lower within the treated groups of both conventionally and screen detected cohorts. Over 50% of screen detected cases remain inadequately treated with a total cholesterol of $>5.0 mmoll^{-1}$ (Table 3.6b).

Table 3.4 Odds Ratios for the frequency of glucose disorders and mean ten-year CVD risk by ethnic groups in modified 40-75 year old ADDITION-Leicester population (n=6041)

Glucose disorder	n (%)	Ethnicity	n (%)	Age-gender adjusted OR [b] (95% CI)	Fully adjusted OR (95% CI)	Adjusted Ethrisk mean % (95% CI)
IFG exclusively	157 (2.6)	WE	125 (2.7)			16.6 (14.9 to 18.2)
		SA	32 (2.4)	1.06 (0.71 to 1.60)	1.03 (0.67 to 1.60)	27.2 (24.0 to 30.4)
IGT exclusively	585 (9.7)	WE	424 (9.1)			15.2 (14.3 to 16.0)
		SA	161 (12.0)	**1.74 (1.42 to 2.13)**	**1.66 (1.33 to 2.06)**	23.5 (22.1 to 24.9)
IGT or IFG	742 (12.3)	WE	549 (11.7)			15.4 (14.7 to 16.2)
		SA	193 (14.3)	**1.61 (1.33 to 1.93)**	**1.53 (1.26 to 1.87)**	24.3 (23.0 to 25.5)
IGT and IFG	118 (2.0)	WE	81 (1.7)			18.9 (17.0 to 2.9)
		SA	37 (2.7)	**2.08 (1.38 to 3.14)**	**1.78 (1.12 to 2.81)**	27.1 (24.2 to 30.0)
Diabetes	196 (3.3)	WE	128 (2.7)			18.3 (16.5 to 20.1)
		SA	68 (5.1)	**2.30 (1.68 to 3.16)**	**2.18 (1.56 to 3.06)**	27.7 (25.2 to 30.2)
Any glucose disorder	1056 (17.5)	WE	758 (16.2)			16.4 (15.7 to 17.1)
		SA	298 (22.0)	**1.93 (1.64 to 2.26)**	**1.80 (1.52 to 2.14)**	25.2 (24.1 to 26.2)

Key

[b] Odds Ratio (OR) for South Asians (SA) versus White Europeans (WE) (Highlighted figures indicate statistical significance). Logistic regression models presented both adjusted for age and gender and adjusted for age, gender, central obesity (using ethnicity specific cut points of waist circumference) and deprivation

Table 3.5 Comparison of conventionally diagnosed (DESMOND control) and screen detected (ADDITION-Leicester) diabetes groups. (Mean values with SD or % indicated)

	Conventionally detected DESMOND n=824	Screen detected ADDITION n=214	p value
Age, Yrs (SD)	59.5 (12.1)	58.9 (10.0)	0.49
Gender, male n (%)	452 (54.9)	126 (58.9)	0.70
Ethnicity n (%)			
White european	725 (90.6)	128 (62.8)	
South Asian	73 (9.2)	76 (37.3)	0.003
Current smoker n (%)	110 (13.4)	29 (13.6)	0.89
Biomedical (SD):			
Height, m	1.68 (0.1)	1.66 (0.11)	<0.0001
Weight, kg	91.7 (19.6)	85.9 (18.5)	<0.0001
Waist, cm	106.1 (14.2)	103.1 (13.3)	0.0003
BMI, kgm^{-2}	32.4 (6.3)	31.0 (5.7)	<0.0001
Systolic BP, mmHg	140.6 (17.6)	148.1 (18.5)	<0.0001
Diastolic BP, mmHg	81.8 (10.5)	88.9 (10.7)	<0.0001
$HbA_{1c\%}$	8.1 (2.1)	7.4 (1.8)	<0.0001
Cholesterol, $mmoll^{-1}$			
Total	5.3 (1.3)	5.6 (1.3)	0.09
LDL	3.2 (1.1)	3.5 (1.1)	0.02
HDL	1.2 (0.4)	1.2 (0.4)	0.81
Triglycerides, $mmoll^{-1}$	2.5 (2.1)	2.2 (1.9)	0.01
Medications n (%):			
BP lowering	493 (60.2)	153 (45.5)	<0.0001
Lipid lowering	335 (40.9)	101 (30.0)	<0.0001
Aspirin	224 (27.4)	50 (14.8)	<0.0001
Ten yr Ethrisk % (SD):			
CHD	13.3 (9.2)	15.4 (11.2)	0.002
Cardiovascular disease	17.2 (12.0)	20.8 (15.6)	0.0001

Table 3.6 Comparison of conventionally diagnosed (DESMOND control) and screen detected (ADDITION-Leicester) diabetes groups by a) anti-hypertensive and b) lipid lowering status. (Mean values with SD or % indicated)

a) Anti-hypertensive status

	Conventionally detected DESMOND control n=819		Screened detected ADDITION-Leicester n=214	
	On antihypertensive	**Not on antihypertensive**	**On antihypertensive**	**Not on antihypertensive**
n (%)	493 (60.2)	326 (39.8)	96 (45.5)	118 (54.5)
Age, years (SD)	63.1 (10.6)	53.9 (12.1)**	60.1 (9.4)	57.9 (10.8)
BMI, kgm^{-2} (SD)	33.4 (9.3)	32.3 (7.1)	31.1 (5.5)	30.4 (5.2)
Blood Pressure:				
Systolic, mmHg (SD)	142.6 (18.7)	137.6 (15.5)**	148.4 (18.6)	145.4 (20.9)
Diastolic, mmHg (SD)	81.6 (11.3)	82.0 (9.4)	88.6 (11.2)	85.3 (11.7)*
>140/90 mmHg, n (%)	240 (48.8)	128 (39.4)*	111 (72.6)	108 (59.0)*

b) Lipid lowering status

	Conventionally detected DESMOND n=819		Screened detected ADDITION-Leicester n=214	
	On lipid-lowering therapy	**Not on lipid-lowering therapy**	**On lipid-lowering therapy**	**Not on lipid-lowering therapy**
n (%)	335 (40.9)	484 (59.1)	64 (30.0)	150 (70.0)
Age, years (SD)	63.0 (10.3)	57.0 (12.6)**	61.2 (9.4)	58.0 (10.4)*
BMI, kgm^{-2} (SD)	33.0 (9.6)	32.9 (7.6)	30.3 (5.3)	30.9 (5.4)
Cholesterol, $mmoll^{-1}$				
Total (SD)	5.0 (1.3)	5.6 (1.3)**	5.2 (1.3)	5.6 (1.1)*
LDL (SD)	2.8 (1.1)	3.5 (1.0)**	3.0 (1.1)	3.5 (0.9)**
HDL (SD)	1.2 (0.4)	1.2 (0.4)	1.2 (0.4)	1.2 (0.3)
Triglycerides, $mmoll^{-1}$	2.4 (1.3)	2.6 (2.5)	2.1 (1.8)	2.3 (1.8)
Total cholesterol >5.0	130 (38.8)	315 (65.1)**	52 (51.5)	162 (68.6)*

Key
*p<0.05
**p<0.001

3.5 Discussion

Ideally, screening should deliver convincing mortality benefits before it is widely endorsed as a key cardiovascular disease prevention strategy in type 2 diabetes. Such improvements probably depend upon the extent of risk reduction that could be achieved within the framework of screening. The comparisons and modelling work described here strengthen existing epidemiological data on screening and cardiovascular risk intervention in early glucose disorders. Novel aspects of this work relate to the feasibility of screening within a multiethnic United Kingdom population, risk profile characterisation of south Asians attending screening and the burden of cardiovascular disease risk across the whole spectrum of screen detected glucose disorders.

3.5.1 Screening uptake and prevalence of glucose disorders

Despite concerted attempts to engage the population, the achieved response rate was lower than other diabetes screening studies, especially within the south Asian population [231-233]. We consider the cultural adaptations of ADDITION-Leicester a significant strength of the study which serves to emphasise the complexities of engaging ethnic minority groups in research and health promotion activities. This should be of particular concern to health authorities and future research programmes aiming to improve health outcomes in ethnic minority groups as despite these measures the uptake in south Asians was low.

Previous studies adopting stepwise screening approaches with questionnaire based risk scores [234]; random capillary testing [235], or a fasting plasma glucose assessment [236] report higher response rates. Assuming more simplistic strategies

tend to attract larger numbers, the decision to characterise post-challenge hyperglycaemia and commit the cohort to an arduous OGTT may have affected our response rate. Whilst acknowledging this as a limitation of the study, the impracticality of screening via this method highlights the importance of capturing IGT or isolated post-challenge hyperglycaemia in other ways. As has been shown in previous studies these classifications are particularly common in south Asians, and are associated with significant, often untreated cardiovascular risk comparable to newly diagnosed diabetes [237]. This is an important finding that should not be overlooked when sensitivity analyses using more utilitarian screening approaches are performed on this population. In this respect, we have recently demonstrated that glycoslated haemoglobin appears to be a more sensitive measure of IGR in south Asians than white Europeans [238].

Although the achieved response may influence our prevalence estimates for newly diagnosed type 2 diabetes and IGR, this source of error is largely unavoidable when reporting undiagnosed cases within population settings and may, in part be off-set by the age of the screened population in ADDITION-Leicester. Typical of previous diabetes screening studies, responders were older with a female preponderance which probably reflects the ability of these groups to commit to a morning three hour appointment. More worryingly it may also indicate a generalised apathy towards screening within perceived “healthy” populations. An unexpected difference in the mean age of eligible and invited populations probably relates to discrepancies in the practice sourced eligibility data and subsequent independent mailing searches conducted by the research team. This may have influenced random sampling and should be considered a potential source of error affecting an otherwise highly representative population. We also acknowledge that it would have been more

appropriate to use a tool measuring an individual's deprivation rather than the geographically determined index employed [219].

After age-gender adjustment the odds ratios for undiagnosed glucose disorders, whether fasting or post-challenge were twice as high in south Asians. A Leicester based study reported similar findings within diagnosed diabetes cases nearly twenty years ago and our data extends this trend into undiagnosed diabetes and IGR ranges [239]. A prevalence of 4.5% for undiagnosed type 2 diabetes is similar to the Indian sample of the Health Survey of England [203].

Whilst the prevalence of IGR was expected to be fifteen to twenty percent, previously undiscovered diabetes frequency was lower than anticipated in both groups. Although this observation could be partly explained by the sampling and ascertainment issues already described, it is also plausible that the number of undiagnosed cases is in decline as a greater effort is made to identify the condition. Although designed prior to its conception, the last year of the screening phase coincided with the implementation of the NHS pay-for-performance quality and outcomes framework (QoF) [240]. This centrally devolved strategy rewards general practitioners for identifying and maintaining good glycaemic control in people with diabetes and may have contributed to increased opportunistic screening activity in primary care over recent years. Our results may reflect a declining population prevalence of undiagnosed diabetes in centres more actively engaged in earlier detection practices. It may also simply be that this relatively discrete Indian population is not as susceptible to glucose disorders as other diasporic south Asian groups.

3.5.2 Burden of CVD risk in the screened population

This study reflects others demonstrating a significant burden of cardiovascular risk in responders to population based screening programmes for type 2 diabetes [227, 234,241,242]. In contrast to other programmes, ADDITION-Leicester provides additional information on risk burden related to glucose intolerance in general. The universal use of OGTT for screening allowed the identification of higher numbers of people with IFG and or IGT in the population than in other programmes. Consequently, a broader range of cardiovascular risk burden was captured, as illustrated by the remarkable similarity of ten-year global cardiovascular disase risk estimates in IGR and screen detected diabetes (ETHRISK 18.0% vs. 21.1%). As response rates for screening programmes remain a major consideration, it is conceivably counterintuitive to potentially reduce the yield of high cardiovascular disease risk cases by implementing strict diagnostic cut-offs derived from micro- and not macrovascular outcomes.

3.5.3 Cardiovascular risk in South Asians

There have been a number of United Kingdom based surveys quantifying cardiovascular risk and CHD within dispersed south Asian communities. To date the largest remains the Southall diabetes study, an ongoing programme describing traditional risk factors and incident vascular events in over 3,000 Bangladeshis and white Europeans [199,209,243]. Similar to our findings the initial cross-sectional analysis of this cohort demonstrated a lower mean plasma cholesterol and smoking tendency in combination with a much higher prevalence of type 2 diabetes in south Asians (19%). Consistently higher rates of CHD in prospective analyses have lead to the assumption that hyperglycaemia and a predisposition to central obesity and its pro-inflammatory consequences probably account for excess vascular disease in this

group. Similarly, the more recent Newcastle Heart project used a robust population based approach to identify new cases of diabetes and compare cardiovascular risk in a mixed ethnicity sample of 325 south Asians and 425 white Europeans [215]. This study employed the OGTT and found more IGT and diabetes in Indians (approximately two-fold), Pakistanis (two-fold) and Bangladeshis (more than three-fold), together with higher rates of CHD compared with white Europeans.

The major finding of these landmark studies is replicated here, with an increased CHD risk and particular predisposition to glucose disorders characterising the Indo-Asian ADDITION-Leicester population. Whilst there have undoubtedly been advances in the awareness and management of cardiovascular disease over the last twenty years, the intervening period since publication of the Southall study appears not to have significantly changed the cardiovascular risk profile of British south Asians. The time may have come to consider changing largely reactive therapeutic approaches and develop culturally sensitive screening programmes aimed at much earlier identification of glucose disorders and vascular disease prevention. Implementation of interventions in people with screen detected non-diabetic hyperglycaemia may be particularly advantageous in ethnic minority populations known to be susceptible to premature diabetes and cardiovascular disease [244].

3.5.4 Comparison of conventionally detected and screened detected type 2 diabetes

There have been few direct comparisons of cardiovascular risk profiles in screen detected and conventionally diagnosed people with diabetes. Uniquely, both studies recruited only newly diagnosed cases enabling direct comparison of untreated hyperglycaemic status. The cohorts were almost identical in terms of age, gender

and smoking status allowing direct comparisons of other biomedical parameters without the need for adjustment for confounding effects. When compared, a number of important differences emerged between these groups. Conventionally identified people with type 2 diabetes have a higher $HbA_{1c\%}$ and are on average seven kilograms heavier than their screen detected counterparts. They are prescribed significantly more lipid lowering and anti-hypertensive medications at diagnosis, reflecting lower mean cholesterol and blood pressure indices. By virtue of these untreated cardiovascular risk factors the screen detected group have a four percent increased risk of a macrovascular event, despite clearly being at an earlier stage of the metabolic disease process.

In the UKPDS trial a mean $HbA_{1c\%}$ of 7.0% in the intensive versus 7.9% in the conventional treatment arm was associated with fewer microvascular complications [245]. Long-term follow-up of this cohort has recently demonstrated that good glycaemic control reduces the frequency of macrovascular events as well [65]. Interestingly, these measures of glucose control are comparable to the 8.1% of the conventionally diagnosed compared to 7.3% in the screen detected group, a difference of 0.8%.

Approximately 65% of individuals identified through screening were hypertensive and or dyslipidaemic, irrespective of treatment. Fewer screen detected cases are prescribed anti-hypertensive or lipid lowering therapies and of those who are treated the majority are above recognised targets for both blood pressure and cholesterol. The implication is that cardiovascular risk intervention in unidentified or latent stage diabetes is inadequate. Earlier diagnosis of glucose disorders will bring adverse cardiovascular risk profiles to the attention of health care professionals facilitating

intervention with proven long-term benefits. Only ten percent of screen detected cases achieve stipulated Joint British Society Guidelines JBS-2 [61] targets for hypertension and dyslipidaemia. It is clear that blood pressure especially is generally under treated and inadequately controlled in both the conventionally and screened detected cohorts. Ten-year estimated CHD of nineteen percent in the population screened ADDITION group is extremely clinically relevant and represents untreated, yet potentially modifiable vascular risk in individuals with readily identifiable latent diabetes.

It should be noted ADDITION-Leicester was regional whereas DESMOND recruited nationally, potentially effecting direct comparability of the studies. Although likely to be representative of clinical practice, our definition of conventionally diagnosed diabetes was reliant upon a physician for diagnosis and subsequent referral to the trial, providing a potential source of selection bias. This group was also limited by a low uptake of Black and Minority Ethnic groups which may have the most to gain from earlier identification of glucose disorders. DESMOND was also not designed to capture self-reported previous medical history at baseline, which would have enabled useful comparisons of macrovascular disease. Although benefits can be inferred from this cross-sectional data, the effectiveness of screening for type 2 diabetes can only be truly judged on its ability to improve vascular outcomes within the confines of a randomised controlled trial.

3.6 Conclusion

A screening strategy for type 2 diabetes incorporating an OGTT captures a significant and potentially reversible burden of cardiovascular disease risk in a multiethnic United Kingdom population. Susceptibility to these complications is potentiated within WHO defined glucose disorders with similar risks for IGR and screen detected diabetes categories. Screening identifies diabetes cases with an adverse and often undertreated cardiovascular risk profile.

South Asian people attending the programme have approximately twice the risk of an undetected glucose abnormality and significantly greater overall cardiovascular disease risk yet are more likely to be prescribed lipid lowering and or anti-hypertensive drugs than their white European counterparts. Novel, culturally sensitive approaches to cardiovascular health screening are urgently needed as unidentified traditional cardiovascular risk factors remain prevalent within this group.

Screening programmes for diabetes should prioritise early identification and aggressive intervention in those at the most risk of macrovascular complications. This will almost certainly require a stratified risk factor based approach including the whole spectrum of hyperglycaemic disorders, not necessarily restricted to the diabetes category.

Chapter 4

Intensive multi-factorial intervention improves modelled CHD risk in screen detected type 2 diabetes mellitus

Results of the ADDITION-Leicester cluster randomised controlled trial

4.1 Summary

Aims: To compare the effects of intensive multi-factorial cardiovascular risk intervention with standard care in screen detected type 2 diabetes.

Methods: Twenty general practices randomly invited 30,950 adults without diagnosed diabetes for screening using 1999 WHO criteria. In a randomised controlled trial, 214 screen detected cases were assigned by practice allocation to receive either intensive, protocol driven cardiovascular risk management, intensive care (IC) (n=95) or standard care (SC) (n=119) according to local guidelines. IC consisted of life-style and pharmacological interventions to achieve an $HbA_{1c\%}$ of ≤6.5%, blood pressure (BP) <130/80mmHg and total cholesterol (T-chol) <3.5mmol^{-1}

Results: There were significant reductions in $HbA_{1c\%,}$ BP and T-chol from baseline in both groups, (mean change for total study population -0.62%, -11.6/10.0mmHg, -1.1mmoll^{-1}). After adjustment for baseline and clustering significant between group differences were observed in mean changes from baseline for $HbA_{1c\%}$ (-0.7% IC vs. -0.6% SC, p=0.001), BP (systolic mmHg: -16.2 IC vs. -8.4 SC, p<0.001), T-chol (mmoll^{-1}: -1.3 IC vs. -1.0 SC mmoll^{-1}, p<0.001) and weight (kg: -3.8 IC vs. -2.2 SC, p=0.01). Five-year CHD risk was reduced by 3.2% and 2.3% respectively (p<0.0001) in favour of IC. IC was associated with more lipid lowering and anti-hypertensive but not glucose lowering medication use (ORs: 2.5 (1.4-4.4), 5.5 (2.4-11.5), 1.6 (0.8-2.3) respectively p<0.001, p=0.003, p=0.65). Treatment satisfaction responses were superior in IC with no increase in self-reported hypoglycaemia.

Conclusion: Intensive multi-factorial cardiovascular risk management in patients with diabetes identified through systematic non-risk factor based screening significantly reduces modelled coronary heart disease risk. This is achieved predominantly with lipid lowering and anti-hypertensive treatments with no adverse effects related to quality of life or hypoglycaemia.

4.2 Introduction

The ADA recommends population screening for type 2 diabetes in everyone over 45 years of age [75] (Chapter 2, Table 2.4). By enabling earlier opportunities for therapeutic intervention, screening theoretically enhances length and quality of life without causing excessive physical or psychological harm [79,106]. Targeted screening in particular identifies populations at significant risk of cardiovascular disease and may be an efficient, highly cost-effective primary prevention strategy [101,227,234].

Populations known to be particularly susceptible to glucose disorders and CHD, such as ethnic minority south Asian groups may benefit the most from such approaches, especially if latent, yet potentially modifiable cardiovascular risk factors are more common than the background “indigenous” population prevalence [72,237]. Intensive multi-factorial cardiovascular risk intervention has been shown to be beneficial in advanced cases of type 2 diabetes with microalbuminuria, but has not yet demonstrated improved macrovascular outcomes earlier in the disease trajectory when preventative therapies may be most effective [66-69].

The aims of this chapter are to report the effects of a twelve month protocol driven intensive multi-factorial intervention on UKPDS five year modelled CHD risk [246] within a randomised multiethnic population identified with type 2 diabetes through screening.

4.3 Methods

4.3.1 Objectives, outcomes and power calculation

The objective of this study was to determine the effects of multi-factorial cardiovascular risk intervention in people with previously undiagnosed type 2 diabetes identified through a non-risk factor based screening programme (ADDITION-Leicester). To achieve this, we designed an intensive intervention (described in detail in Chapter 2 and Section 3.3.3) and compared it to routine practice within the confines of a randomised controlled trial. By necessity, the "control" population here was more of a comparator and received standard community based care available at the time of diagnosis. This was based on the assumption that this would be less intensive than the active intervention arm of the study.

The primary outcome was a biomedical composite of modelled five-year UKPDS CHD risk one year after diagnosis through screening. Secondary outcomes included individual measures of $HbA_{1c\%,}$ blood pressure, serum lipids, weight, self-reported hypoglycaemia and diabetes specific quality of life assessments (short form (SF)-12), diabetes treatment satisfaction) [222,223]. Assuming a prevalence of undiagnosed diabetes of 4.5% [239], we calculated a target of 7,000 volunteers over three years sufficient to identify a screen detected diabetes cohort of n=225 demonstrating a six percent difference (80% power, alpha 0.05) in UKPDS modelled CHD risk between routine and intensive groups at one year assuming a cluster co-efficient of 0.14 and loss to follow up of 15%.

4.3.2 Participants

Systematic screening identified previously undiagnosed type 2 diabetes within random samples of 40-75 year olds (25-75 if of “self-reported” south Asian ethnicity) registered across twenty socio-economically diverse general practices. The study adhered strictly to WHO diagnostic criteria for fasting and two hour post-challenge plasma glucose concentrations with newly diagnosed diabetes cases enrolled within a week of screening [21,226].

The screening phases of the study are described in Chapters 2 and 3. Briefly, inclusion criteria included the age range specified, screen detected type 2 diabetes and sufficient understanding of the study to comply with standard operating procedures. South Asians aged 25-40 years at screening were eligible. Exclusion criteria included pre-existing diabetes, pregnancy, severe mental illness, and steroid induced hyperglycaemia.

4.3.3 Intensive and control intervention groups

The screen detected diabetes intervention group received bi-monthly contact from a team specifically tasked with delivering a structured, multi-factorial cardiovascular risk intervention. ADDITION-Leicester adopted a pragmatic, target driven approach to evidence based optimisation of blood glucose, blood pressure and lipid profiles (Table 2.3). Moderation by an independent steering committee ensured the intensive treatment protocol was continuously updated in line with current national recommendations for the management of patients with type 2 diabetes. Treatment targets for the intervention were based on a complex intervention with proven efficacy in more advanced diabetes; glycosylated haemoglobin ($HbA_{1c\%}$) <7.0%, with

initiation of treatment at 6.5%, blood pressure <130/80mmHg, and total cholesterol <3.5 $mmoll^{-1}$[66].

The screen detected control group received the normal standards of care provided by community practitioners at the time of diagnosis. Participating primary care teams were provided with published national standards for type 2 diabetes which on study entry advised an $HbA_{1c\%}$ <7.0%, blood pressure <140/85 mmHg and total cholesterol <4.0 $mmoll^{-1}$.

4.3.4 Study process and biomedical measurements

Baseline and twelve month measurements were performed within a single centre by independent researchers (University Hospitals of Leicester) blind to treatment allocation. Management changes were occasionally recommended during the final one year assessment where it was deemed clinically necessary to intervene; otherwise general practitioners were sent an ethically approved generic pro-forma detailing the annual review assessment.

Anthropometric measurements and blood pressure were performed according to standard operating procedures detailed in Chapter 2. Medical history and prescribed medication were recorded at the time of screening. Self-reported vascular diseases were noted and included the terms angina, myocardial infarction, cerebrovascular accident, heart attack, stroke, CABG and angioplasty.

4.3.5 Adverse events and quality of life assessments

All participants in the intensive arm of the study were provided with an Asensia contour® glucometer, Bayer Healthcare, Berkshire, United Kingdom and testing strips. Although not specifically part of a structured education programme, instruction in the use of capillary glucose testing aimed to empower self-management and life-

style change. It was not a pre-designed safety measure, as the anticipated frequency of hypoglycaemia with the ADDITION-Leicester glucose lowering algorithm was low. Symptomatic hypoglycaemia was graded as minor or major according to reported symptoms (minor), self-treatment (minor), or the requirement of a third party (major) during the one year assessment. No objective measures of hypoglycaemia were collected.

At the end of the study participants completed the Diabetes Treatment Satisfaction (DTSQc) questionnaire and Short Form (SF-12) health survey [222,223]. DTSQc measures patients' satisfaction with their treatment regimen. It consists of eight domains, six measuring satisfaction, convenience, flexibility, and understanding and two measuring perception of hypoglycaemia and hyperglycaemia. SF-12, an abbreviated form of standard SF-36 is a validated instrument of self-evaluated health-related quality of life encompassing a spectrum of domains with scores ranging from zero (worst health) to one hundred (best health).

4.3.6 Statistics

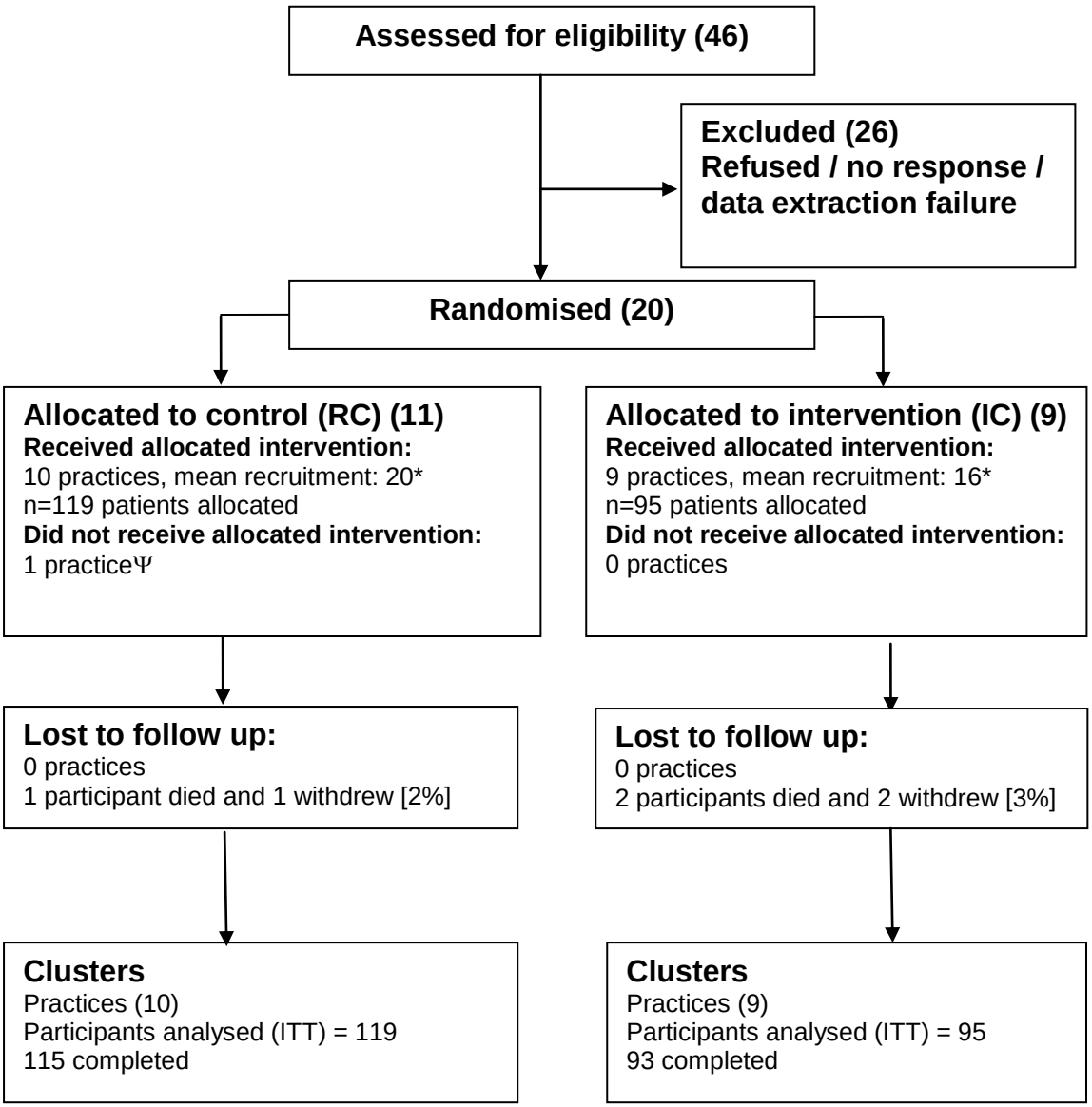
Statistical analysis was carried out by intention to treat. Continuous variables are given as mean and standard deviation and categorical variables are given as counts and percentages. To adjust for cluster we used robust generalised estimating equations with an exchangeable correlation structure. For binary outcomes we used a logit link with a binomial distribution for the outcome and for continuous outcomes we used an identity link with a normal distribution. Adjustment for baseline value and ethnicity was made in all models (apart from treatment satisfaction and quality of life which were not recorded at baseline). The analysis of CHD and cardiovascular disease risk was repeated for white Europeans and south Asians separately. Statistical significance was set at five percent. All analysis was carried out in Stata

(version 10.0). All statistical tests were two sided and performed at a significance level of p=0.05 or less. ADDITION-Leicester adhered to the CONSORT guidelines for cluster randomised controlled trials [247].

4.4 Results

The trial profile and flow of participants within cluster randomised practices are shown in Figure 4.1. Two hundred and fourteen volunteers with screen detected type 2 diabetes were identified from an invited and eligible population of 30,950 sourced from twenty general practices (Section 3.3). One intensive intervention practice withdrew consent shortly after randomisation and did not participate in the screening phase of the study. Remaining practices (ten intensive intervention and nine standard care controls) were well matched for size, deprivation status and underlying frequency of known diabetes (Figure 2.2 and Appendix C). An index of deprivation was constructed from practice based demographics and disease reporting using standardised criteria [219]. The response rate for screening was 22% and calculated prevalence of undiagnosed diabetes was 4.0%. No newly identified screen detected cases subsequently refused to participate in the intervention phase.

Figure 4.1 Flow of clusters and participants through the ADDITION-Leicester intervention study.



*****mean number of recruits per practice (RC range 7-44, IC range 6-34,)
IC: Intensive care group **RC**: Routine care group **ITT**: Intention to treat
( ) signifies number of practices participating
Ψ practice withdrew shortly after randomisation (chapter 3)

95 patients were enrolled with practices randomised to receive intensive multi-factorial intervention and 119 to practices delivering standard levels of care. Mean duration of follow up at the time of analysis was 1.1 years with a standard deviation (SD) of 0.15.

4.4.1 Baseline characteristics the study population

Table 4.1 depicts the baseline cardiovascular risk profile and anthropometrics of the intensive intervention and standard care control groups. Mean age for the whole study population was 59.8 (SD 10.0) years. Demographically the groups were well matched with no statistically significant differences in age, gender or ethnicity. The number of smokers and individuals with pre-existing cardiovascular disease were much the same in both groups, but more patients in the intensive group were taking anti-hypertensive medication before entering the study. This did not result in significant differences in mean systolic or diastolic blood pressure measurements at baseline. The groups were otherwise matched for serum lipids, auxology and $HbA_{1c\%.}$ Using the UKPDS equation, mean five-year risk of coronary heart disease was 11.9% (SD 8.9) with no difference between treatment groups

4.4.2 Intervention effects

During twelve months of follow-up, one patient died in the intervention group and two in the control group. New self-reported cardiovascular events were recorded for six patients, two in the intervention group and four in the standard care group. None of these differences were statistically significant. Three patients withdrew from the study but were included in the intention to treat analysis. Mean parameter changes within intensive intervention and standard care groups are outlined in Table 4.2 Values are adjusted for cluster effects, ethnicity and baseline difference. There was

a significant reduction in the primary outcome (five-year UKPDS CHD risk) in the intensive intervention group from baseline [-1.49% (-2.20 to -0.77), p<0.0001]. This effect was even greater for a ten-year modelled CHD risk score [-3.1% (-4.60 to -1.63)]. A statistically significant greater reduction in $HbA_{1c\%}$ [-0.20% (-0.31 to -0.08)], total cholesterol [-0.56 $mmoll^{-1}$ (-0.87 to -0.25)], LDL cholesterol [-0.47$mmoll^{-1}$ (-0.71 to -0.23)] systolic [-10.41mmHg (-14.82 to -6.00)] and diastolic blood pressures [-6.21mmHg (-8.27 to -4.16)] was seen in the intensive intervention group. Temporal changes in $HBA_{1c\%}$, blood pressure, lipids, weight and serum hepatic alanine transaminase (ALT) are depicted in Figures 4.2, 4.3 and 4.4. Biomedical changes and primary outcomes were similar in white Europeans and south Asians (ethnicity stratified data shown as Appendix D).

Table 4.1 depicts the proportion of people on different medications in the intervention and control group at twelve months. A significantly higher proportion (Odds ratio intensive vs. standard care) of participants were on anti-hypertensive [OR: 2.46 (1.37 to 4.41)], lipid-lowering [OR: 5.53 (2.44 to 12.53)] and anti-platelet therapy [OR: 8.27 (3.64 to 18.81)] in the intensive intervention group. There was a similar pattern of anti-hypertensive medication use with ACE inhibitors and or ARBs being prescribed most frequently in both groups (intervention 61% versus control 27%), followed by diuretics, calcium channel blockers, beta-blockers and alpha-blockers. Metformin was prescribed more in the intensive intervention group (38.5% vs. 53.9%, p<0.001). There was no statistically significant difference in the use of sulfonylurea therapies or insulin between the groups.

Table 4.1 Baseline and one year biomedical characteristics of the ADDITION-Leicester population (n=214)

	Standard care n (%) =119 (57.7)		**Intensive care** n (%) = 95 (42.2)	
	Baseline	1 year	Baseline	1 year
Age (Years)	60.0 (10.0)	-	59.4 (10.0)	-
Gender, male n (%)	116 (58.3)	-	83 (56.9)	-
Ethnicity n (%)				
White European	74 (62.3)	-	49 (52.7)	-
South Asian	44 (37.2)	-	44 (43.8)	-
Other	1 (0.5)	-	2 (3.4)	-
Pre-existing CVD n (%)	36 (18.1)	-	25 (17.1)	-
Weight (kg)	87.8 (18.7)	86.4 (20.0)	84.8 (18.6)	81.1 (18.5)χ
BMI (kgm^{-2})	31.5 (5.7)	29.4 (9.1)	31.0 (5.9)	29.7 (5.8)
Waist circumference (cm)	104.4 (13.9)	102.3 (13.3)	102.9 (12.7)	100.3 (12.8)
Current smoker n (%)	20 (10.2)	20 (10.9)	22 (15.2)	18 (13.2)
$HbA_{1c\%}$	7.3 (1.8)	6.8 (1.1) χ	7.2 (1.5)	6.4 (0.5)χ
Total Cholesterol ($mmoll^{-1}$)	5.6 (1.3)	4.7 (1.1) χ	5.3 (1.2)	4.0 (1.1)χ
LDL cholesterol ($mmoll^{-1}$)	3.5 (1.0)	2.6 (0.9)	3.2 (1.0)	2.1 (0.7)
HDL cholesterol ($mmoll^{-1}$)	1.2 (0.3)	1.2 (0.3)	1.2 (0.4)	1.2 (0.3)
Triglycerides ($mmoll^{-1}$)	2.1 (1.4)	1.9 (1.4)	2.1 (1.9)	1.7 (2.3)
Systolic BP (mmHg)	148.4 (20.5)	140.1 (17.2)	143.7 (18.5)	129.3
Diastolic BP (mmHg)	89.5 (10.7)	82.0 (9.5) χ	87.8 (10.4)	75.2 (9.9)χ
Mean Arterial Pressure	109.1 (10.2)	101.3 (9.8) χ	106.4 (10.3)	93.2 (9.8) χ
Medication use (%)				
Anti-hypertensives	27.1	42.2 χ	40.4*	65.8χ
Lipid lowering	2.5	49.2 χ	4.8	82.5χ
Aspirin	4.0	28.2 χ	4.1	71.3χ
Metformin	-	38.5	-	53.9
Sulfonylurea	-	15.1	-	7.7
Insulin	-	1.7	-	2.1
Five-Year CHD risk (%)	12.6	10.1 χ	11.3	7.6χ

*Baseline between group difference p<0.05

χ between group difference at one year p<0.05

Table 4.2 Five and ten-year modelled CVD risk reduction and biomedical outcomes (adjusted for baseline variables, ethnicity and clustering effects)

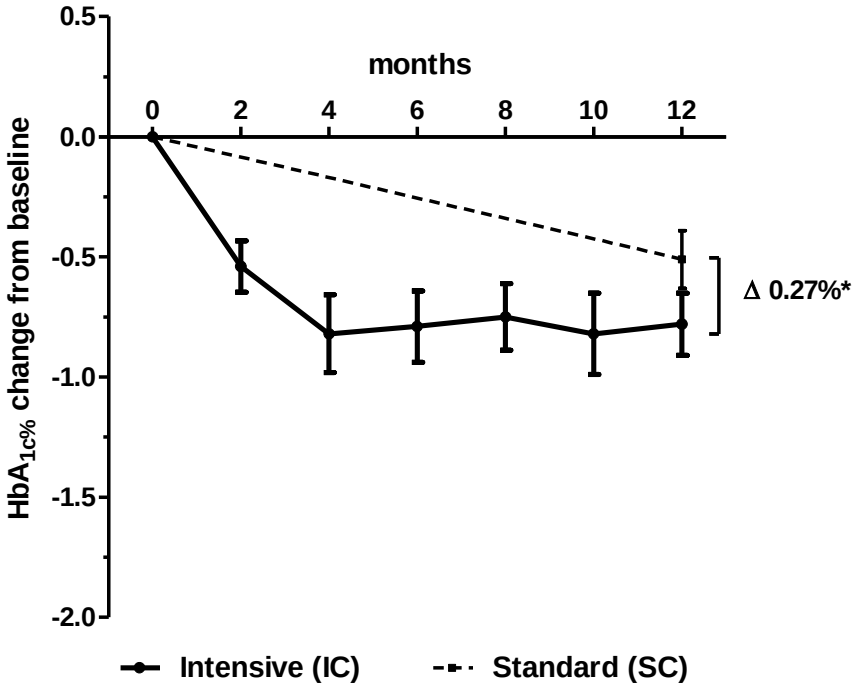
	Mean intervention effect (95% Confidence Interval)		Model summary	
	Standard care	Intensive care	Coefficient (95% CI)	P value
CHD five year risk (%)	-2.3 (4.8)	-3.2 (3.8)	-1.49 (-2.20 to -0.77)	<0.001
CHD ten year risk (%)	-4.5 (8.1)	-6.8 (7.6)	-3.11 (-4.60 to -1.63)	<0.001
$HbA_{1c\%}$	-0.6 (1.6)	-0.7 (1.4)	-0.20 (-0.31 to -0.08)	0.001
Weight (kg)	-2.2 (5.5)	-3.8 (5.5)	-1.78 (-3.05 to -0.50)	0.01
BMI (kgm^{-2})	-2.2 (6.5)	-2.3 (2.0)	0.64 (-0.60 to 1.90)	0.31
Waist circumference (cm)	-1.6 (6.0)	-2.8 (6.0)	-1.36 (-2.78 to 0.07)	0.06
Total cholesterol ($mmoll^{-1}$)	-1.0 (1.2)	-1.3 (1.3)	-0.56 (-0.87 to -0.25)	<0.001
LDL cholesterol (mmoll-1)	-0.9 (1.0)	-1.1 (1.0)	-0.47 (-0.71 to -0.23)	<0.001
HDL cholesterol ($mmoll^{-1}$)	0.02 (0.3)	-0.01 (0.2)	-0.03 (-0.08 to 0.02)	0.29
Triglycerides ($mmoll^{-1}$)	-0.2 (1.2)	-0.4 (2.5)	-0.21 (-0.56 to 0.15)	0.25
Systolic blood pressure (mmHg)	-8.4 (18.6)	-16.2 (19.6)	-10.41 (-14.82 to -6.00)	<0.001
Diastolic blood pressure (mmHg)	-7.9 (11.4)	-13.0 (11.4)	-6.21 (-8.27 to -4.16)	<0.001

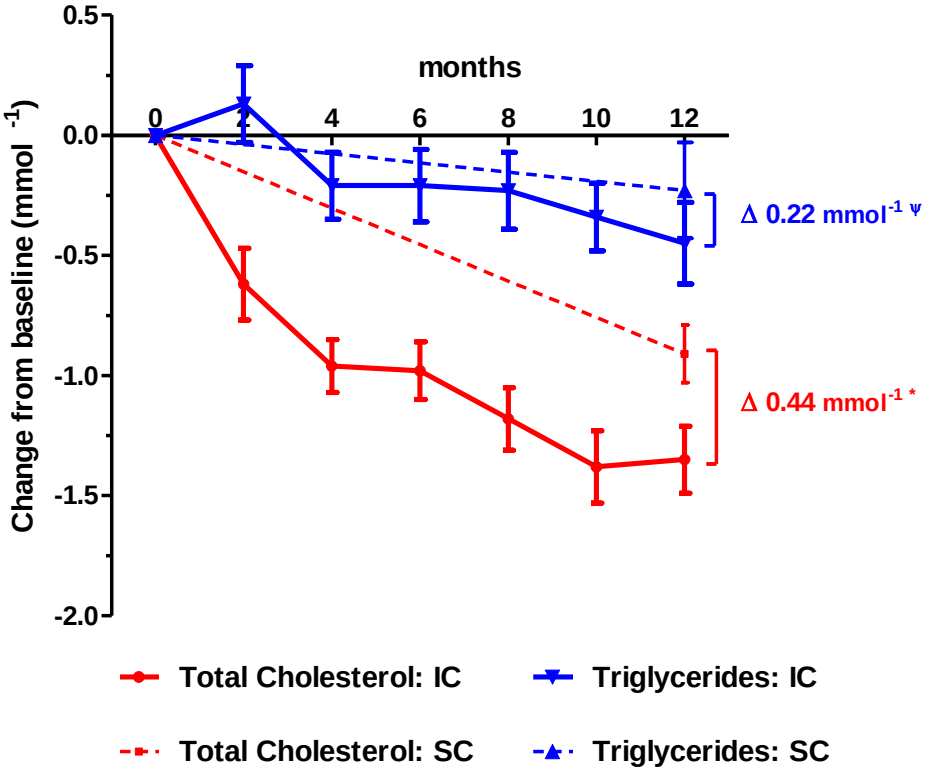
Table 4.3 Treatment satisfaction, perceived glucose control and quality of life

	Intervention mean (95% Confidence Interval)		Model summary	
	Standard care	Intensive care	Co-efficient	p value
DTSQc*	8.5 (7.24 to 9.71)	13.7 (12.8 to 14.7)	5.22 (3.67 to 6.77)	<0.0001
Hyperglycaemia†	-0.02 (-0.33 to 0.29)	-0.30 (-0.39 to 0.04)	-0.26 (-0.68 to 0.15)	0.21
Hypoglycaemia†	-0.02 (-0.28 to 0.24)	-0.57 (-0.90 to -0.24)	-0.53 (-0.88 to -0.18)	0.03
SF-12				
Physical score	38.5 (37.1 to 40.0)	39.0 (37.4 to 40.5)	0.54 (-1.27 to 2.35)	0.56 0.45
Mental score	39.2 (36.5 to 41.9)	38.2 (35.2 to 41.2)	-0.98 (-3.52 to 1.55)	

*High score reflects greater treatment satisfaction
† Based on the questions “how often have you felt that your blood sugars have been unacceptably high/low recently?” Low score reflects better control

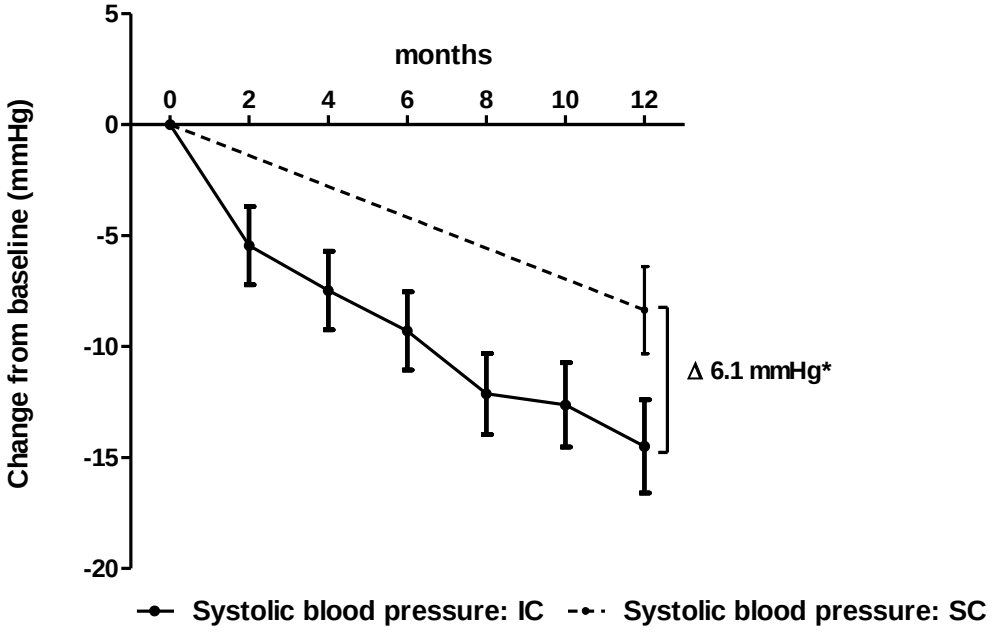
Figure 4.2 Change (unadjusted mean ±SE) in $HbA_{1c\%}$ (upper) and lipid indices (lower) within Intensive (IC) and Standard (SC) care intervention groups of the ADDITION-Leicester study

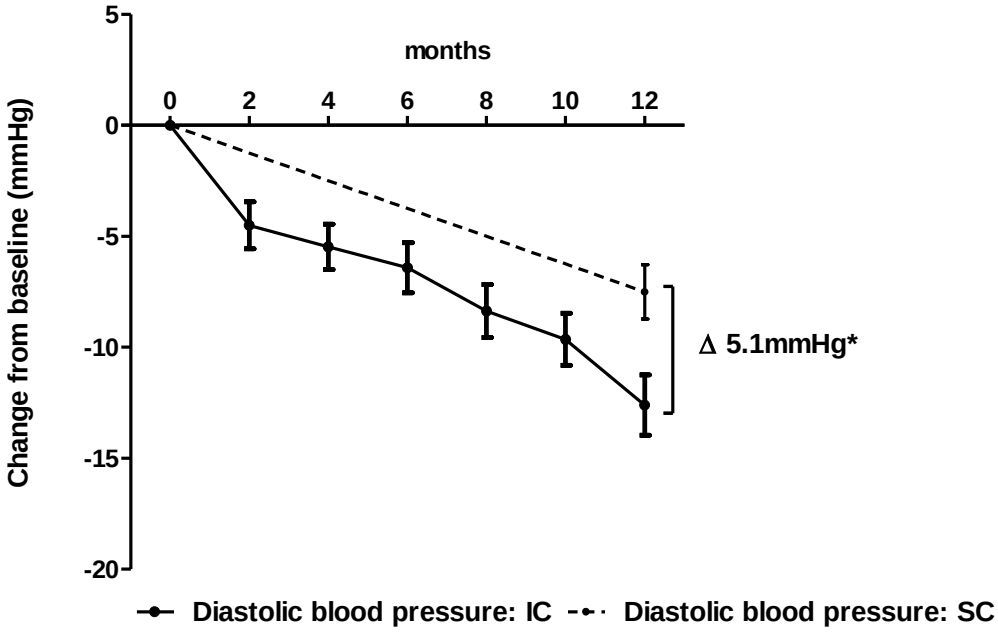




*p<0.05 Ψ: not significant
NB. SC data collected at baseline and 12 months only

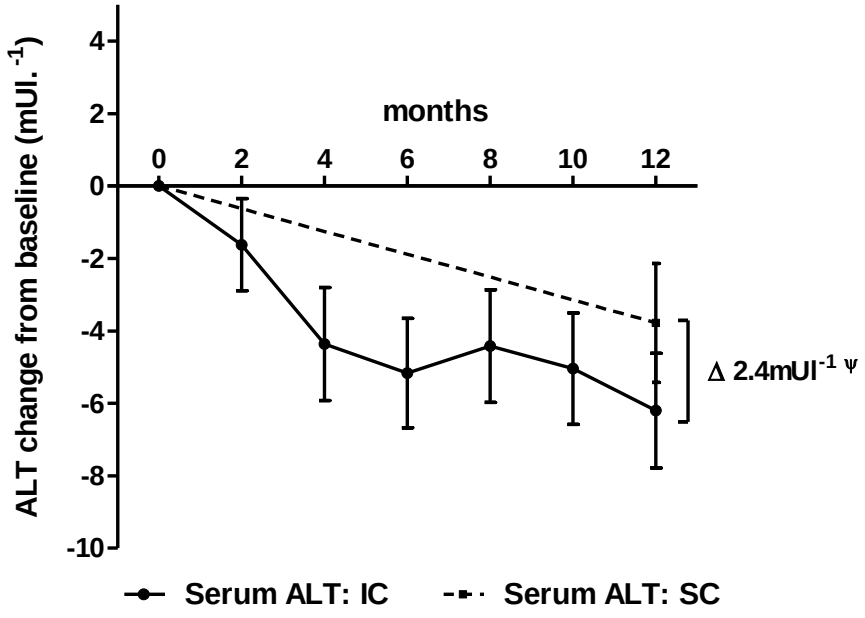
Figure 4.3 Change (unadjusted mean ±SE) in systolic (upper) and diastolic blood pressure (lower) within Intensive (IC) and Standard (SC) care intervention groups of the ADDITION-Leicester study

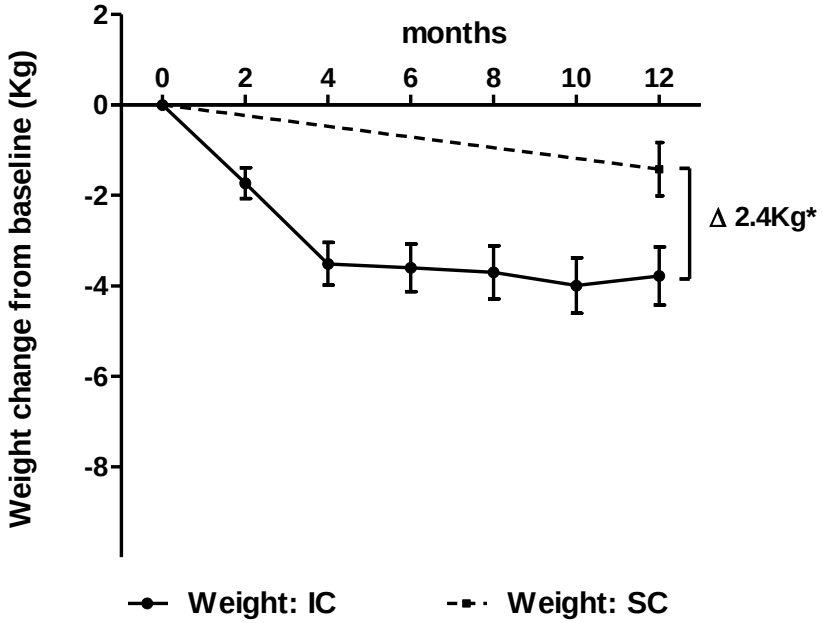




*p<0.005
NB. SC data collected at baseline and 12 months only

Figure 4.4 Change (unadjusted mean ±SE) in serum Alanine Transaminase (ALT) (upper) and weight (lower) within Intensive (IC) and Standard (SC) care intervention groups of the ADDITION-Leicester study





*p<0.05 Ψ: not significant
NB. SC data collected at baseline and 12 months only

4.4.3 Adverse effects: hypoglycaemia

There were nine mild symptomatic hypoglycaemic episodes reported in the intensive intervention arm and sixteen in the routine care arm (p=0.17). No major hypoglycaemic events were recorded for either group during the study.

4.4.4 Quality of Life data

Scores for DTSQc and SF-12 questionnaires are displayed in Table 4.3. There was a highly significant difference in overall treatment satisfaction in favour of the intensive intervention group. Perceived frequency of hypoglycaemia also decreased significantly in this group. There were no significant differences in SF-12 quality of life physical or mental scores between the two groups at twelve months.

4.5 Discussion

Expert consensus recommends establishing firm evidence of benefits arising from the early identification of diabetes through screening. Implicit here is a requirement for randomised trials of cardiovascular risk intervention in screen detected cases, preferably with patient centred clinical endpoints relating to macrovascular events. The disappointing results of recent glucose lowering cardiovascular outcome trials in established type 2 diabetes suggest that the time-frame, size and delivery of a study for newly diagnosed screen detected patients may be logistically challenging [62-64]. The ADDITION-Europe study, a multi-centered (ADDITION-Denmark, Netherlands, Cambridge and Leicester, United Kingdom) randomised trial in over 2,000 such cases will

report five year composite cardiovascular outcomes in late 2010. Until then and possibly beyond we will be reliant upon shorter more manageable studies using surrogate cardiovascular outcomes relevant to particular populations. Whilst adhering to the target-driven intervention protocol of the collaborative study, participating centres (including Leicester) were individually powered to assess modelled cardiovascular risk reduction and were free to style their own intensive intervention and screening methods.

ADDITION-Leicester was specifically designed to assess the feasibility of non-risk factor based screening and used the glucose tolerance test to capture early post-challenge hyperglycaemia particularly prevalent within insulin resistant migrant south Asian populations. The composition of the study population (43% south Asian in intensive group) indicates that intensive cardiovascular risk intervention in screen detected type 2 diabetes is feasible within multiethnic Western populations.

The ADA currently recommends regular population screening starting at 45 years of age. Our results should therefore be particularly applicable to the North American experience as the study adopted a universal approach to screening and did not stratify high risk volunteers.

ADDITION-Leicester used peripatetic clinics run by a dedicated specialist team (specialty doctor and diabetes educator) to deliver a community based intervention. This is in contrast to other ADDITION centres which relied upon the incumbent primary care team to achieve the targets set out in the intensive management protocols. Published data from ADDITION-Netherlands, which adopted a similar approach to Leicester, demonstrated equally dramatic improvements in cardiovascular risk [248]. Comparison of

our one year results with other centres using less pragmatic approaches will assist in the identification of an optimal treatment strategy for screen detected cases.

Although the current concept of multi-factorial intervention in type 2 Diabetes has validity within selected, usually high risk groups there is little direct evidence that this approach is effective in population settings where most of the disease is located and managed. Whilst cumulative extrapolation of the results of outcome trials targeting individual risk factors has credence, it could be argued studies examining the effects of multiple interventions within highly representative populations are just as important. A major strength of ADDITION-Leicester is its attempt to recruit and then realistically manage a non-selected population personifying everyday general practice. The study has additional important strengths, including independent in house data collection, individualised prescription counts, a remarkably low dropout rate and extremely robust screening methodology. The proportion of south Asians entering the study should also be regarded as a success given the recognised difficulties of engaging often vulnerable ethnic groups in preventative rather than curative medicine.

ADDITION-Leicester is not sufficiently powered to demonstrate a difference in vascular events at one year and the results, based on modelled outcomes should therefore be interpreted with caution. There is little doubt that primary care practice is becoming more intensive as national guidelines recommend tighter control of cardiovascular risk factors in diabetes. It is therefore possible the impressive biomedical differences seen at this stage will be attenuated by more aggressive intervention in the “control group” in future comparisons. In

this respect it could be argued our control group is in fact not a true “control” rather a reflection of a comparable treatment approach.

Although there were no major differences observed in the magnitude of risk reduction between south Asians and white Europeans, ADDITION-Leicester was neither designed or powered as an inter-ethnic comparison study and should not be interpreted as such. By virtue of differing entry criteria (25-75 years for south Asians and 40-75 years for white Europeans) it would be necessary to adjust this data for the resultant difference (7.8 years) in mean age between the groups and this should be acknowledged as a potential limitation of this secondary analysis.

A criticism of population level screening studies is selection bias and the perception that screening is less likely to engage high risk groups with the most to gain from earlier diagnosis and treatment. Those attending ADDITION-Leicester were older and more likely to be female than those invited (56.7 versus 51.7 years, 50.1% versus 47.7% respectively unpublished data), an observation previously reported in screening programmes for diabetes [231-235]. Whilst acknowledging that this must be taken into account when reporting prevalence and outcomes we feel our sample approximated the background population as closely as possible and is a true representation of a major multiethnic screening programme in the United Kingdom. This pattern of recruitment was consistent across practices and did not influence the intervention as intervention and control groups were well matched at baseline.

4.6 Conclusion and implications for clinical practice

The results of this study demonstrate that intensive, multi-factorial treatment for people with type 2 diabetes identified through community based non-selective screening is feasible and reduces modelled cardiovascular risk without adverse effects on quality of life or hypoglycaemia. Cardiovascular risk reductions were achieved with available life-style interventions and pharmacotherapy with comparable effects in white Europeans and south Asians. These impressive one year effects on surrogate cardiovascular risk factors would be expected to ultimately improve “hard” clinical outcomes for people with type 2 diabetes and strengthen the case for screening and earlier latent phase intervention.

Chapter 5

PACE 1

Impact of metabolic indices on central artery stiffness

5.1 Summary

Aims/hypothesis: Non invasive measures of aortic stiffness reflect vascular senescence and predict outcome in diabetes. Glucose mediated elastic artery sclerosis may play an integral role in the development of macrovascular complications. We used carotid-femoral PWV ($_{cf}$PWV) to quantify independent associations with 1) fasting glucose 2) post-challenge glucose and 3) derived Insulin Resistance (HOMA-IR) with aortic stiffness.

Methods: $_{cf}$PWV was measured using a MicroMedical PT4000 platform within newly identified, age-gender matched Normal Glucose Metabolism (NGM) (n=176), Impaired Glucose Regulation (n=219) (IGR) and diabetes populations (n=175)

Results: Before and after multivariate adjustment IGR and diabetes were associated with significant aortic stiffening compared with NGM (adjusted $_{cf}$PWV ± se (ms^{-1}): NGM: 9.15±0.12, IGR: 9.76±0.11 (p=<0.001), diabetes: 9.89±0.12 (p<0.001). IGR stratification indicated impaired fasting (IFG) (9.71±0.12) and post challenge (IGT) (9.82±0.24) glucose categories have similar $_{cf}$PWV (p=0.83). Modelled predictors of $_{cf}$PWV were used to assess independent metabolic associations with arterial stiffness. Fasting glucose concentration (β=0.10 (95%CI: 0.05, 0.18) p=0.003), 2 hour post-challenge glucose (β=0.14 (95%CI: 0.02, 0.23) p<0.001) and HOMA-IR (β=0.20 (95%CI: 0.05, 0.53) p<0.001) independently related to $_{cf}$PWV after adjustment for age, gender, MAP, heart rate, BMI, renal function and anti-hypertensive medication.

Conclusion: IGR characterised by fasting or post-challenge hyperglycaemia is associated with significant vascular stiffening. 2 hour post-challenge glucose and HOMA-IR are the most powerful metabolic predictors of arterial stiffness, implying hyperglycaemic excursion and insulin resistance play important roles in the pathogenesis of arteriosclerosis.

5.2 Introduction

There is a continuous relationship between fasting and post-challenge plasma glucose concentration and incident cardiovascular events well below existing diagnostic thresholds for diabetes [249]. As the more predictive index of mortality, 2 hour plasma glucose following a standard OGTT (2-HPG) may contribute to the pathogenesis of macrovascular disease more than fasting plasma glucose values (FPG) [33].

Premature conduit vessel arteriosclerosis predicts mortality in type 2 diabetes [124] and may be attenuated by therapeutic approaches with direct glucose or blood pressure lowering potential [159-165,250-252]. Independent association of FPG and 2-HPG indices with the haemodynamic consequence of early arteriosclerosis (specifically conduit vessel stiffening) implies potentially modifiable structural mechanisms connect hyperglycaemia with vascular complications even within “pre-diabetes” categories of IGT and IFG [136-140, 253]. These relationships are mostly reported individually or as a component of the NCEP Metabolic Syndrome [141-143, 254-258] and do not specifically address relative strengths of FPG and 2-HPG associations with measures of arterial stiffness.

Establishing the role of insulin resistance in the pathogenesis of thrombotic vascular disease has renewed significance because recent outcome trials utilising glucose lowering endpoints have failed to prevent cardiovascular events [62-64]. Insulin resistance mediated premature vascular sclerosis may be an important early feature of sub-clinical atherosclerosis and in part explain

observed gender disparity in population stiffness measures and cardiovascular morbidity [259-265].

The aim of this study was to compare independent associations of glucose measures, namely FPG and 2-HPG concentration, insulin resistance (HOMA-IR) and $HbA_{1c\%}$ with carotid-femoral Pulse Wave Velocity ($_{cf}$PWV), a validated, non-invasive measure of central aortic stiffness. We sought to characterise premature vascular stiffening in non-diabetes range glucose disorders and compare the independent contribution of glucose indices to this process within a treatment naïve age and gender matched population.

5.3 Methods

5.3.1 Study population

A sub-group was randomly selected from the screening phase of the ADDITION-Leicester study. As described in Chapters 2 and 3, this program characterised cardio-vascular risk and glucose status in 6,749 volunteers recruited from twenty general practices between August 2005 and December 2009. Single session repeated $_{cf}$PWV measurements were obtained within four weeks of a standard OGTT, lipid profile, medical history and anthropometric assessment in individuals screened through ADDITION-Leicester and consenting to another vascular assessment study (PACE). Details of the recruitment process for PACE 1 are described in Chapter 2 and Figure 5.1. As it was impossible to offer every volunteer a $_{cf}$PWV appointment within the study timeframe, an eligible population was randomly selected to provide an adequately powered, matched sample evenly distributed across

the glucose spectrum. The operator was blind to this process and participants specifically asked not to reveal the results of screening prior to the completion of vascular tests. Resultant $_{cf}$PWV measurements were then returned to the independent researcher and matched to glucose data.

5.3.2 $_{cf}$PWV measurements

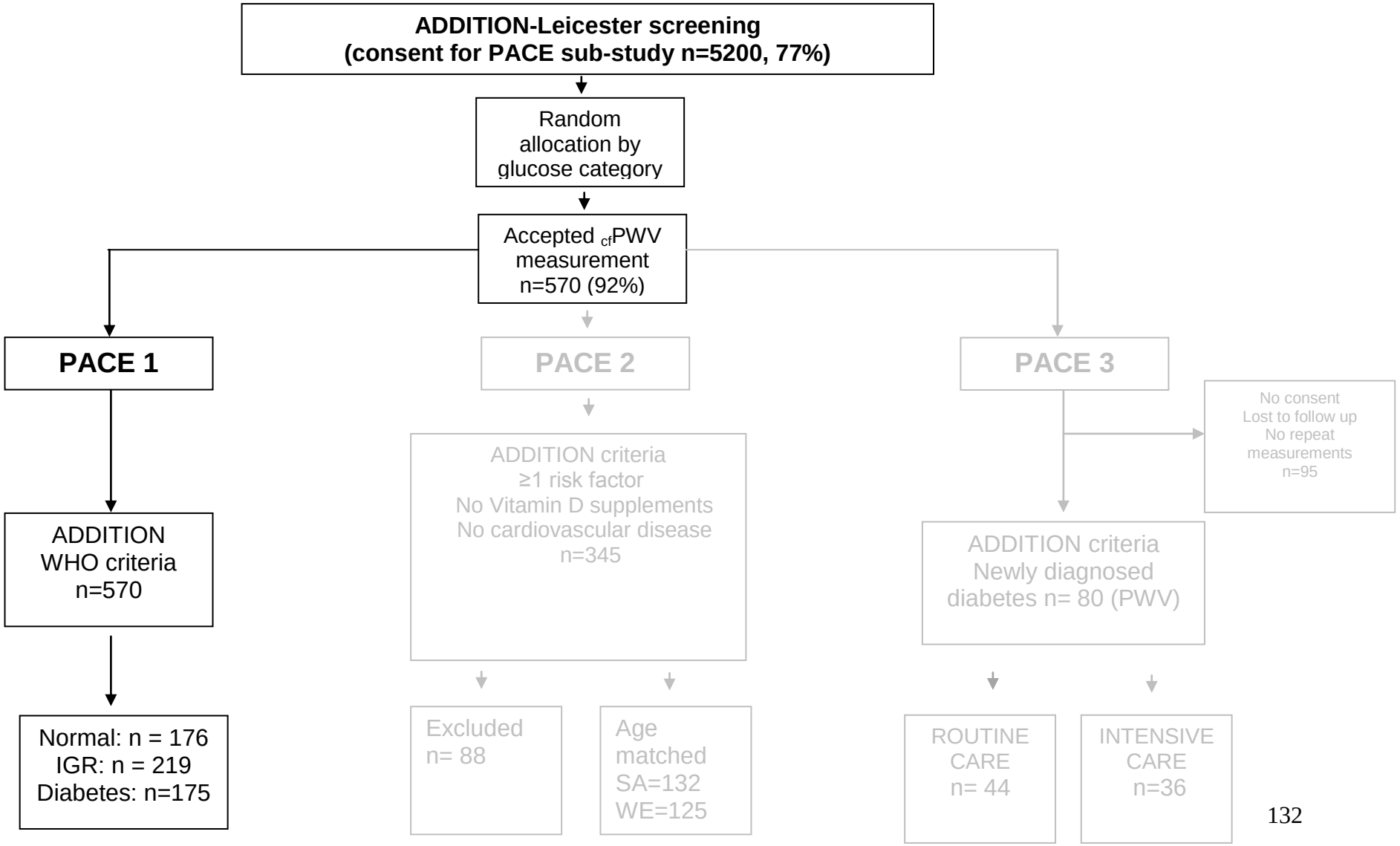
A single operator blinded to glucose status performed all arterial measurements within a single site research facility at the University Hospitals of Leicester. For further standardisation, an intra-operator reproducibility study was performed on a group of volunteers participating in the one year intervention trial (Chapter 7) and a $_{cf}$PWV measurement protocol strictly adhered to (Appendix B). Participants were fasted, rested supine and asked to refrain from smoking or consuming caffeine based beverages prior to $_{cf}$PWV assessment. Supine brachial artery blood pressure and heart Rate were recorded using a semi-automated oscillometric device (OmronMS-I). After ten minutes rest these were reassessed and $_{cf}$PWV measurements only performed once haemodynamic stability was achieved. Subjects were supine whilst $_{cf}$PWV was measured using a 4MHz continuous-wave doppler ultrasound probe (Micro Medical Pulse Trace PT4000). The cutaneous distance between the site of the femoral pulsation and the sternal notch was repeatedly measured and entered into the device according to manufactures guidelines detailed in Chapter 3. After archiving a minimum of three ten second continuous waveforms, data was processed using existing software and mean $_{cf}$PWV calculated. Captured sequences with waveform $_{cf}$PWV variation greater than 5% were rejected according to manufacturer's

recommendations. $_{cf}$PWV (ms^{-1}) was calculated by dividing measured surface difference by the respective ECG derived transit time.

5.3.3 Biochemical measurements

Glucose status was determined by fasting and post-challenge (120 minutes) plasma levels according to WHO criteria [21,226]. Newly diagnosed type 2 diabetes was defined by either a fasting glucose ≥7,0 or post-challenge ≥11.1$mmoll^{-1}$ on two occasions at least 48 hours apart. IFG and IGT diagnostic cut-offs are described in Chapter 1 together with the composite IGR term. For clarity subjects within the normal glucose range are referred to in text and diagrams as normal glucose metabolism (NGM). As an aim of the study was to characterise the independent effects of fasting and post-challenge glucose on $_{cf}$PWV, subjects with combined intermediate levels (i.e. Fasting 6.1-6.9 and post-challenge 7.8-11.1$mmoll^{-1}$) were categorised separately within an IFG/IGT term. Glucose was analysed via the hexokinase method (NADPH production at 340nm Abbott Aeroset) and $HbA_{1c\%}$ using DCCT aligned BIO-rad Variant II HPLC system**.** Quantitve analysis of serum Insulin was carried out using the AutoDelfia time-resolved fluoro-immunoassay (Perkin Elmer AutoDelfia1235). Insulin intra-assay co-efficient of variation (CV) was <10%. The Homeostatic Model Assessement for Insulin Resistance (HOMA-IR) score was used as a validated surrogate of insulin sensitivity obtained through a euglycaemic glucose clamp [266].

Figure 5.1 Schematic illustration of recruitment flow for the PACE 1 sub-study



Microalbuminuria was defined as a urine albumin:creatinine ratio in a random sample of more than 2.5 $mgmmol^{-1}$ in men and 3.5 $mgmmol^{-1}$ in women. Estimated Mean Arterial Pressure (MAP) was calculated by adding diastolic and one third pulse pressure (systolic – diastolic) measurements recorded during the $_{cf}$PWV session.

5.3.4 Power Calculation and Statistical Analysis

The study was powered to determine differences between IGR and diabetes as well as sub-group categories of IGR. A minimum of 202 (101 per group) subjects were required to demonstrate a clinically significant $_{cf}$PWV difference (>0.8ms^{-1}) between IGR and diabetes groups at 80% power and (2-tailed) alpha 0.05, based on a conservatively estimated dependent variable standard deviation of 2.5 [124]. From available data (using brachial-ankle PWV) 90 (45 per group) subjects were required for similar IFG and IGT comparisons (80% power, alpha 0.05 $_{cf}$PWV SD1.5) [267]. Data was analysed using SPSS software (version 14) and assessed using Kolmogorov-Smirnov tests. Normally distributed continuous data was compared using ANOVA or Student's independent t-test, and categorical data was analysed using a chi-square test to determine univariate between group differences. Adjusted linear regression models were constructed to assess the influence of glucose indices, and HOMA-IR, as independent predictors of $_{cf}$PWV. Parameters selected for the stepwise method of regression were based on univariate analyses and variables known to be associated with arterial stiffness. The significance level was set at $P<0.05$. Data is presented as mean +/- standard deviation, percentages, counts or ratio and all tests are two-tailed.

5.4 Results

5.4.1 $_{cf}$PWV acquisition

Overall 77% of those participating in ADDITION-Leicester also consented for the arterial measurement sub-study. Ninety-two percent of waveform sequences were considered of sufficient quality for inclusion, with recognised limitations of the $_{cf}$PWV technique accounting for the majority of discounted cases. Thirty-eight subjects were excluded as a result of morphological variability of waveform capture (n=22), electrocardiographic rhythm disturbance (frequent ventricular ectopic activity, extreme heart rate variability, atrial fibrillation) (n=12), or blood pressure instability (n=4). Patients with diabetes or IGR were equally represented in the excluded cases group (chi squared, p=0.83).

5.4.2 Biomedical characteristics

Glucose comparisons are presented as both categorical and continuous data. Table 6.1 describes biochemical and anthropometric characteristics of the selected NGM (n=176), IGT (n=142), IFG (n=47), IFG/IGT (n=30) and diabetes (n=175) $_{cf}$PWV study population. As there were no significant differences between IGT and IFG baseline characteristics, these are pooled as an IGR (n=219) composite term.

Groups were matched for age, gender and ethnicity. There were anticipated differences in glucose, lipid and anthropometric measures but no statistically significant difference in reported history of cardiovascular disease. Despite more frequent use of anti-hypertensive therapy brachial systolic pressures were higher in both IGR and diabetes groups. There were no significant differences in the nature of anti-hypertensive prescribed, ACE-Inhibitors / angiotensin receptor blockers were

used most frequently (57%), followed by diuretics (43%), beta-blockers (41%) and calcium channel antagonists (28%) (p value trend = 0.47, 0.78, 0.88 respectively for NGM, IGR, diabetes)

5.4.3 Glucose comparisons

Thoraco-abdominal aortic stiffness as determined by non-invasive $_{cf}$PWV increased with deteriorating glucose metabolism before and after adjustment for age, gender, MAP, BMI and self-reported cardiovascular disease (Table 5.1 and Figure 5.2a). Significant differences were observed between NGM and IGR categories, (adjusted mean $_{cf}$PWV ± standard error of mean: 9.15±0.12 ms^{-1} vs. 9.76±0.11 ms^{-1} p=<0.001 respectively), and NGM and diabetes categories (9.15±.0.12 ms^{-1} vs. 9.81±0.12 ms^{-1} p<0.001 respectively) (figures 5.2 and 5.3). This relationship was maintained after stratification of IGR into WHO defined impaired fasting (IFG (9.82±0.22 ms^{-1}) p=0.006 vs NGM) and post-challenge (IGT (9.71±0.14 ms^{-1}) p=0.002 vs NGM) hyperglycaemic categories. There was no significant difference between IGR and diabetes mean $_{cf}$PWV (Figure 5.2a (p=0.18)). Mean $_{cf}$PWV was significantly elevated within isolated FPG (FPG abnormality alone 9.95±0.22 ms^{-1} vs NGM 9.15±0.12 ms^{-1} p<0.001) or 2-HPG (2-HPG alone abnormality 9.77±0.12 ms^{-1} vs NGM 9.15±0.12 ms^{-1} p<0.001) hyperglycaemic categories (Figure 5.4).

Table 5.1 Baseline demographics and biomedical characteristics of age and gender matched $_{cf}$PWV PACE 1 study population

	NGM n=176	**IGR n=219**	**DM** n=175	**p***
Age (Years)	58.2 ± 8.9	59.4 ± 10.4	58.7 ± 9.8	0.51
Gender (%M)	55	53	57	0.58
Ethnicity (%WE)	53	57	59	0.58
Fasting Glucose ($mmoll^{-1}$)	5.0 ± 0.4	5.7 ± 0.6	7.7 ± 2.5	<0.005
Post-Load Glucose ($mmoll^{-1}$)	5.5 ± 1.1	8.5 ± 1.5	13.8 ± 4.0	<0.005
$HbA_{1c(\%}$	5.7 ± 0.4	6.0 ± 0.5	7.1 ±1.5	<0.005
HOMA-IR (arb. units)	2.07(0.91-2.17)	3.01(1.46-3.70)	4.57(2.48-6.62)	<0.005
Total Cholesterol ($mmoll^{-1}$)	5.4 ± 1.1	5.5 ±1.1	5.5 ± 1.3	0.78
HDL-C ($mmoll^{-1}$)	1.33 ± 0.3	1.26 ± 0.3	1.18 ± 0.4	<0.005
Triglycerides ($mmoll^{-1}$)	1.39(0.9-1.6)	1.65(1.0-2.0)	2.20(1.2-2.4)	<0.005
C-Reactive Protein (mgl^{-1})	3.03 ± 4.45	3.78 ± 5.22	6.92 9.64	<0.005
White Cell Count ($x10^{9}l^{-1}$)	6.50 ± 1.71	7.16 ±1.64	7.27 ± 1.64	<0.005
ALT (IUl^{-1})	21.3 ± 10.1	24.6 ± 15.5	30.0 ± 17.7	<0.005
Systolic BP (mmHg)	132.1 ± 17.0	133.9 ± 15.3	136.7± 17.9	0.03
Diastolic BP (mmHg)	80.1 ± 9.4	81.6 ± 8.3	82.6 ± 9.3	0.25
Pulse Pressure (mmHg)	51.1 ± 12.5	52.3 ± 12.0	54.1 ± 13.6	0.08
MAP(mmHg)	98.0 ± 10.9	99.0 ± 9.6	100.6 ± 11.1	0.03
Heart Rate (min^{-1})	63.5 ± 10.3	64.7 ± 9.9	66.8 ± 11.1	0.01
Weight (kg)	77.7 ± 15.9	79.8 ± 15.2	85.8 ± 17.4	<0.005
BMI (kgm^{-2})	28.2 ± 4.4	29.0 ± 4.6	31.1 ± 5.3	<0.005
Waist Circumference (cm)	94.7 ± 12.3	97.3 ± 11.3	102.8 ±11.9	<0.005
Lipid Lowering (%)§	20.1	20.2	21.4	0.99
Anti-hypertensive (%)$	24.7	37.4	38.2	0.01
Active Smoking (%)	11	11	10	0.35
Cardiovascular Disease (%) †	9	12	11	0.08
Microalbuminuria (%)	5	10	13	0.03
$_{cf}$PWV (ms^{-1})	8.91 ± 1.69	9.76 ± 2.06	10.04 ± 2.09	<0.005

*p: ANOVA (continuous) / chi^2 (categorical) within groups trend.
§ Reported prescription of HMG-CoA Reductase or PPAR-α receptor inhibitor therapies
$ Reported prescription of ACE inhibitor, Angiotensin Receptor Blocker , Calcium channel blocker or beta blocker therapies
† Reported history of Cardiovascular Disease (CVD): Ischaemic Heart Disease, myocardial infarction, stroke or peripheral vascular disease

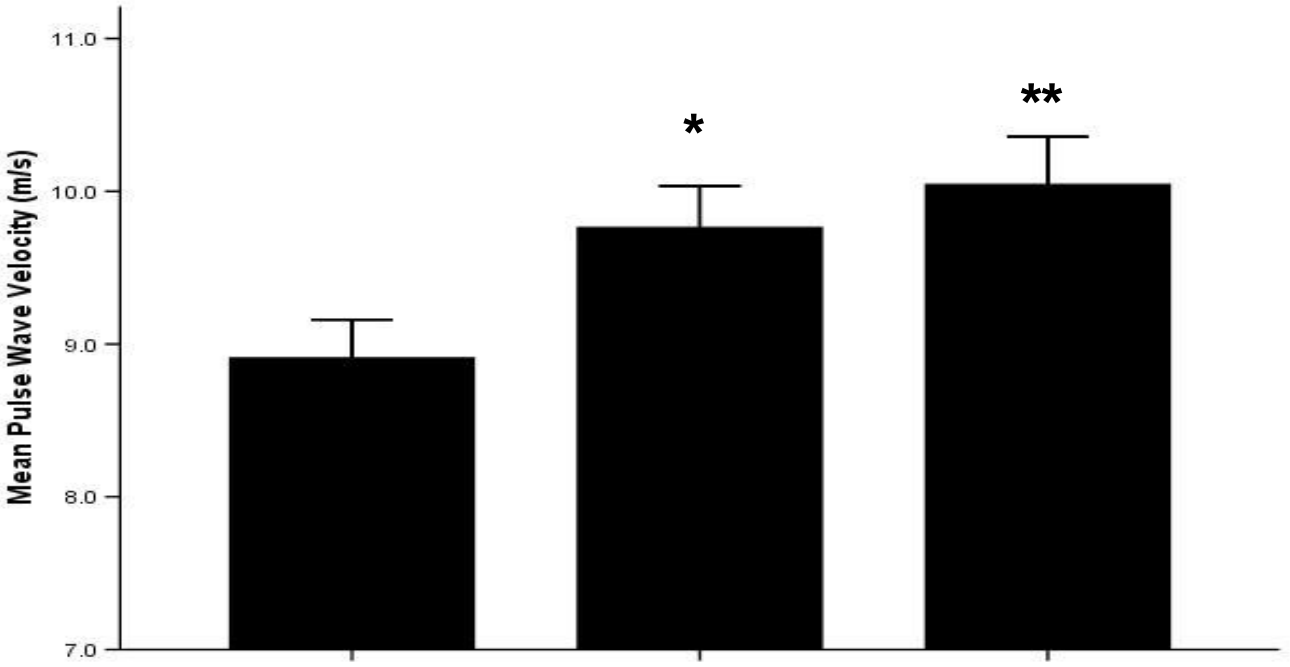
5.4.4 Univariate and multivariate analysis

Univariate analyses were performed to determine the strength of $_{cf}$PWV correlations with FPG, 2-HPG, HOMA-IR and a range of other variables (Table 5.2). Age (β 0.58, p<0.01), MAP (β 0.52, p<0.01), anti-hypertensive medication (β 0.23, p<0.01), self reported cardiovascular disease (β 0.21 <0.01), lipid lowering therapy (β 0.18 p=0.01), microalbuminuria (β 0.14 p=0.01), female gender (β 0.10 P=0.01), BMI (β 0.09, p=0.04), FPG (β 0.09, P=0.03), 2-HPG (β 0.15, P<0.01), and log HOMA-IR (β 0.14 p<0.01) were associated with $_{cf}$PWV.

In order to control the number of covariates entered and limit the effects of co-linearity we constructed three multivariate models to assess correlations of $_{cf}$PWV with FPG, 2-HPG, HbA1c% and HOMA-IR after adjustment for other factors. Model 1 included FPG, 2-HPG glucose or HOMA-IR index, age, MAP, self-reported cardiovascular disease (CVD), gender, BMI, Heart Rate (HR) and anti-hypertensive therapy. Lipid lowering therapy and microalbuminuria were added in place of anti-hypertensive therapy as independent variables in models 2 and 3 respectively. The strongest positive predictors of $_{cf}$PWV in all three multivariate models were age, MAP, gender, and heart rate (eg. standardised β-coefficients in FPG analysis: 0.57, 0.22, 0.15, 0.15 (all p=<0.01)). All 3 models demonstrated strong relationships between $_{cf}$PWV and FPG (model 1: β=0.09, model 2: β=0.09, model 3: β=0.10 all p<0.01) 2-HPG (all 3 models β=0.14 p<0.001) and HOMA-IR (model 1: β=0.15, model 2: β=0.16, model 3: β=0.20 all p<0.001). The strength (as measured by standardised β coefficients) of 2-HPG and HOMA-IR associations were comparable to that of directly measured haemodynamic covariates (MAP and heart rate). These analyses are shown in abbreviated form in Table 5.4 with only the independent

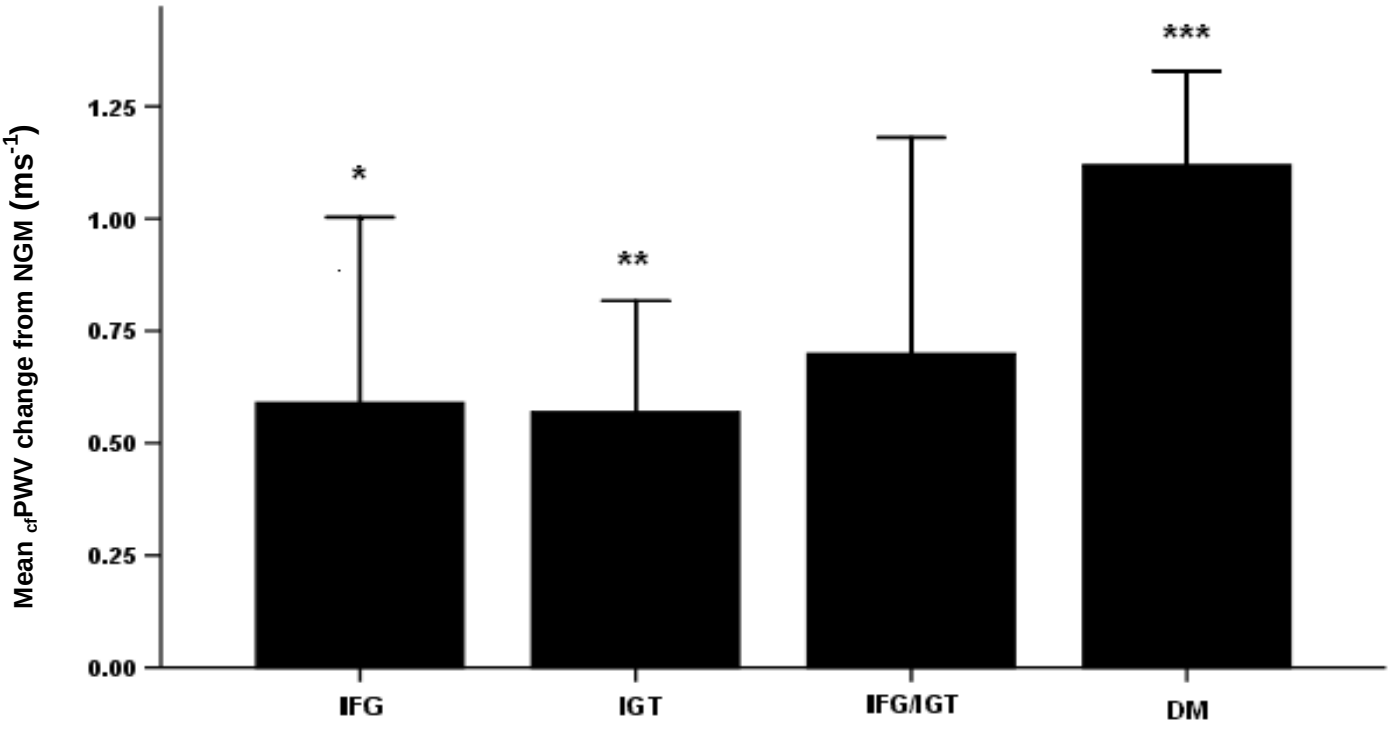
effects of the parameters of interest indicated. Parameter estimates and cumulative effects of model 1 are included in Appendix E as an example of working. Multivariate regression within age quartiles made little difference to the contribution of glucose indices to these linear models and this was confirmed with logistic and complex non-linear regression (not included in this thesis). Figure 5.5 illustrates forced linear regression and comparable slopes for adjusted $_{cf}$PWV versus FPG and $_{cf}$PWV versus 2-HPG lines.

Figure 5.2a Unadjusted mean $_{cf}$PWV ± SEM in Normal Glucose Metabolism (NGM), Impaired Glucose Regulation (IGR) and diabetes (DM) categories



*** NGM vs IGR p<0.0001** **IGR vs DM p=0.18** **** NGM vs DM p<0.0001**

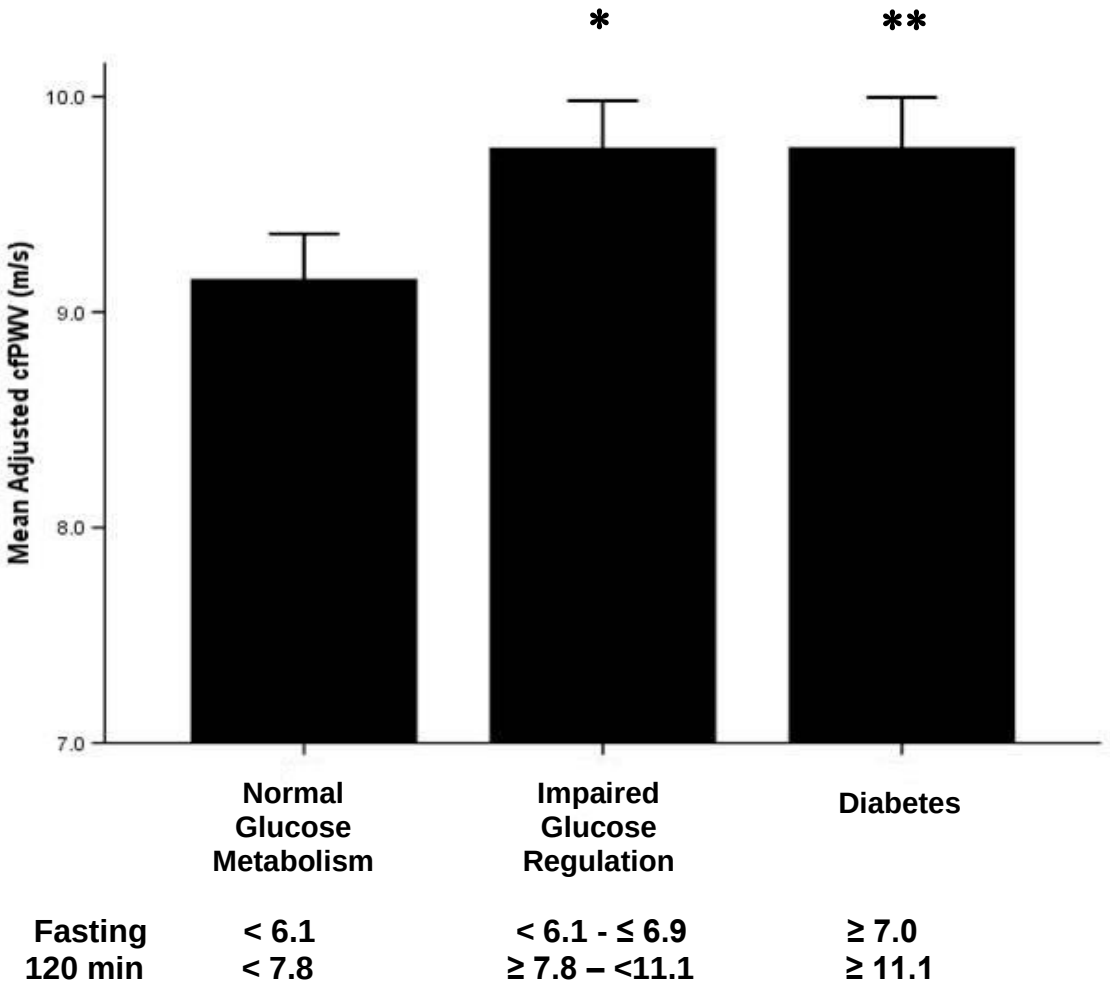
Figure 5.2b Effects of IFG, IGT and diabetes range hyperglycaemia on mean $_{cf}$PWV (depicted as mean PWV change ± SEM from NGT)



***NGM vs IFG (p=0.001)** ****NGM vs IGT or IFG/IGT (p=<0.001)** *****NGM vs DM (p<0.001)**

IFG vs IGT, IFG vs DM, IGT vs DM: not significant

Figure 5.3 Adjusted mean $_{cf}$PWV±SEM (§) in Normal Glucose Metabolism (NGM), Impaired Glucose Regulation (IGR) and diabetes (DM) categories



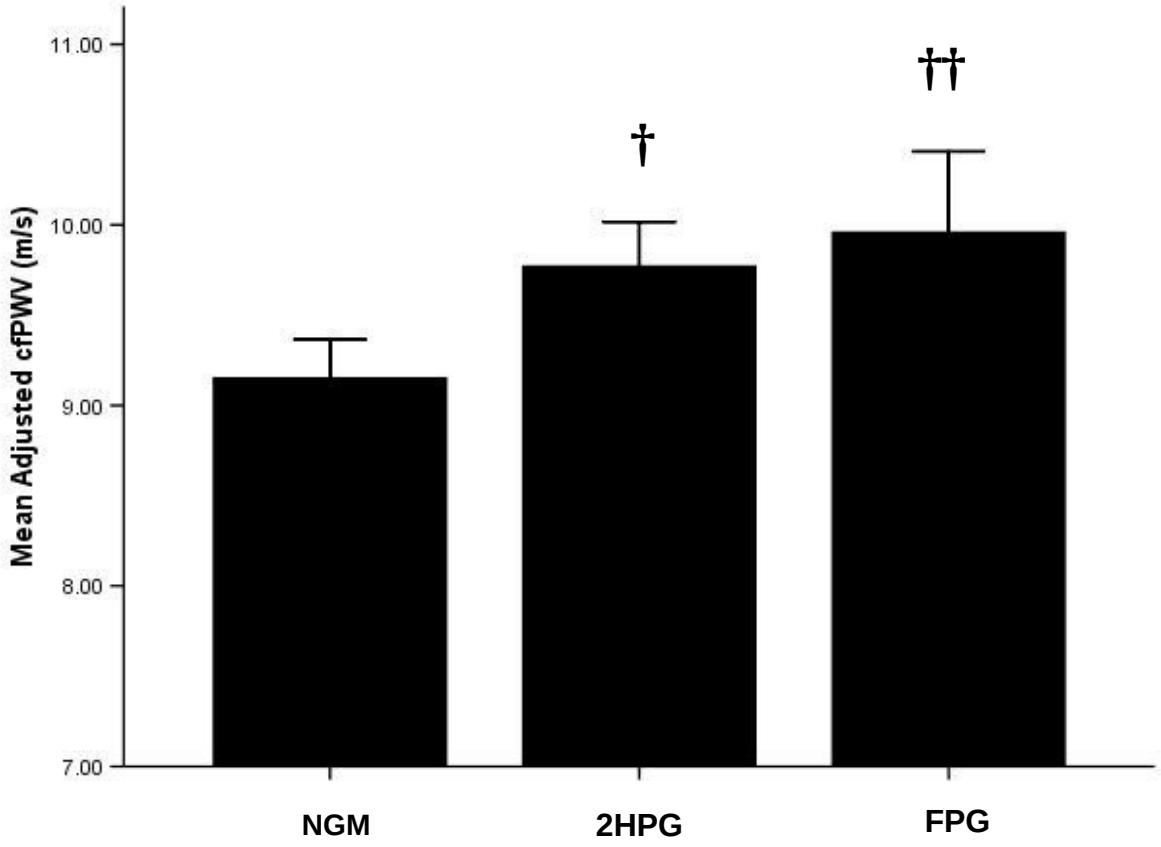
* NGM (9.15± 0.12 m/s) vs IGR (9.76 ± 0.11 m/s) p<0.001

**NGM (9.15 ± 0.12 m/s) vs DM (9.81 ± 0.12 m/s) p<0.001

$ Adjusted for age, gender, MAP, BMI, and CVD

NGM: Normal Glucose Tolerance
IGR: Impaired Glucose Regulation
DM: Diabetes

Figure 5.4 Adjusted mean PWV (ms^{-1}) ±SEM (§) of isolated abnormal post challenge (2HPG) (n=174) and fasting glucose (FPG) (n=60) groups



Fasting	**< 6.1**	**< 6.1**	**≥ 6.1**
120 min	**< 7.8**	**≥ 7.8**	**< 6.1**

† NGM (9.15 ± 0.12 ms^{-1}) vs. abnormal 2HPG (9.77 ± 0.12 ms^{-1}) p <0.001

†† NGM (9.15 ± 0.12 ms^{-1}) vs. abnormal FPG (9.95 ± 0.22 ms^{-1}) p<0.001

$ Adjusted for age, gender, MAP, BMI, and CVD

Table 5.2 Linear Regression analysis demonstrating standardised parameter associations in a univarate model with $_{cf}$PWV as dependent variable

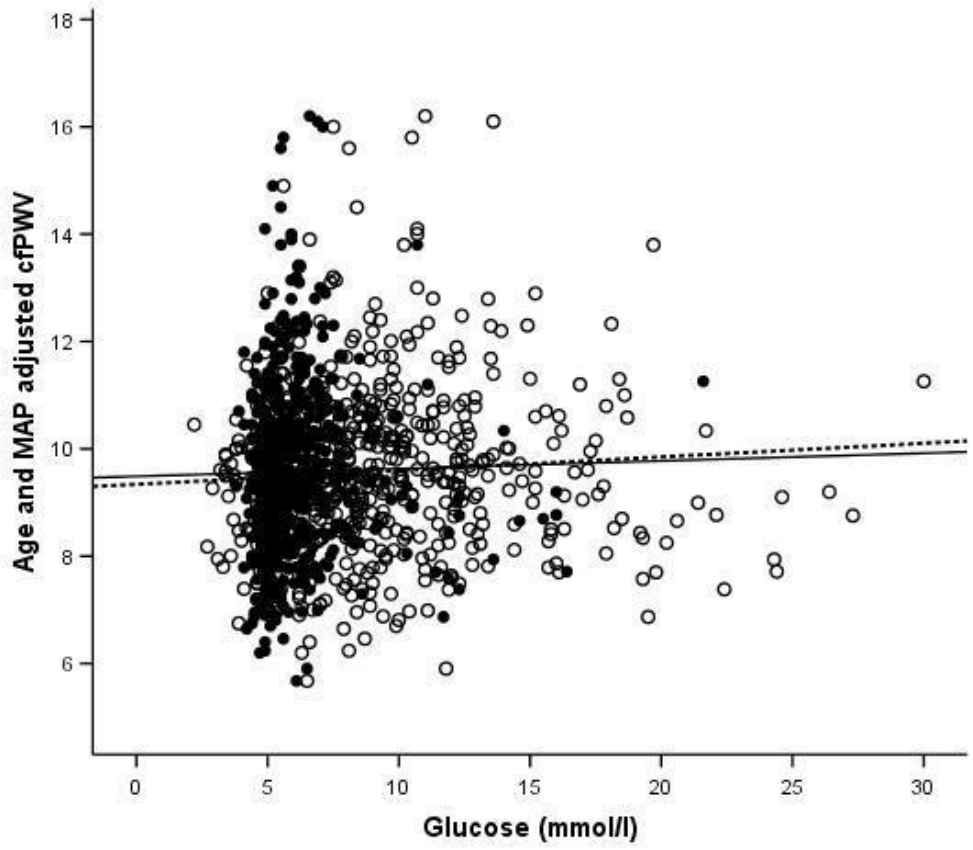
	Univariate analysis			
	β (95% CI)	Standardised β	p-value	R^2
Age (Years)	0.12 (0.11- 0.14)	0.58	<0.01	0.34
MAP (mmHg)	0.08 (0.07- 0.09)	0.52	<0.01	0.27
Antihypertensive therapy	0.09 (0.66- 0.13)	0.23	<0.01	0.23
Cardiovascular Disease	1.24 (0.67- 1.8)	0.21	<0.01	0.17
Lipid Lowering therapy	0.67 (0.26-1.09)	0.18	0.01	0.12
Microalbuminuria	0.14 (0.08 - 0.19)	0.14	0.01	0.14
Gender (Female)	0.41 (-0.74 to -0.07)	0.10	0.01	0.01
Body mass Index (KGm^{-2})	0.04 (0.01 - 0.07)	0.09	0.04	0.01
Heart Rate (min^{-1})	0.01 (-0.01 - 0.03)	0.07	0.15	0.01
Total Cholesterol ($mmoll^{-1}$)	0.02 (-0.13 - 0.63)	0.01	0.84	<0.01
Active smoking	0.10 (-0.6 - 0.45)	0.02	0.71	<0.01
FPG ($mmoll^{-1}$)	0.10 (0.01 - 0.19)	0.09	0.03	0.09
2-HPG ($mmoll^{-1}$)	0.08 (0.03 - 0.11)	0.15	<0.01	0.14
HbA1c%	0.13 (0.02 - 0.29)	0.07	0.08	0.07
Log HOMA-IR	0.04 (0.02 - 0.11)	0.14	<0.01	0.15

Table 5.3 Individual glucose / HOMA-IR effects in three multivariate models incorporating combinations of co-factors influencing $_{cf}$PWV.

	Model1[a]			Model 2[b]			Model 3[c]		
	β (95% CI)	p	R^2	β (95% CI)	p	R^2	β (95% CI)	p	R^2
FPG	0.09 (0.01-0.14)	0.015	0.48	0.09 (0.03-0.17)	0.021	0.44	0.10 (0.05- 0.18)	0.018	0.48
2-HPG	0.14 (0.02-0.16)	<0.001	0.51	0.14 (0.04-0.10)	<0.001	0.46	0.14 (0.02- 0.23)	<0.001	0.51
$HbA_{1c\%}$	0.08 (0.05- 0.25)	0.007	0.48	0.10 (0.07- 0.30)	0.002	0.44	0.11 (0.02- 0.28)	0.001	0.48
$HOMA\text{-}IR_{log}$	0.15 (0.05-0.88)	<0.001	0.51	0.16 (0.04-1.50)	<0.001	0.51	0.20 (0.05- 0.53)	<0.001	0.51

a) Model 1 adjusted for age, MAP, CVD, gender, BMI, HR, anti-hypertensive therapy
b) Model 2 adjusted for age, MAP, CVD, gender, BMI, HR, lipid lowering therapy
c) Model 3 adjusted for age, MAP, CVD, gender, BMI, HR, microalbuminuria

Figure 5.5 Linear regression: $_{cf}$PWV and FPG / 2-HPG associations



Regression plot co-efficient:

FPG: y= 2.58 + 0.09x
2-HPG: y=3.93 + 0.15x

p = 0.28

● FPG
o 2-HPG
——— **FPG**
-------- **2-HPG**

5.5 Discussion

The results of this study add to the body of evidence advancing premature arteriosclerosis as an important vasculopathic mechanism in latent diabetes and IGR. Undiagnosed, common metabolic diseases clinically characterised by fasting or post-challenge hyperglycaemia are associated with significant thoraco-abdominal aortic stiffness. We found dichotomising FPG and 2-HPG data into normal or abnormal glucose categories resulted in similar evidence of premature conduit artery stiffness in both isolated fasting and post-challenge hyperglycaemic groups.

Importantly this study also demonstrates strong relationships between glucose concentration and arterial stiffening independent of other known risk factors for premature vascular disease. It appears the structural integrity of the vascular wall is determined by a number of diverse and potentially modifiable pathogenic mechanisms. Impaired Glucose Regulation characterised in particular by post-challenge hyperglycaemia and insulin resistance is a potentially reversible pathway promoting large artery stiffness.

All three glucose indices contributed an additional three to six percent of the variance in $_{cf}$PWV after adjustments for age and mean arterial pressure, although two hour post-challenge plasma glucose was found to be a stronger independent predictor of $_{cf}$PWV than fasting values. These results imply a dose effect, with a higher mean glucose concentration explaining the more significant contribution of post-prandial glucose to $_{cf}$PWV variance. Direct hyperglycaemia-related non-enzymatic glycation of matrix proteins resulting in accumulation of glycation end products within the vessel wall would provide an explanation for these observations. It is also plausible insulin resistance is characterised by abnormalities in the physiological properties of insulin which normally preserve arterial wave reflections and central

haemodynamics. In this population 2-HPG and HOMA-IR may reflect visceral fat induced pro-inflammatory signals altering insulin function and possibly structural integrity of the vascular wall. We did not attempt to quantify the relative contribution of wave reflections to elevated $_{cf}$PWV although recognise this may be an important determinant of central haemodynamic dysfunction in insulin resistant states.

As has been previously reported, increased $_{cf}$PWV in latent asymptomatic diabetes or even earlier in the pathogenesis of insulin resistance is an important patho-physiological finding [268]. Implied central artery stiffening and its attendant vascular consequences clearly develop well before conventionally diagnosed diabetes is manifest. Until there is vascular outcome data available from randomised controlled trials of screened cases, surrogate indices of atherosclerosis could reliably inform screening strategy. In order to prevent macrovascular complications our data suggests early identification and intervention in the IGR range is advisable. Mean $_{cf}$PWV in this group is indistinguishable from newly diagnosed type 2 diabetes cases. Screening methodology will presumably need to reflect the influence both fasting and post-challenge hyperglycaemia within this range have upon vascular stiffening.

The ADDITION-Leicester $_{cf}$PWV PACE sub-study is one of the largest cross-sectional analyses of central artery stiffness conducted within fasting and post-challenge glucose intolerant states. The study population is tagged for cardiovascular outcome and glycaemic status comprehensively described with universal OGTT testing. Accepted measurements adhered to strict standards with two operators blinded to glucose status minimising variability and ensuring robust translation of waveform data. The use of a non-invasive, reproducible gold-standard measurement ensured accurate regional representation of central (aortic) stiffness.

Detailed phenotyping allowed comprehensive regression analyses incorporating a range of covariates influencing $_{cf}$PWV. The absence of potentially confounding glucose lowering therapies in our diabetes group adds strength to the studies aim of assessing the independent effects of glucose on arterial stiffness. An important feature of the study was its population based approach introducing clinically relevant $_{cf}$PWV estimates.

Dyslipidemia, more prevalent within IGR and NDM categories appeared not to play an important role in arteriosclerosis, contrasting with the ARIC study [262] which reported a synergistic effect of serum glucose and triglyceride concentrations on stiffness measures. It is plausible hyperlipidemia and foam cell driven atherosclerotic plaque formation may manifest effects on vascular wall integrity at a later stage in the pathogenic process.

The independent yet relatively minor contribution of glucose indices to $_{cf}$PWV variance corresponds with previous studies [142, 254, 255] and is predictable given the nature of variables entered into the multivariate analysis. Ageing inevitably dominates determinants of haemodynamic structure and function due to its profound effects on vascular wall integrity, rheology, and central aortic pressure. Concerted efforts to recruit a matched study population will have reduced the impact of this variable but it remained necessary to adjust for its effects in multivariate modelling. When the contributions of irreversible determinants of $_{cf}$PWV are considered, potentially modifiable glucose-specific effects become more relevant. Our study population was relatively young (mean Age 59 years) which will reduce potential selection bias resulting from the death of older diabetic individuals with particularly

stiff arteries but conversely may make extrapolation of the results to older higher risk populations problematic.

Other recognised limitations of the study include potential inaccuracies of waveform acquisition and estimated carotid-femoral distance in overweight individuals. These factors, together with the accepted higher prevalence of ectopic activity, arrthymia and extreme heart rate variability in people with type 2 diabetes may have resulted in unavoidable selection bias. Subjects excluded due to poor waveform concordance did not have a significantly higher waist circumference or diabetes prevalence compared with the study population, suggesting abdominal obesity *per se* was not a major confounding influence. Individuals with unacceptable waveforms due to irregular chronotropic activity were evenly distributed across the glycaemic spectrum, and conceivably reflect favourable cardiac function in “early” diabetes.

Our study is limited by its cross-sectional design, which does not enable us to draw conclusions in terms of causal relationships. We are therefore limited to speculating on the importance of observed independent associations. The natural course of glucose disorders to develop simultaneous fasting and post-prandial hyperglycaemia, in combination with the clinical requirement to intervene once diabetes thresholds are reached would make implementation and interpretation of a prospective comparison extremely difficult.

The clearly complex interactions of FPG, 2-HPG and insulin resistance and the demonstrated independent contributions of these indices to sub-clinical arterial change would probably necessitate a prohibitively large sample size.

5.6 Conclusion

This data provides evidence of significant central artery stiffness in IGR characterised by either fasting or post-challenge hyperglycaemia. As measures of arterial compliance have been shown to have prognostic importance in people with established type 2 diabetes, the significance of this relationship in individuals at earlier stages of metabolic disease is clear. Demonstration of “pre-diabetic” glucose and hypertension mediated macrovascular damage via accelerated senescence is mechanistically plausible and requires further investigation preferably with intervention studies aimed at ameliorating arterial stiffness. This hypothesis is supported by recent vascular outcome studies in type 2 diabetes implying glucose lowering may only be effective if introduced early in the natural history of the disease. In this representative glucose treatment naïve population post-challenge glucose concentration and HOMA-IR were more powerful predictors of arterial stiffness than fasting measures, implying hyperglycaemic excursion or central obesity induced insulin resistance are particularly important determinants of vascular wall sclerosis.

Chapter 6
PACE 2

25-Hydroxyvitamin D independently associates with conduit vessel arteriosclerosis in south Asians

6.1 Summary

Aims: South Asians migrating to northern latitudes are more susceptible to premature cardiovascular disease than expected for given levels of blood pressure. Vitamin D deficiency is common in this group and may play an important role mediating vascular wall senescence in response to central pressure effects.

Methods: A cross-sectional association study. South Asian and white European participants were recruited from a population based diabetes screening programme. Carotid-femoral Pulse Wave Velocity ($_{cf}$PWV), biochemistry (25-Hydroxyvitamin D, insulin, glucose), anthropometrics, resting blood pressure and a physical activity measure (IPAQ) were measured under controlled conditions.
Participants: 257 matched south Asians and white Europeans not taking Vitamin D supplements with a risk factor for diabetes but no overt cardiovascular disease.

Results: Age (mean south Asian: 55.7 vs white European: 56.0 years), mean arterial pressure (MAP: 97.4 vs 99.5 mmHg) and 5-year calculated CVD risk (10.4 vs 9.7%) were similar in both groups. $_{cf}$PWV was 9.32 ± 0.18 ms^{-1}± SEM in South Asians and 8.68 ± 0.13 ms^{-1}±SEM in white Europeans (p=0.001). Conversely (25-Hydroxyvitamin D ($nmoll^{-1}$ ± IQR) was lower in south Asians compared to white Europeans (21.29 ± 1.22 vs. 52.58 ± 2.28, p<0.001). 25-Hydroxyvitamin D independently associated with $_{cf}$PWV in multivariate modelling adjusted for age, MAP, gender, glucose, heart rate and ethnicity (R-squared=0.73, adjusted β = -0.19, p=0.004). Age, MAP, anti-hypertensive treatment, female gender, insulin resistance, and 25-Hydroxyvitamin D concentration were all associated with $_{cf}$PWV in south Asians after adjustment in multivariate modelling (R^2 0.63-0.69). Vitamin D levels below the 50th centile of the study population (<31.7 $nmoll^{-1}$) were associated with a larger increase in age-adjusted $_{cf}$PWV per unit change in MAP.

Conclusion: Aortic stiffness is significantly increased in south Asians without vascular disease despite overall risk profiles which are comparable to age-matched white Europeans. This effect may be mediated by a greater pressure-dependent increase in stiffness in individuals with vitamin D insufficiency.

6.2 Introduction

South Asians appear particularly susceptible to classic environmental determinants of cardiovascular disease [237]. More frequent use of tobacco, the “Western” sedentary lifestyle, poor nutrition and social deprivation probably contribute to unacceptably high rates of premature coronary and cerebrovascular thrombo-embolic disease within south Asians migrating to the United Kingdom [196,212,269]. Adverse outcomes within this diaspora are linked to intra-abdominal fat deposition, pro-inflammatory cytokine activity and insulin resistance as part of the so-called “metabolic syndrome” [209,237,270]. Whilst genetic predisposition to central obesity and its patho-physiological consequences are clearly important in determining risk, even when combined with other traditional risk factors they fail to entirely account for observed variation in vascular events [199]. Certain reversible nutritional deficiencies, including disorders of vitamin D metabolism, are particularly common within this ethnic group and may augment accelerated atherosclerotic processes in Asians migrating to Northern latitudes [169].

There has been a resurgence of interest in the recognition and treatment of vitamin D deficiency beyond established roles in metabolic bone disease. Epidemiological studies implicate abnormal vitamin D and calcium homeostasis in a range of non-skeletal immune-based chronic diseases [169,180]. Some observational cohorts and intervention trials examining mainly bone related outcomes demonstrate inverse relationships between serum 25-Hydroxyvitamin D concentration and incident vascular disease, hypertension or diabetes [170-172,271-273]. Others do not report strong associations [274,275] however, and meta-analyses of supplementation data have so far failed to convincingly demonstrate positive effects on metabolic [276] or cardiovascular outcomes [173,277] in mainly healthy and relatively Vitamin D replete

populations. Those examining blood pressure are equally controversial, [186,274,278] suggesting a simple cause and effect relationship between vitamin D and vascular wall disease in otherwise healthy individuals is unlikely.

Vitamin D deficiency independently associates with measures of arterial stiffness, abnormal conduit function and vascular outcome in healthy subjects [279] as well as those with advanced renal disease [184]. Available data suggests that vitamin D exerts a biphasic 'dose response' on vascular calcification with potentially deleterious effects above and below a currently unknown “optimal” range. Although the pathogenesis remains unclear and in the case of end stage renal disease likely multi-factorial, plausible mechanisms implicate intimal and medial layer calcification in the development of arterial stiffening [177]. Aortic calcification contributes to poor vascular compliance in isolated systolic hypertension and vitamin D supplementation has been shown to lower age-associated systolic blood pressure [280,281].

There are a limited number of inter-racial comparisons of arterial calcification and stiffness, with the majority of studies reporting blood pressure mediated elevated pulse wave velocity in Afro-Caribbean migrants to the United Kingdom or United States [282-287]. Three studies within healthy south Asian populations have demonstrated significantly elevated stiffness and or augmentation index, whilst a third failed to demonstrate a statistically significant difference in pulse wave velocity in south Asian men with established type 2 diabetes [285,288,289]. A recent study using magnetic resonance imaging and conducted within British south Asians of mainly Indian descent independently associated circulating 25-Hydroxyvitamin D concentration with pulse wave velocity in the descending aorta [290].

PACE2 tested the hypothesis that central arteriosclerosis is more advanced in United Kingdom residing south Asians compared with similar white Europeans and that this discrepancy relates to circulating vitamin D status independent of other factors influencing arterial stiffness.

6.3 Methods

6.3.1 Experimental design and protocol

An age-matched study population was recruited from the ADDITION-Leicester screening programme. Detailed in Chapters 2 and 3, ADDITION-Leicester is a National Health Service initiative specifically designed to determine the feasibility and effectiveness of screening for type 2 diabetes mellitus within a United Kingdom multiethnic population. There are an estimated 65,000 people of mostly Indian (Gujarati) (>85%) descent in Leicester but as indicated, the term “south Asian” included the small number of self-reported Pakistani, Sri Lankan, and Bangladeshi minority groups also invited for screening. Consent was obtained for further vascular measurements during ADDITION-Leicester screening as the PACE sub-study and arrangements made for a single-session carotid-femoral Pulse Wave Velocity ($_{cf}$PWV) assessment within two weeks of screening.

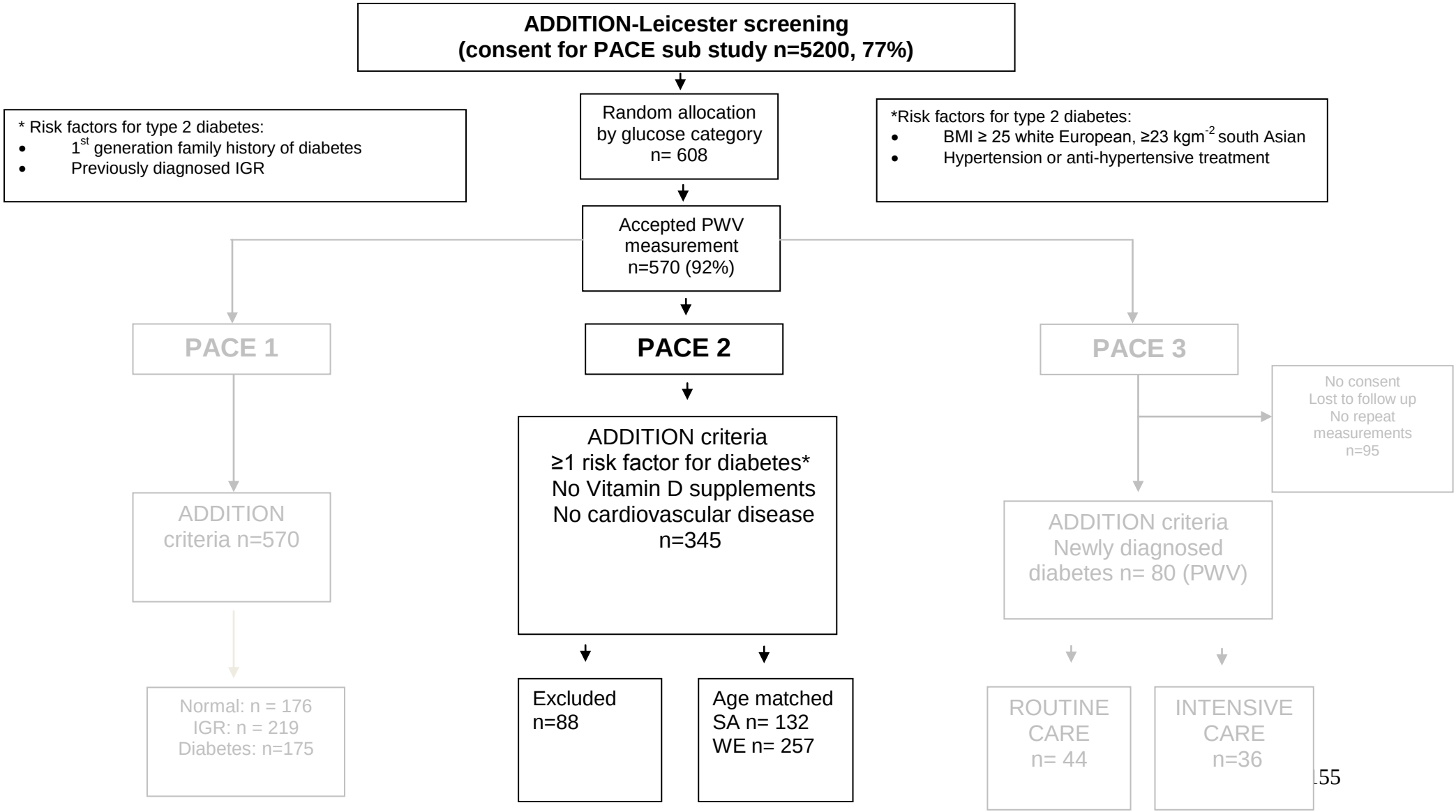
For PACE 2, study groups were defined by self-reported ethnicity as Indian (Gujarati) south Asian or British white European. Other Asian or European groups, Afro-Caribbean’s and those of mixed race were excluded. In the future it is likely that population screening will preferentially target high-risk groups, so we decided to select individuals with at least one risk factor for diabetes for inclusion in this study (Figure 6.1). This served to focus clinical relevance, as a population at increased vascular and metabolic risk but no demonstrable cardiovascular disease was

studied. Defined risk factors were a family history (first generation) of type 2 diabetes, previously diagnosed IGR or “pre-diabetes” (IFG or IGT) a BMI of ≥25 (white Europeans) or ≥23 (south Asians) and history of hypertension or its treatment. Cardiovascular disease was defined as any of the following general practice diagnostic codes or clinical information recorded as part of the ADDITION-Leicester baseline survey; ischaemic heart disease, myocardial infarction, coronary artery bypass graft, cerebrovascular accident, stroke, and peripheral vascular disease. Subjects taking any form of Vitamin D or calcium supplementation (prescribed or otherwise) or with a history of active parathyroid or significant renal disease (Chronic Kidney Disease (CKD) stage 3 or greater (eGFR ≤45 $ml^{-1}min^{-1}$ x 1.73) were also excluded.

Prescribed anti-hypertensive (defined as angiotensin converting enzyme inhibitor, angiotensin receptor blocker, alpha or beta-blocker, calcium antagonist or thiazide diuretic) or lipid lowering (defined as statin or fibrate) therapies were categorised together as cardiac medication for subsequent analyses.

Volunteers attended this second appointment in a fasted state and were asked to refrain from smoking or consuming caffeine based drinks. Further fasting plasma samples were obtained for 25-Hydroxyvitamin D, bone profiling and insulin estimation during this session.

Figure 6.1 Schematic illustration of recruitment flow for the PACE 2 sub-study



6.3.2 PWV measurement

A single operator performed all arterial measurements within a single site research facility at the University hospitals of Leicester. A standard protocol for $_{cf}$PWV, described in Chapters 2, 5 and Appendix B was followed for all measurements.

6.3.3 Serum biomarker and standardised biomedical measurements

An aliquot of whole blood was centrifuged, the serum fraction removed after clotting and immediately stored at -80^{o}C. 25-Hydroxyvitamin D measurements were performed by a competitive EIA (Enzyme immunoassay), range 7.3 – 384 mMl^{-1} (mid range intra- and inter assay co-efficient of variation (CV) 6% (IDS, Boldon Business Park Tyne and Wear, United Kingdom). Glucose was analysed via the hexokinase method (NADPH production at 340nm Abbott Aeroset) and $HbA_{1c\%}$ using DCCT aligned BIO-rad Variant II HPLC system**.** Quantitve analysis of serum Insulin was carried out using the AutoDelfia time-resolved fluoroimmunoassay (Perkin Elmer AutoDelfia1235). Insulin intra assay co-efficient of variation (CV) was <10%.

A standard protocol ensured participants were fasted and rested supine prior to $_{cf}$PWV assessment [144]. Baseline supine brachial artery Blood Pressure and heart rate were recorded using a semi-automated oscillometric device (Omron MS-I). After ten minutes rest these were re-assessed and $_{cf}$PWV measurements taken once haemodynamic stability achieved (defined as two readings within Systolic (+/- 9mmHG), Diastolic (+/- 6mmHG) and heart rate (+/- 8 beatsmin^{-1})$_{\cdot}$ Blood pressure and heart rate concordance was ensured prior to subsequent $_{cf}$PWV measurements (Chapter 3). Estimated MAP (mmHg) was calculated by adding diastolic and one third pulse pressure (systolic – diastolic) measurements recorded during the $_{cf}$PWV

session. Insulin Resistance was estimated using the homeostasis model assessment (HOMA-IR) [266]. Microalbuminuria was defined as a urine albumin : creatinine ratio on a random sample of more than 2.5mgmmol^{-1} in men and 3.5mgmmol^{-1} in women. ten-year cardiovascular disease (CVD) risk was estimated from an equation derived from Framingham data adjusted for the effects of ethnicity.

6.3.4 Statistics

To compensate for the imbalance in age between south Asians and white Europeans entering the study (by virtue of ADDITION-Leicester inclusion criteria: 25-75 years for south Asians and 40-75 years for white Europeans) age-matched groups were generated after completion of the study by an independent statistician blinded to $_{cf}$PWV results. Measurements were then compared by ethnicity using the student t-test for normally distributed continuous data and chi-squared (χ^2) for categorical data. Skewed variables were log transformed before analysis and data presented as mean ± standard error (SEM) or inter-quartile range (IQR), percentages or counts. Pearson's correlation tested the relationship between $_{cf}$PWV and various factors in a univariate analysis. Forward logistic regression was used to determine the independent contribution of key factors to $_{cf}$PWV variance within the combined population and individual ethnic groups, with various models adjusting for the effects of age, gender, MAP, cardiac medication, heart rate, glucose, insulin resistance (HOMA-IR), BMI, waist circumference, physical activity and 25-Hydroxyvitamin D. The gradient of regression lines for $_{cf}$PWV and MAP in individuals above and below the 50th centile of plasma 25-Hydroxyvitamin D concentration was visualised after adjusting for age. This cut-point (31.7 nmoll^{-1}) was chosen as a potentially realistic therapeutic goal using available ergo- or cholecalciferol preparations. All analyses were performed using SPSS v16 (SPSS Inc. Chicago, Illinois, USA) or

GraphPadPrism v5 (San Diego, California, USA). When missing data occurred participants were excluded. Significance was considered at $p<0.05$ with 95% confidence intervals (CIs) included were appropriate.

6.4 Results

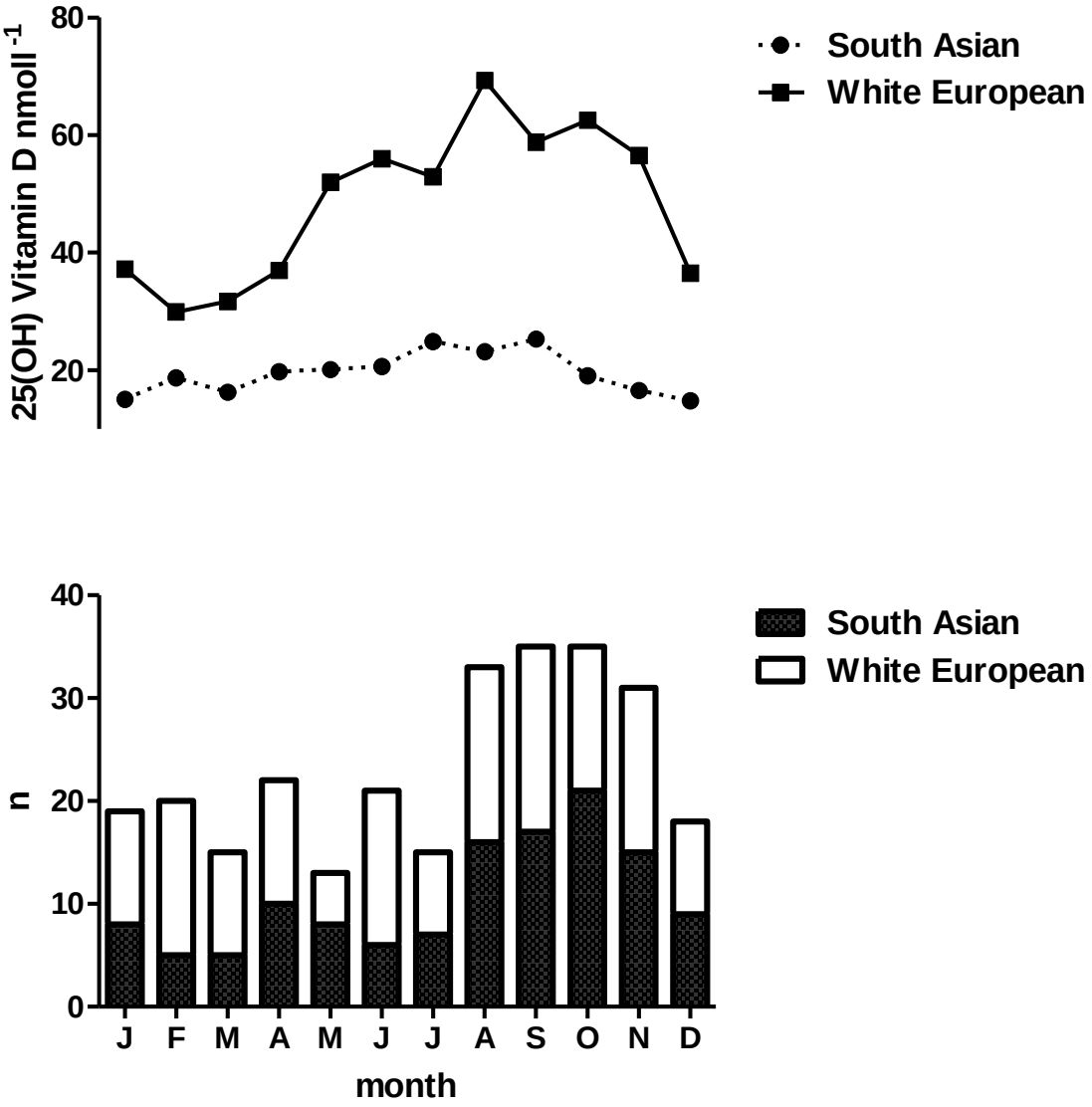
6.4.1 Recruitment from the PACE cohort

After age-matching, 132 Indian south Asians (58% Male) and 125 white Europeans (56% Male) fulfilled the inclusion criteria for PACE 2. All those recruited had at least one designated risk factor for diabetes but no self-reported medical history of cardiovascular disease. South Asians were more likely to report a first degree relative with diabetes (40% versus 25% $p<0.01$). The cumulative frequency of other risk factors for diabetes was similar between the groups (ANOVA trend $p=0.55$). Recruitment flow is depicted in Figure 6.1.

6.4.2 Vitamin D measurements

The study was conducted over twelve months with no seasonal differences in the rate of south Asian and white European recruitment ((defined as a dichotomous winter or summer variable: $x^2=0.09$, $p=0.76$) (Figure 6.2). The mean 25-Hydroxyvitamin D level was significantly higher in summer compared with winter in white Europeans (June – November mean 57.6 $nmol^{-1}$, December – May mean 46.9 $nmol^{-1}$ $p<0.01$) but even at its lowest remained significantly higher than the maximum mean monthly concentration in south Asians (52.59 vs 21.29 $nmol^{-1}$). White Europeans exhibited more variation in 25-Hydroxyvitamin D, with peak mean values over the summer and a typical nadir over the winter months (Figure 8.2). Serum 25-

Figure 6.2 Monthly recruitment (n) (lower) and mean 25-Hydroxyvitamin D concentration (25-Hydroxyvitamin D ($nmoll^{-1}$) (upper) within south Asian and white European ethnic groups. (Mean vitamin D concentration adjusted for age, BMI and physical activity)



Hydroxyvitamin D concentration was lower in south Asians before and after adjustment for age, gender, physical activity and season. International consensus suggests circulating 25-Hydroxyvitamin D levels of less than 30 $nmol^{-1}$ (12 $ngml^{-1}$) constitute a deficiency state with a normal reference range of 50-150 nmol-1 (20-60 $ngml^{-1}$) [291].

6.4.3 Biomedical characteristics

Ethnicity stratified anthropometric, haemodynamic, and biochemical characteristics are displayed in Table 6.1. Urine albumin:creatinine ratio (ACR), 25-Hydroxyvitamin D, IPAQ physical activity and HOMA-IR calculated insulin resistance were log transformed. No statistically significant differences in age, waist circumference, physical activity, renal function, glucose or calcium indices were found between south Asians and white Europeans. Calculated mean cardiovascular disease risk estimates were similar (south Asian 10.4% versus white European 9.7%, p=0.45), reflecting comparable brachial blood pressure and serum cholesterol indices, self-reported smoking activity and cardiac treatments. The prescription frequency of individual cardiovascular medications (not shown), calculated insulin resistance (HOMA-IR) and microalbuminuria were similar between the groups. Anthropometrically, the groups differed in respect to height and BMI but not waist circumference or waist-hip ratio. South Asians were shorter (162 ± 0.07 versus 170 ± 0.07 cm, p<0.01) more tachycardic (77 ± 0.9 versus 75 ±1.0 min^{-1}, p=0.05), and 25-Hydroxyvitamin D deficient (21.29 versus 52.58 $nmol^{-1}$ p<0.01) than white Europeans. The south Asian group had a significantly higher $_{cf}$PWV (9.32 ± 0.14 versus 8.68 ± 0.13 ms^{-1}, p<0.01) (Figure 6.3). $_{cf}$PWV correlated with cardiovascular

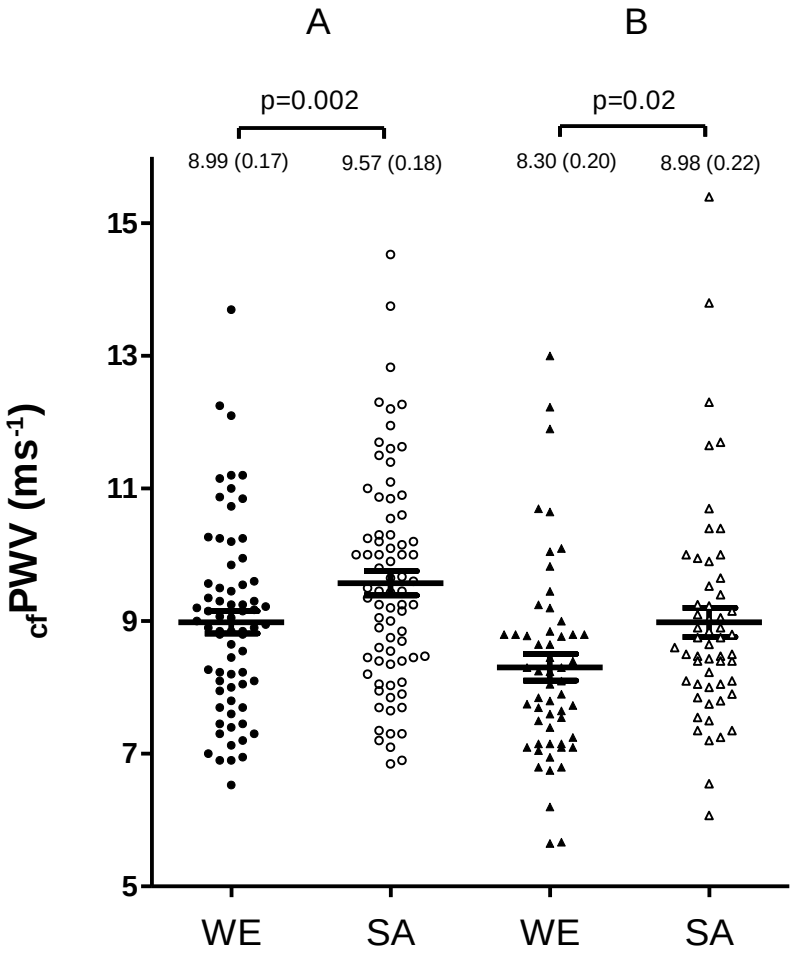
Table 6.1 Calculated means, percentages or absolute values of measured characteristics by ethnic group

Measurement	South Asian (n=132)	European (n=125)	p value*
Anthropometric:			
Age (years)	55.7 (0.8)	55.8 (0.8)	0.94
Gender M:F (n)	77:55	70:55	0.80*
Active smoking (%)	8.3	9.6	0.72*
Height (cm)	162 (0.01)	170 (0.01)	<0.01
BMI (kgm^{-2})	28.4 (0.4)	30.1 (0.4)	<0.01
Waist circumference (cm)	77.8 (12.2)	79.4 (14.2)	0.93
Waist : hip ratio	0.91 (0.01)	0.91 (0.01)	0.94
†Physical activity ($metmin^{-1}wk^{-1}$)	4507 (395)	3806 (522)	0.11
Haemodynamic:			
SBP (mmHg)	130.0 (1.3)	132.9 (1.5)	0.15
DBP (mmHg)	81.0 (0.76)	82.9 (0.87)	0.12
MAP (mmHg)	97.4 (0.88)	99.5 (1.01)	0.11
HR (min^{-1})	77 (0.90)	75 (0.98)	0.05
$_{cf}$PWV (ms^{-1})	9.32 (0.14)	8.68 (0.13)	<0.01
Biochemical:			
Fasting glucose ($mmoll^{-1}$)	5.9 (0.12)	6.0 (0.19)	0.82
2hr post-challenge glucose ($mmoll^{-1}$)	8.9 (0.36)	8.3 (0.39)	0.30
†Insulin Resistance (HOMA-IR)	3.12 (0.33)	2.75 (0.41)	0.39
Total cholesterol ($mmoll^{-1}$)	5.35 (0.09)	5.80 (0.09)	0.01
†Triacylglycerides ($mmoll^{-1}$)	1.60 (0.07)	1.68 (0.11)	0.55
†ACR ($mgmmol^{-1}$)	1.29 (0.19)	1.55 (0.35)	0.52
Calcium ($mmoll^{-1}$)	2.43 (0.02)	2.47 (0.01)	0.08
Creatinine ($mmoll^{-1}$)	86 (17)	87 (14)	0.45
eGFR (MDRD) ($mlmin^{-1}$)	79.7 (21)	77.8 (18)	0.13
†25-Hydroxyvitamin D ($nmoll^{-1}$)	21.29 (1.22)	52.58 (2.28)	<0.01
Cardiac medication:			
Anti-hypertensive / statin (%)	19.6	14.7	0.15*
Framingham five year CVD risk (%)	10.4 (0.6)	9.7 (0.5)	0.43

Data expressed as mean±standard error (SEM) for normally distributed or inter-quartile range for log transformed data† or n (%).Groups compared using unpaired t-test or chi-squared (X^2)*

Physical Activity via IPAQ (International Physical Activity Questionnaire), **SBP**: Systolic Blood Pressure, **DBP**: Diastolic Blood Pressure, **MAP**: Mean Arterial Pressure, **HR**: Heart Rate, **$_{cf}$PWV**: carotid femoral Pulse Wave Velocity, **ACR**: single estimation urine Albumin: creatinine Ratio

Figure 6.3 Comparison of mean unadjusted $_{cf}$PWV (ms^{-1}) between south Asians (SA) and white Europeans (WE) A) Males (n=147) B) Females (n=110)



Individual data displayed with horizontal lines indicating mean ± SEM

disease (CVD) risk score in both ethnic groups: $_{cf}$PWV: south Asians r=0.62 ($p<0.01$), white Europeans r=0.57 ($p<0.01$).

6.4.4 Univariate and multivariate analyses

Univariate associations for the combined study population are displayed in Table 6.2. Age, MAP, cardiac medication, HOMA-IR, female gender, south Asian ethnicity, heart rate, 25-Hydroxyvitamin D concentration and waist circumference were significantly associated with $_{cf}$PWV.

After adjustment for age, MAP, gender, fasting glucose and heart rate; south Asian ethnicity and 25-Hydroxyvitamin D were inversely related to $_{cf}$PWV in multivariate modelling (Table 6.3). Entering 25-Hydroxyvitamin D and ethnicity simultaneously, attenuated but did not completely remove the south Asian $_{cf}$PWV interaction. Combinations of haemodynamic (MAP or cardiac medication) or metabolic (HOMA-IR, fasting glucose or 120 minute post challenge glucose) parameters had little additional effect. Those incorporating age, gender, heart rate, MAP and fasting glucose are depicted in Table 6.3.

Age, MAP, cadiac medication, gender and 25-Hydroxyvitamin D were consistent independent predictors of $_{cf}$PWV in south Asians (Table 6.4). R^2 calculations for the four models ranged from 0.54 to 0.61. In white Europeans 25-Hydroxyvitamin D independently associated with $_{cf}$PWV in a model adjusting for the effects of age, female gender, MAP, post-challenge glucose and heart rate (Table 7.4 model 2). The output summary of the multivariate linear regression analyses for all 3 models is shown in Appendix F.

In a third linear regression model assessing the effect of MAP on age-adjusted $_{cf}$PWV, a difference in the gradient of the regression lines was demonstrated when

Table 6.2 Univariate associations between $_{cf}$PWV and selected anthropometric, haemodynamic and biochemical parameters of the combined PACE 2 study population

	$_{cf}$PWV	
	r	p
Age (years)	0.55	<0.01
MAP (mmHg)	0.32	<0.01
Cardiac medication	0.30	<0.01
Insulin resistance (HOMA-IR)	0.23	<0.01
Gender (Female)	-0.20	<0.01
Ethnicity (south Asian)	0.20	<0.01
Heart rate (min^{-1})	0.17	<0.01
25-Hydroxyvitamin D ($nmol^{-1}$)	-0.15	0.02
Waist circumference (cm)	0.11	0.04
Albumin:creatinine Ratio ($mgmmol^{-1}$)	0.10	0.05
Active smoking	0.08	ns
Fasting glucose ($mmoll^{-1}$)	0.08	ns
Height (cm)	-0.07	ns
2hr post-challenge glucose ($mmoll^{-1}$)	0.05	ns
Physical activity ($metmin^{-1}wk^{-1}$)	0.04	ns
Total cholesterol ($mmoll^{-1}$)	0.01	ns

Table 6.3 Linear regression model demonstrating an independent association of $_{cf}$PWV with 25-Hydroxyvitamin D

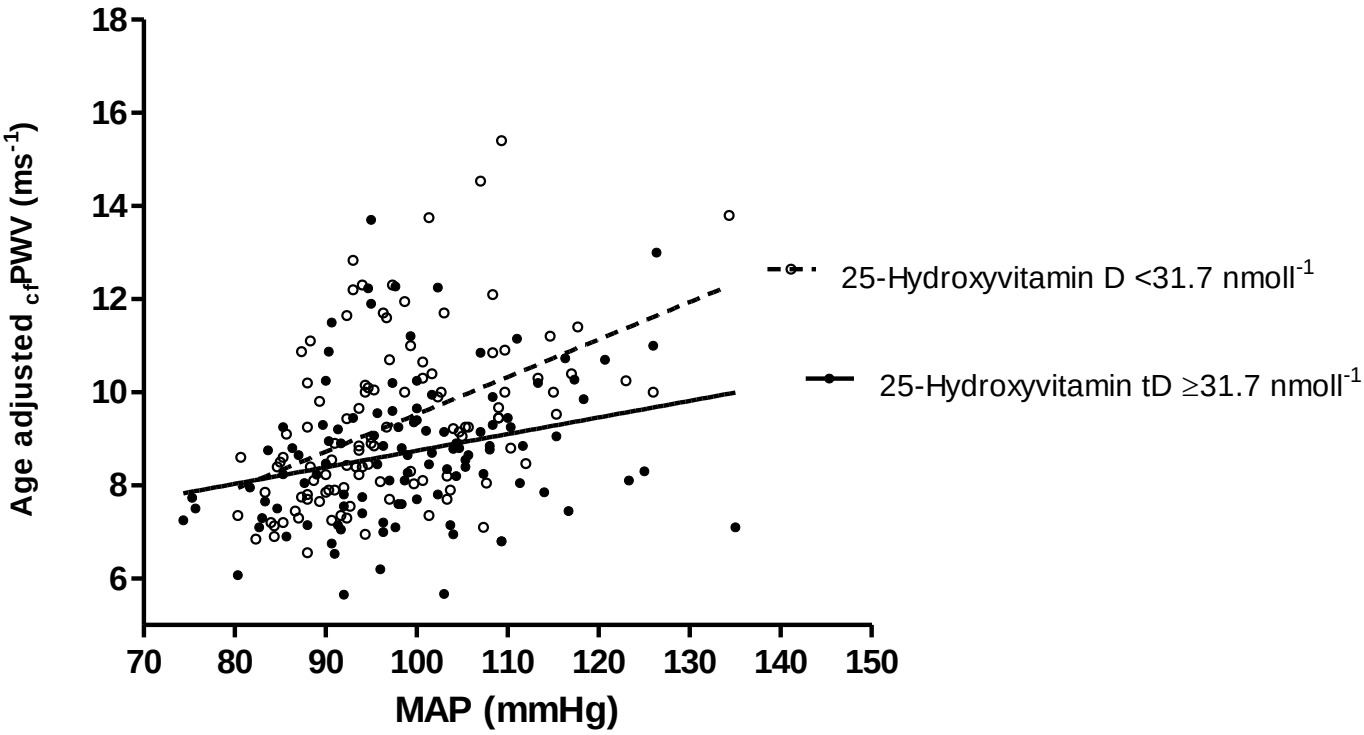
	Model 1 (R^2= 0.69) Basic		**Model 2** (R^2= 0.73) Basic + 25(OH) Vitamin D	
	β (se)	p	β (se)	p
Age (years)	0.10 (0.008)	<0.001	0.11 (0.009)	<0.001
Mean Arterial Pressure (mmHg)	0.05 (0.007)	<0.001	0.05 (0.007)	<0.001
Gender (female)	0.43 (0.15)	0.005	0.35 (0.16)	0.03
Fasting glucose ($mmoll^{-1}$)	0.14 (0.04)	<0.001	0.12 (0.04)	0.003
Heart rate (min^{-1})	0.02 (0.007)	0.01	0.01 (0.008)	0.17
Ethnicity (south Asian)	0.71 (0.15)	<0.001	0.42 (0.21)	0.04
25-Hydroxyvitamin D ($nmoll^{-1}$)	---	---	- 0.44 (0.15)	0.004

Table 6.4 Independent effect of 25-Hydroxyvitamin D in south Asians in four multivariate models incorporating selected combinations of co-factors influencing $_{cf}$PWV variability

	South Asians			White Europeans		
	Model R^2	vitamin D β (95% CI)	p	Model R^2	vitamin D β (95% CI)	p
Model 1	0.61	-0.18 (-0.94, -0.08)	0.02	0.50	-0.11 (-0.96, -0.07)	0.14
Model 2	0.54	-0.16 (-097, -0.13)	0.04	0.47	-0.13 (-0.75, 0.08)	0.12
Model 3	0.69	-0.15 (-1.00, -0.09)	0.03	0.56	-0.16 (-0.96, 0.06)	0.04
Model 4	0.55	-0.19 (-1.39, -0.31)	0.02	0.45	-0.03 (-0.55, 0.67)	0.70

Included co-factors:
Model 1: Age, gender, MAP, HOMA-IR, heart rate, 25-Hydroxyvitamin D
Model 2: Age, gender, anti-hypertensive treatment, heart rate, fasting glucose, waist circumference, 25-Hydroxyvitamin D
Model 3: Age, gender, MAP, two-hour post challenge glucose, heart rate, 25-Hydroxyvitamin D
Model 4: Age, gender, anti-hypertensive treatment, heart rate, HOMA-IR, physical activity, 25-Hydroxyvitamin D

Figure 6.4 Association between $_{cf}$PWV and mean arterial pressure (MAP) above and below the 50th population centile of 25-Hydroxyvitamin D concentration



subjects were categorised above and below the fiftieth centile of 25-Hydroxyvitamin D concentration. These results suggest that for a given MAP those with a low Vitamin D concentration (< 31.7 $nmoll^{-1}$) have a higher $_{cf}$PWV even after adjustment for the effects of age (Figure 6.4)

6.5 Discussion

The PACE 2 study tests the hypothesis that common nutritional deficiencies play an important role in the development of arteriosclerosis. There are two major findings. Firstly, compared with indigenous white Europeans, aortic pulse wave velocity is significantly elevated within a specific Indo-Asian (Gujarati) population relocating to a northern latitude Western environment. Secondly, geographical position and a vegetarian diet presumably predispose this group to profound circulating 25-Hydroxyvitamin D deficiency, and this measure inversely associated with central artery stiffness independently of known confounders. We believe this is the first documentation of a putative role for vitamin D in the development of arteriosclerosis within an otherwise overtly healthy population at inherent risk of premature cardiovascular disease.

6.5.1 Arterial stiffness measures in migrant populations

Although data is scare and that available possibly subject to publication bias, we have identified similar studies comparing arterial stiffness within migrant Afro-Caribbean's [282-287,292] or South Asians [285,288-290] and white European controls. Significant racial variation was demonstrated in central stiffness measurements in eight studies with peripheral arterial or small vessel differences reported in the remainder [285,292]. The implications of this research base are

uncertain but would appear to support our findings of significant racial differences in accepted measures of arteriosclerosis. Three previous studies within healthy south Asians have demonstrated significantly elevated stiffness and / or augmentation indices [288-290]. Pinto *et al.* failed to demonstrate a statistically significant difference in $_{cf}$PWV when sixteen south Asian men with established type 2 diabetes were compared to forty-one similar white Europeans and Afro-Caribbean's [285]. It is plausible a differential effect occurs within certain genetically predisposed groups, whereby known and unknown factors exert a greater influence on vascular wall haemodynamic properties, and effectively lower the threshold for deleterious change. It has previously been proposed that beneficial haemodynamic responses to vitamin D supplementation may be mediated through rennin dependent blood pressure lowering [293-295], although interestingly in this study, vitamin D was associated with aortic stiffness independent of brachial artery pressures or glucose. In patients with advanced renal disease, accelerated vascular calcification and arterial stiffness occur independently of blood pressure indices, relate to vitamin D concentration and predict cardiovascular mortality [184]. Vitamin D deficiency may contribute to premature aortic stiffness through pathways other than direct pressure effects, with possible mechanisms including pro-inflammatory responses ordinarily regulated by vitamin D and disordered mineral metabolism associated with chronic insufficiency states.

6.5.2 Plausible mechanisms linking vitamin D deficiency and arteriosclerosis

It is plausible vitamin D deficiency augments deleterious changes in the properties of elastic arteries normally produced by the cyclical effects of elevated pulse pressure, a theory supported by a number of studies reporting significant ethnic variation in the

impact of blood pressure on measures of arterial stiffness. Comparable systolic and MAP measurements between our populations suggest changes in the haemodynamic properties of the vascular wall occur at lower blood pressures in south Asians. Diagnostic thresholds for hypertension mainly applicable to white European populations may therefore not be suitable for United Kingdom ethnic groups at increased risk of cardiovascular disease.

The independent association of 25-Hydroxyvitamin D concentration with $_{cf}$PWV in south Asians is interesting and may suggest profoundly low circulating levels of this vitamin contribute to observed early haemodynamic changes. We hypothesise the lack of association in white Europeans may relate to a much higher protective circulating vitamin D concentration in this group. We propose there may be a threshold concentration below which vitamin D no longer “protects” the vascular wall from adverse physical and chemical stressors associated with blood pressure and other cardiovascular risk factors. A vitamin D receptor knockout model appears to develop target organ disease with moderate levels of hypertension, suggesting an important biological function in maintaining haemodynamic structural integrity [296].
A pro-inflammatory state generated by genetic predisposition to intra-abdominal fat accumulation in south Asians may further impair endothelial cell function and expose underlying intimal smooth muscle cells to mediators promoting functional change. Vascular smooth muscle cells have been shown to exhibit osteoblast-like behaviour and secrete hydroxyapatite in response to oxidative stress and pro inflammatory activation [297]. Vitamin D deficiency is associated with metallo-matrix protein deposition and endothelial dysfunction possibly mediated via an established immuno-modulatory role regulating innate responses to inflammatory stimuli [298-

300]. Vitamin D receptors and 1α-hydroxylase activity are present in endothelial and vascular smooth muscle cells, and 1,25-Hydroxyvitamin D stimulates endothelial growth factor and prostacyclin, a potent vasodilator [301]. Emerging exploratory intervention studies suggest correcting vitamin D deficiency suppresses pro-inflammatory cytokines, improves endothelial function and has a directly beneficial effect on arterial stiffness [178,190,302]. In a study of black Americans, a vitamin D dose of 2000IU/day for sixteen weeks decreased $_{cf}$PWV by 0.3ms^{-1} compared with an increase of 0.4ms^{-1} in a control population taking a daily dose of 400IU [190]

6.5.3 Strengths and weakness of the study

There are a number of strengths and weaknesses of this study that merit attention. By virtue of its cross-sectional design, causation can only be in implied and therefore the role of vitamin D in the pathogenesis of conduit vascular stiffening remains purely speculative. We believe our findings to be of sufficient interest to warrant a prospective trial of vitamin D intervention within this particularly deplete population with outcomes focusing upon haemodynamic and structural measures of vascular wall integrity. Any demonstrable ameliorative effect may represent an important and readily implementable therapeutic opportunity, as measures of vascular stiffness have been shown to powerfully predict vascular mortality across a range of diseases and populations.

Although recruitment occurred at a consistent rate over the study period, it could be argued that seasonal variation in vitamin D levels and other unknown confounders may have introduced a degree of inaccuracy. Whilst acknowledging this as a potential source of error, concerted efforts were made to standardise available

measures and the study population was robustly characterised for a range of factors potentially contributing to $_{cf}$PWV variation. 25-Hydroxyvitamin D was measured via a competitive immunoassay and not gold-standard liquid chromatography tandem mass spectrometry. Measurement of serum parathyroid hormone (PTH) would have been useful due to the probable higher rate of secondary hyperparathyroidism in south Asians. PTH has been shown to independently associate with augmentation index in a small study of vitamin D replete white Americans [303].

To our knowledge this is the largest description of central artery stiffness within a "westernised" south Asian population and the two study populations were clearly well matched for comparative purposes. The exclusion of individuals with known cardiovascular disease allowed important conclusions to be drawn about a population at risk of cardiovascular disease. A criticism commonly levelled at studies targeting ethnic minority groups is assumed genetic and cultural heterogeneity of the population. The PACE study cohort was derived exclusively from the Leicester south Asian population and further screening ensured only Indo-Asians were recruited. It is estimated over 90% of south Asian participants were of Guajarati descent.

6.6 Conclusion

Conduit vessel arteriosclerosis is more advanced within overtly healthy United Kingdom south Asians at risk of diabetes and cardiovascular disease, indicating clinically relevant ethnic variation in arterial stiffness. Carotid femoral pulse wave velocity may be an important marker of subclinical vascular disease in this group. This population represents an under-researched, high risk phenotype in urgent need of targeted interventions simultaneously redressing obvious health care inequalities

and exploring novel environment-disease interactions contributing to the pathogenesis of premature vascular disease.

25-Hydroxyvitamin D is associated with pulse wave velocity independently of cardiovascular risk factors known to influence central artery stiffness. There may be a threshold concentration below which vitamin D is associated with deleterious changes in vascular wall integrity and premature arterial stiffness. A prospective vitamin D supplementation trial with outcomes related to central vascular stiffness would establish causation and guide future therapeutic intervention.

Chapter 7

PACE 3

Effects of intensive cardiovascular risk management on measures of arterial stiffness and vascular inflammation in screen detected type 2 diabetes

7.1 Summary

Introduction: Vasculopathic consequences of premature aorto-sclerotic stiffening and inflammation are important features of diabetes related large vessel disease. Some commonly used therapeutic interventions improve arterial compliance in established type 2 diabetes and may be even more effective if introduced earlier in the course of disease. Identification through screening allows earlier therapeutic opportunities which may ultimately improve outcomes in people with type 2 diabetes. We hypothesised that established markers of arteriosclerosis ($_{cf}$PWV) and vascular inflammation (CRP, TNFα, IL6, adiponectin) are improved by aggressive cardiovascular risk management in screen detected cases.

Methods: White European or south Asian volunteers with newly diagnosed diabetes were recruited from a population based screening programme and randomised to receive either standard care (Routine care group–RG) or target driven multi-factorial cardio-vascular risk lowering (Intensive care group–IG). Biomarker measurements were performed at baseline and 12 post intervention in both groups.

Results: At baseline, RG (n=44) and IG (n=36) groups did not differ in respect to age, blood pressure, lipids, cardiovascular disease, CRP or $_{cf}$PWV ($p>0.05$). After the study, improvements in blood pressure, total cholesterol (TC) and $HBA_{1c\%}$ were seen in both RG and IG. IG was associated with an 10% reduction in MAP (101 to 91mmHg), a 5% reduction in $HbA_{1c\%}$ (6.8% to 6.5%) and a 25% reduction in TC (5.4 to 4.0mmoll^{-1}) due to increases in prescribed anti-hypertensive, lipid and glucose lowering medication. $_{cf}$PWV (-0.24 ms^{-1}), and CRP (-1.90 mgl^{-1}) decreased whilst adiponectin (+1.50 ugml^{-1}) increased. Smaller changes in blood pressure, TC, $_{cf}$PWV and CRP were observed in RG. There was a statistically significant between group difference in CRP but not $_{cf}$PWV, IL6, TNFα or adiponectin. Multivariate analysis demonstrated intensive treatment was independently associated with CRP change in a model incorporating age and pre-existing cardiovascular disease.

Conclusion: Existing multi-factorial therapeutic approaches improve circulating inflammatory biomarkers in screen detected type 2 diabetes. Intensive management of cardiovascular risk factors over twelve months improves CRP but not $_{cf}$PWV.

7.2 Introduction

Large artery stiffness and chronic inflammation characterise a number of common chronic conditions associated with an increased risk of cardiovascular disease [114, 129,139,141,253,265]. Indirect measures of these processes have proven to be powerful time-integrated markers of risk, predicting vascular mortality in established type 2 diabetes, hypertension and renal disease [123,124,304]. As signals of ensuing arterial complications, therapeutic approaches targeting markers of premature arteriosclerosis and inflammation may slow or even prevent the development of vascular disease in IGR and diabetes [305].

It is currently unknown whether arterial stiffness and inflammation are inter-related, and contribute to atherosclerosis via common mechanisms. For example, accelerated age related effects on the vascular wall may be mediated by a number of factors in addition to well characterised cyclical distending central pressure stresses imposed by hypertension. There is some evidence that angiotensin converting enzyme inhibitors (ACE inhibitors) [155,156], 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors (statins) [158,159,251] and peroxisome proliferator receptor agonists (thiazolidinediones) [164,165,250,255, 306,307] improve measures of arterial compliance independent of their respective blood pressure, lipid and glucose lowering effects.

Conceptually, sustained administration of Simvastatin, the most commonly prescribed statin in the United Kingdom may reduce arterial stiffness through direct inhibition of intima collagen formation, bradykinin degradation and pro-inflammatory vascular smooth muscle cell proliferation [308]. Emerging evidence suggests that statins have beneficial pleiotropic actions, possibly mediated through anti-

inflammatory effects on oxidative stress, nitric oxide availability and free fatty acid generation [251,309]. Similarly, an attractive proposed feature of Thiazolidinediones has been a consistent beneficial effect on a range of surrogate measures of cardiovascular risk independent of glucose lowering capacity [168,310].

Because of the insidious nature of the processes described, to be effective these agents may need to be introduced as early as possible in the natural history of a complex disease such as type 2 diabetes. Identification through screening allows opportunities for earlier therapeutic intervention which may ultimately improve outcomes in a disease which remains a major cause of premature vascular death.

We aimed to establish whether intensive multi-factorial cardiovascular risk intervention improves markers of central artery stiffness (carotid-femoral PWV) and vascular inflammation (CRP, Interleukin-6, TNF-α and adiponectin) in people with type 2 diabetes identified through screening.

7.3 Methods

7.3.1 Experimental design and protocol

The PACE 3 study cohort was recruited from a large population based screening programme for type 2 diabetes (ADDITION-Leicester), described in detail in Chapters 2, 3 and Appendix B. Briefly, 30,950 volunteers from twenty general practices across Leicestershire were invited for a community based diabetes and cardiovascular risk assessment. 6749 (22% response rate) adults aged 40-75 years (25-75 if south Asian) were screened with an OGTT. Anthropometric measurements and a detailed medical history were obtained in addition to a comprehensive biomedical profile. Consent for further venesection and vascular measurements was obtained as part of the PACE sub-study, and a second appointment made within two

weeks of screening. Individuals identified with newly diagnosed (screen detected) diabetes were subsequently invited to enter a randomised controlled trial of multi-factorial cardiovascular risk intervention (ADDITION-Leicester intervention phase – Chapters 2 and 4). Validated measurements of arterial stiffness ($_{cf}$PWV) and inflammation (CRP, IL6, TNFα, adiponectin) were performed within two weeks of baseline and at one year assessments of the trial. An intra-observer validation study was performed on a second population drawn from the ADDITION-Leicester cohort, with repeated $_{cf}$PWV measurements two and four weeks after screening.

7.3.2 ADDITION-Leicester multi-factorial cardiovascular risk intervention

The intervention phase of ADDITION-Leicester is a randomised controlled trial comparing intensive multi-factorial cardiovascular risk management with routine care in general practice according to national guidelines. Randomisation is performed at practice level by an independent steering committee provided with population demographics, deprivation status and approximates of known or diagnosed diabetes prevalence. The screen detected diabetes control group (routine care group) receive an expected management standard within a standard primary care setting, according to national recommendations for management of diabetes and prevention of cardiovascular disease. These participants are reviewed one and five years post diagnosis, when anthropometric and biochemical data is collected. The screen detected diabetes intervention group (intensive care group) are introduced to dedicated, specialist physicians and nurses who provide a structured, intensified, protocol-driven, mult-factorial approach again within the primary care setting (Chapter 2).

7.3.3 $_{cf}$PWV measurement

A single operator, where possible blinded to treatment allocation, performed all arterial measurements within a single site research facility at the University Hospitals of Leicester. A standard protocol for $_{cf}$PWV, described in Chapters 2, 5 and Appendix B was followed for baseline, reproducibility and one year measurements.

7.3.4 Serum Biomarker measurements

In order to assess the anti-inflammatory effects of multi-factorial intervention in PACE 3, consent was obtained for venesection to measure the following battery of serum biomarkers; C-reactive protein (CRP), adiponectin, Tumour Necrosis Factor-alpha (TNF-α) and Interleukin-6 (IL6). All biomarker measurements were performed on fasting samples after spinning and storage at -70^{O}C. Biomarker analyses took place at the Unilever Discover Laboratory, Colworth House, Sharnbrook, Bedfordshire, United Kingdom. Details of the assays used are described in Chapter 2.

7.3.5 Statistics and power calculation

Independent samples t-tests were used to assess baseline differences in subject characteristics between intensive and routine care diabetes groups. Variables displaying a non-normal distribution (as assessed by the Kolmogorov-Smirnov test) were log transformed before statistical analyses were performed. Variables for which log-transformation was necessary are indicated but untransformed data is reported for clarity. Independent t-tests were used to test for differences in the magnitude of change in structural and circulating biomarker indices at baseline and twelve months post intervention in both groups. For the purposes of this study anti-hypertensive

medication is defined as any of the following; angiotensin converting enzyme inhibitor (ACE), angiotensin receptor blocker (ARB), calcium channel antagonist, alpha/beta blocker or thiazide diuretic. Lipid lowering medication is defined as any statin (HMG-CoA reductase inhibitors) or fibrate (PPAR-α receptor agonist). Glucose lowering therapy is defined as any of the following; biguanide (metformin), sulphonylurea, insulin, Thiazoldinedione or GLP-1 agonist.

The effects of relevant variables (baseline biomarker parameters, MAP change, presence of cardiovascular disease and intensive treatment) were analysed using a multivariate regression analysis. Within observer differences between the first (baseline), second (two weeks) and third (four weeks) measurements in the reproducibility sample were assessed by the method of Bland-Altman using a 95% limits of agreement approach (mean difference ± 2 standard deviations). All data are expressed as mean ± standard error (SEM) with statistical significance set at $p<0.05$. Analyses were performed on SPSS v16 and graphics completed using graph pad prism v5. Assuming a standard deviation of PWV of 2.0 ms^{-1} and a clinically relevant difference of 1.0ms^{-1} a total of 63 screen detected diabetes subjects were required to test the hypothesis that multi-factorial treatment improves $_{cf}$PWV at a significance level of 0.05 and power of 80%. This sample size is consistent with intervention trials using $_{cf}$PWV and inflammatory outcome measures in subjects with diabetes [159,164].

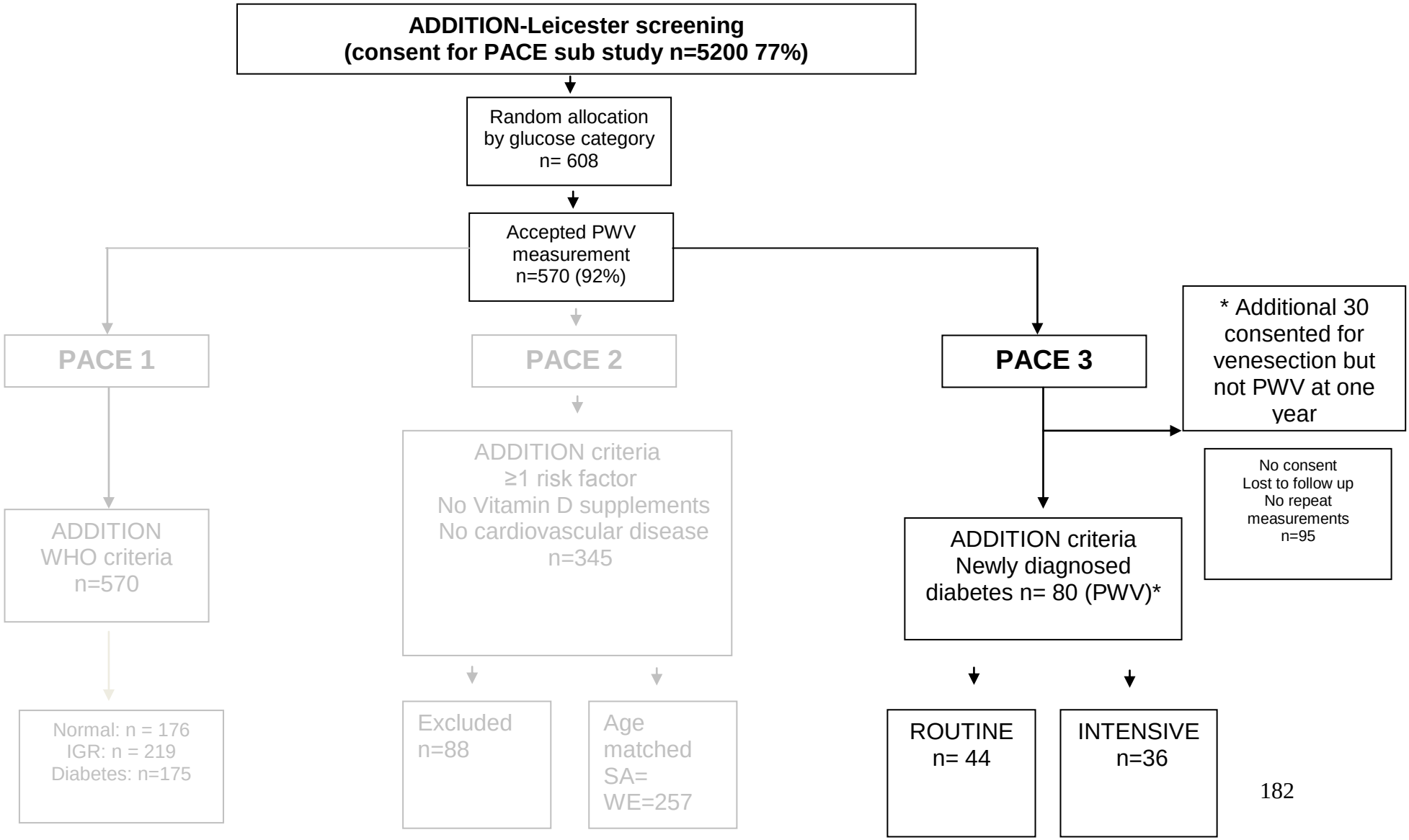
7.4 Results

The screening phase of ADDITION-Leicester identified 214 new cases of diabetes (Chapter 3). Of these, 175 (81%) consented for the PACE sub-study and recorded

acceptable baseline PWV waveforms (chapter 5). 80 volunteers (46%) returned for a second twelve month post intervention $_{cf}$PWV assessment and an additional 30 consented only to further venesection for circulating biomarkers (total biomarker study population n=110) (Figure 7.1). There were no statistically significant biomedical differences between the screened cases with diabetes (n=214) either not consenting to, or subsequently failing waveform capture (n=39) and those recording baseline $_{cf}$PWV measurements (n=175) (Table 7.1).

Follow up populations differed only in ethnic makeup (Table 7.2). South Asians with a baseline $_{cf}$PWV measurement were less likely than white Europeans to attend the follow up assessment (south Asian PACE 3 cohort n=27 (37%) vs south Asian no 1-year follow up n=45 (63%, p=0.04). Otherwise, the final $_{cf}$PWV study population (n=80) was statistically similar to the group only providing baseline measurements (Table 7.2).

Figure 7.1 Schematic illustration of recruitment flow for the PACE 3 sub-study



7.4.1 Reproducibility assessment

$_{cf}$PWV measurements were repeated in nineteen volunteers two and four weeks after baseline assessment in PACE 1. Seven of these had newly diagnosed diabetes, six had IGR and six had normal glucose tolerance. The mean age and MAP of this group was 58.4 ± 9.7(SD) years, and 105.0 ± 10.7(SD) mmHg respectively. Three (15%) had pre-existing cardiovascular disease and seven were prescribed either anti-hypertensive or lipid lowering medications (37%). The within observer differences in $_{cf}$PWV at two and four weeks are shown in Table 7.3. The intra-class Pearson correlation coefficient for the two comparisons were 0.97 (two weeks) and 0.94 (four weeks) respectively (both $p<0.001$). Both measurements had a negative bias (-0.09 ms^{-1} and -0.04 ms^{-1} for two and four week analyses respectively) but Bland-Altman plots indicated no values greater than two standard deviations from the mean $_{cf}$PWV difference (Table 7.3, Figure 7.2a and 7.2b). Variability was consistent across both plots with no obvious trend in the diabetes group.

Table 7.1 Biomedical characteristics of diabetes cases included in the PACE sub-study (n=175) and those screened through ADDITION-Leicester but subsequently excluded or not consenting to $_{cf}$PWV analysis (n=39)

	PACE n=175	Excluded n=39	
Age (Years)	58.7 ± 1.0	57.6 ± 1.4	All comparisons non-significant
Gender (% male)	57	50	
Ethnicity (%white)	59	65	
$HbA_{1c\%}$	7.1 ± 0.2	7.2 ± 0.4	
BMI (kgm^{-2})	30.9 ± 0.5	31.6 ± 0.8	
MAP (mmHg)	101.1 ± 0.9	99.5 ± 1.5	
CVD (%)	11.5	10.2	

Table 7.2 Baseline biomedical characteristics of the PACE 3 sub-study population attending follow up $_{cf}$PWV measurements (n=80) and those with a baseline $_{cf}$PWV assessment but no follow up assessment (n=95)

	PACE 3 n=80	No 1 year assessment n=95
Age (Years)	60.9 ± 1.3	58.6 ± 0.9
Gender (% male)	64	54
Ethnicity (%white)	72	55*
$HbA_{1c\%}$	7.1 ± 1.9	7.1 ± 1.8
BMI (kgm^{-2})	30.0 ± 0.7	31.3 ± 0.5
MAP (mmHg)	101.3 ± 1.6	100.5 ± 1.0
CVD (%)	8.7	10.1

*p=0.04
All other comparisons non-significant

Table 7.3 Within-observer reproducibility of $_{cf}$PWV two and four weeks after baseline (PWV_0) measurement (n= 19)

Measurement	Correlation coefficient	Mean difference from PWV_0 (ms^{-1}) + 95% CI	2 Standard Deviations (2SD)	95% Limits of agreement
2 weeks	0.97	-0.09 (-0.58, +0.38)	0.96	-0.85 to +1.03
4 weeks	0.94	-0.04 (-0.56, +0.66)	1.24	-1.26 to +1.18

Figure 7.2 Bland-Altman plots illustrating intra-observer differences against mean $_{cf}$PWV for a) two week and b) four week (wks) measurements

Figure 7.2 a) 2 week measurements

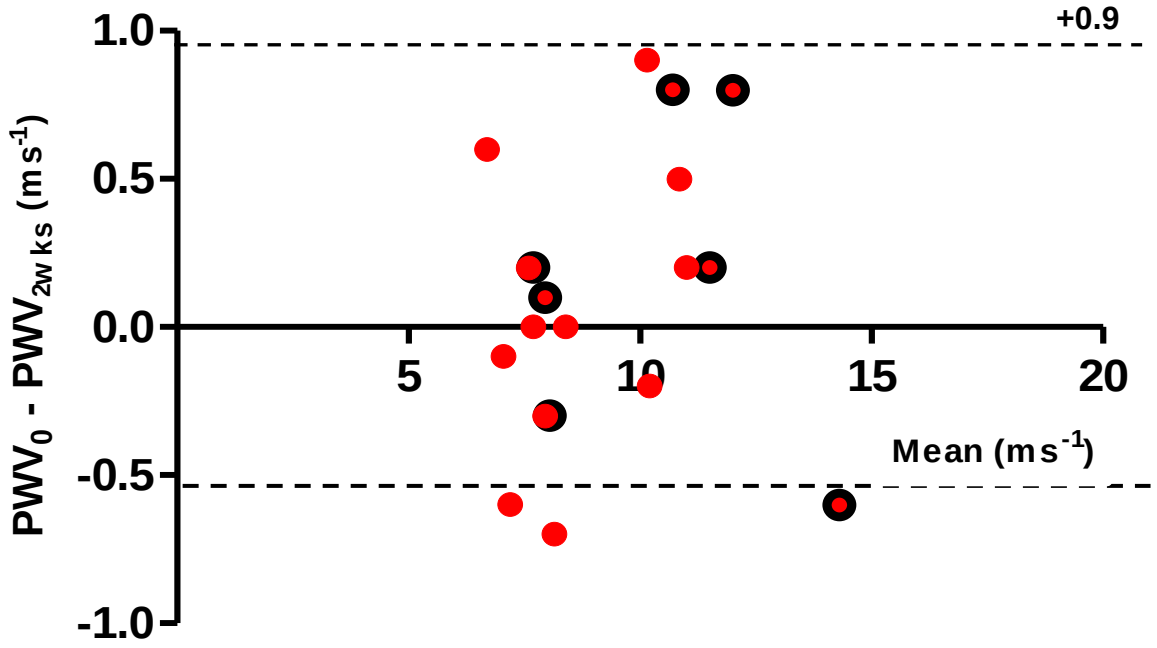
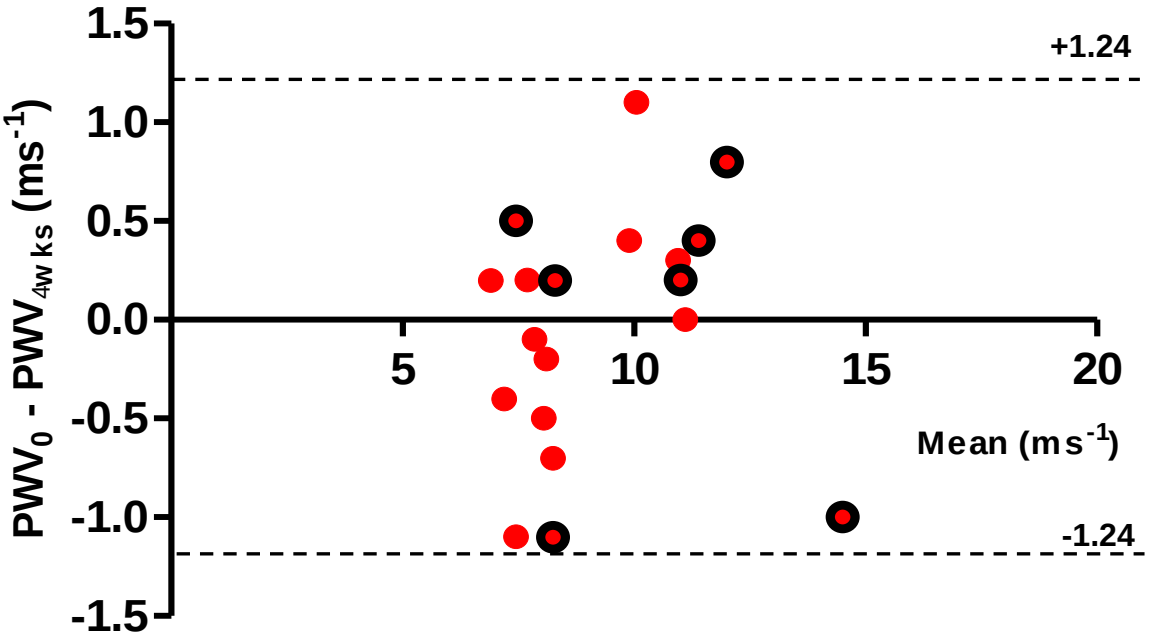


Figure 7.2 b) 4 week measurements



Dotted line represents 2 standard deviations from mean value
Diabetes cases indicated by symbols with a black border

7.4.2 Effects of multi-factorial cardiovascular risk intervention

Baseline and post intervention characteristics of the 80 subjects enrolled in the trial and undergoing repeat $_{cf}$PWV measurements are displayed in Table 7.4. The 44 and 36 subjects randomised to routine or intensive multi-factorial interventions respectively were similar in respect to age, gender, anthropometrics, biomedical characteristics and brachial pressure indices.

After twelve months of multi-factorial cardiovascular risk intervention reductions in glucose ($HbA_{1c\%}$: -0.3 ± 0.2), blood pressure (eg. MAP [-7.9 ± 1.9 mmHg]), lipid (eg. total cholesterol [-1.1 ± 0.35 $mmoll^{-1}$]) and other metabolic (eg. weight [-2.7 ± 2.3 Kg], serum Alanine Transaminase (ALT) [-3.5 ± 2.7 IUl^{-1}]) parameters were observed in both groups. Total cholesterol (-0.7 vs -1.4 $mmoll^{-1}$), MAP (as a consequence of both systolic [-7.4 vs 16.8 mmHg] and diastolic blood pressure [-2.0 vs -8.0 mmHg] components) and weight (-1.4 vs -4.0kg) were reduced significantly more in the intensive intervention group (Tables 7.4 and 7.5). These improvements in cardiovascular risk profile corresponded to increases in anti-hypertensive, lipid and glucose lowering medications in both groups. Cardiovascular protection therapies were more frequently prescribed in the intensive arm (eg. 50% vs 82% for lipid lowering treatments). The most commonly used class of anti-hypertensive were ACE inhibitors, followed by ARBs, thiazide diuretics and calcium channel antagonists. A statin was the lipid lowering treatment of choice in the majority of cases (85%), with less than 5% on fibrate therapy alone. Glucose lowering medications used were metformin (76%), Gliclazide, Glibenclamide, and Insulin (3%). There were no statistically significant differences in the choice of prescribed medications between the groups.

Table 7.4 Baseline biomedical characteristics of routine and intensive intervention groups of the $_{cf}$PWV study population (n=80)

	ROUTINE (n=44)		INTENSIVE (n=36)	
	Baseline	One Year	Baseline	One Year
Age (Years)	61.5 ± 1.9	-	60.5 ± 1.9	-
Gender (% male)	60	-	65	-
Ethnicity (% white)	63	-	70	-
Fasting Glucose ($mmoll^{-1}$)	8.0 ± 0.5	-	7.5 ± 0.3	-
Post-Load Glucose ($mmoll^{-1}$)	13.7 ± 0.8	-	12.7 ± 0.5	-
HBA1c (%)	7.2 ± 0.3	6.9 ± 0.3	6.8 ± 0.2	6.5 ± 0.2
HOMA-IR (arb. units)$	4.65 (1.46-11.9)	4.14 (1.10-10.9)	3.95 (0.26-9.7)	3.5 (0.24-9.9)
Total cholesterol ($mmoll^{-1}$)	5.7 ± 0.3	5.0 ± 0.3	5.4 ± 0.1	4.0 ± 0.1†
HDL-C ($mmoll^{-1}$)	1.2 ± 0.1	1.1 ± 0.1	1.3 ± 0.1	1.4 ± 0.1
Triglycerides ($mmoll^{-1}$)$	2.4 (0.9-1.6)	2.2 (0.9-1.6)	2.9 (1.0-2.0)	2.3 (1.0-2.1)
White Cell Count	7.1 ± 0.4	-	7.1 ± 0.4	-
ALT (IUl^{-1})	37.4 ± 3.8	33.8 ± 3.0	33.4 ± 2.9	29.9 ± 3.0
Systolic BP (mmHg)	137.8 ± 3.4	130.4 ± 3.6	140.2 ± 4.2	123.2 ± 4.9†
Diastolic BP (mmHg)	81.1 ± 1.6	79.1 ± 1.6	83.1 ± 1.8	75.1 ± 1.8†
Pulse Pressure (mmHg)	56.7 ± 3.3	51.3 ± 3.6	57.1 ± 2.6	48.1 ± 2.3†
Mean Arterial Pressure(mmHg)	101.2 ± 2.0	95.9 ± 1.8	101.3 ± 2.1	90.94 ± 2.0†
Heart Rate (min^{-1})	63.5 ± 2.3	63.9 ± 2.6	65.4 ± 1.7	64.4 ± 2.2
Weight (kg)	85.2 ± 3.1	83.8 ± 2.9	81.5 ± 2.9	77.5 ± 2.3†
BMI (kgm^{-2})	30.5 ± 0.9	29.2 ± 1.0	29.7 ± 0.9	28.0 ± 0.9
Waist Circumference (cm)	104.8 ± 2.2	102.5 ± 2.0	99.2 ± 2.3	96.8 ± 1.7
Medications:				
Lipid Lowering (%)	10	50	8	82†
Anti-hypertensive (%)	23	43	35	69†
Glucose Lowering (%)	0	45	0	69
Cardiovascular Disease (%)	10	10	9	10
Active Smoking (%)	10	9	17	14
PWV (ms^{-1})	10.70 ± 0.4	10.59 ± 0.3	10.15 ± 0.4	9.91 ± 0.4
CRP (mgl^{-1}) $ (n=110)	6.2 ± 1.1	5.3 ± 1.6	5.8 ± 1.2	3.9 ± 1.8†
TNFα (pgl^{-1}) (n=95)	2.29 ± 0.17	2.28 ± 0.19	2.14 ±0.12	2.05 ±0.12
IL6 (pgl^{-1}) (n=95)	2.91 ± 0.19	2.72 ± 0.19	3.20 ± 0.28	2.58 ± 0.28
Adiponectin (ngl-1) (n=101)	10.5 ± 0.90	11.6 ± 1.0	11.2 ± 0.90	12.7 ± 0.90

Values expressed as mean ± SEM or where not normally distributed as IQR
Variables analysed using log transformed values are indicated $
Between group comparisons: baseline all p>0.05, †one year p<0.05

Figure 7.3 Mean biomarker change (± SEM) between routine and intensive groups 12 months after diagnosis of type 2 diabetes

Figure 7.3a $_{cf}$PWV change (ms^{-1}) and expected population age related incremental increase

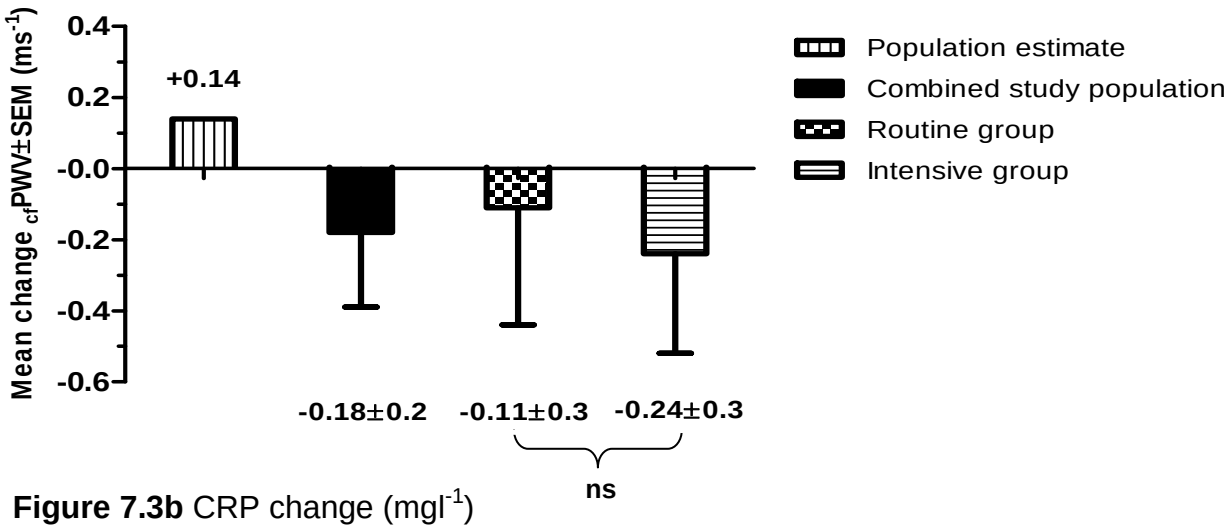


Figure 7.3b CRP change (mgl^{-1})

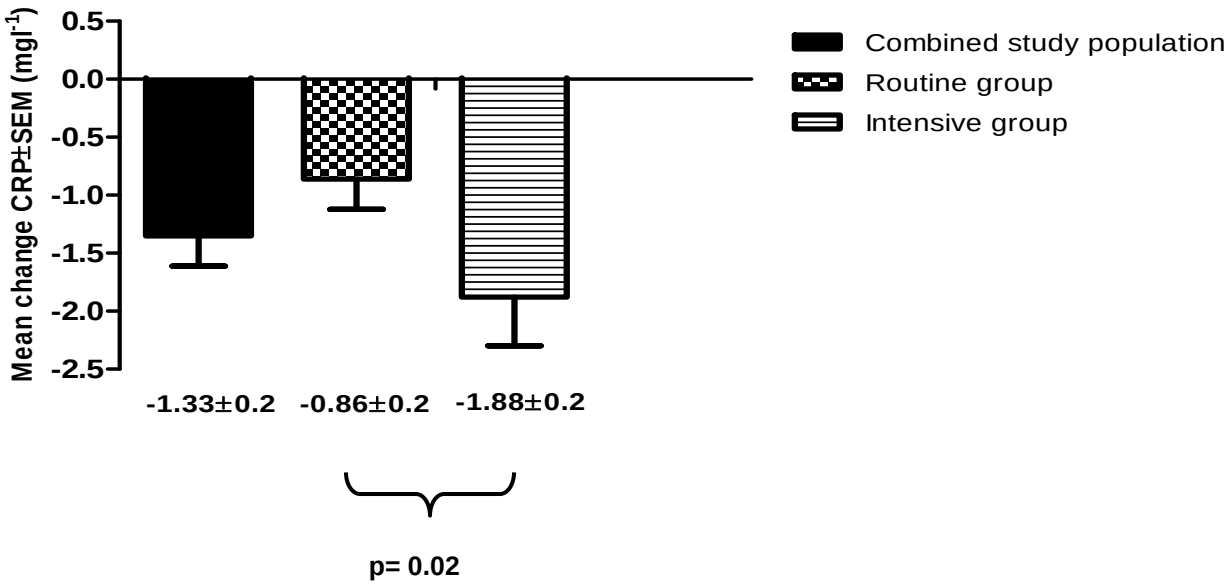


Figure 7.3c Adiponectin change (ugml^{-1})

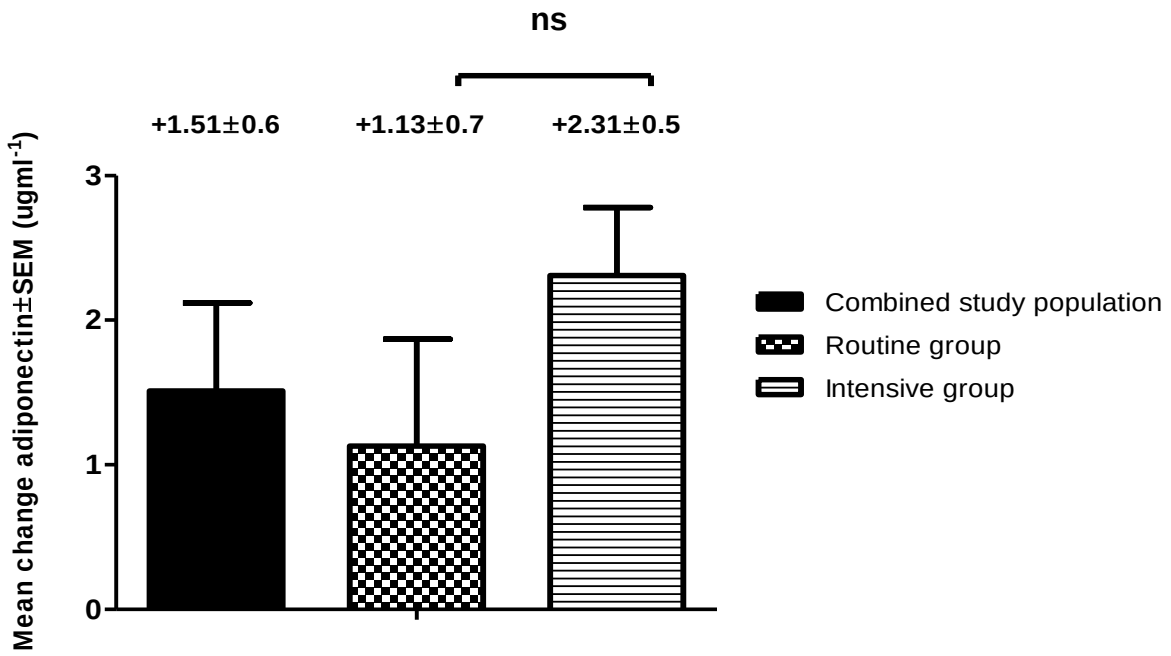


Figure 7.3d IL6 change (pgml^{-1})

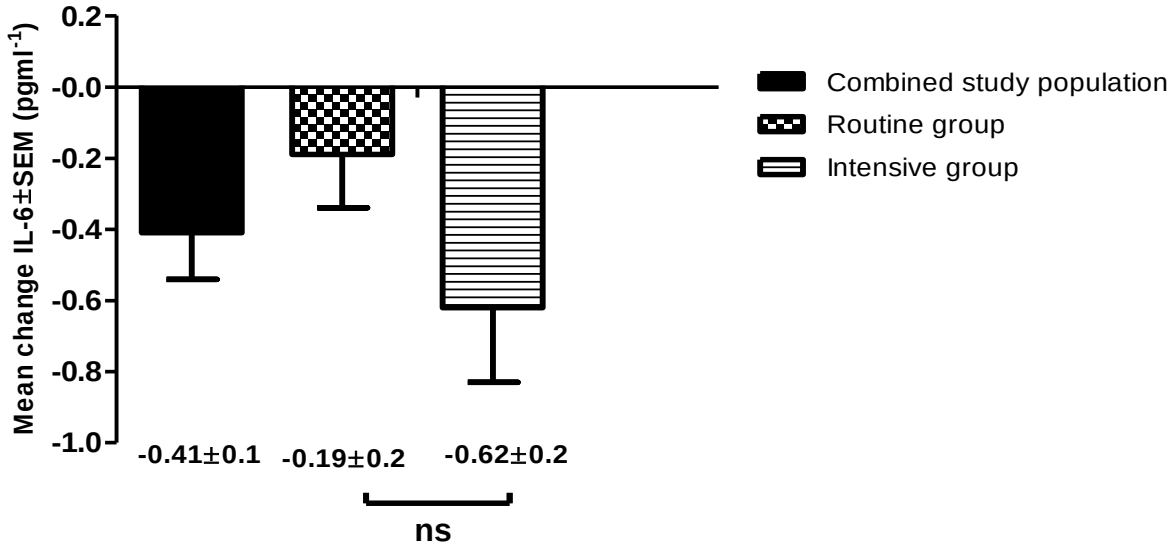


Figure 7.3 e TNF-α change (pgml^{-1})

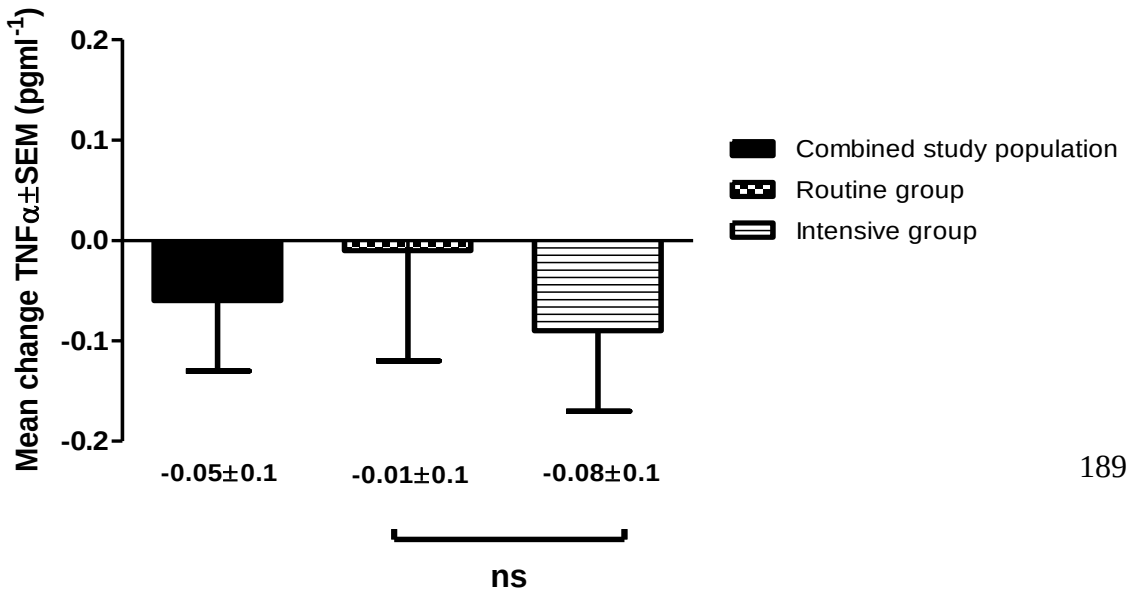


Table 7.5 and figure 7.3 demonstrate mean changes in structural and circulating biomarkers over the study period. Carotid-femoral $_{cf}$PWV decreased by 0.11 ms^{-1} in the routine and 0.24 ms^{-1} in the intensive study arm (p=0.36). CRP was reduced significantly more by intensive multi-factorial treatment whilst adiponectin increased in both groups. There was no significant change in TNFα, IL-6 or adiponectin between the groups. A stepwise multivariate regression analysis (Table 7.6) demonstrated that CRP change over the study period significantly related to intensive multi-factorial treatment, independent of baseline biomarker values, age or pre-existing cardiovascular disease. Intensive treatment did not independently associate with $_{cf}$PWV (Table 8.6), TNF α, IL-6 or adiponectin.

Table 7.5 Mean change (±SEM) in biomedical and biomarker indices from baseline in routine and intensive intervention groups

	Total (n=80)	Routine (n=44)	Intensive (n=36)	p
Biomedical:				
$HbA_{1c\%}$	-0.3 ± 0.24	-0.3 ± 0.23	-0.3 ± 0.24	0.45
T-cholesterol ($mmol^{-1}$)	-1.1 ± 0.35	-0.7 ± 0.37	-1.4 ± 0.28	<0.01
MAP (mmHg)	-7.9 ± 1.9	-5.3 ± 1.8	-10.4 ± 2.0	<0.01
Biomarker:				
PWV (ms^{-1})	-0.18 ± 0.21	-0.11 ± 0.33	-0.24 ± 0.28	0.36
CRP (mgl^{-1})	-1.35 ± 0.26	-0.86 ± 0.26	-1.88 ± 0.42	0.02
TNFα ($pgml^{-1}$)	-0.06 ± 0.07	-0.01 ± 0.11	-0.09 ± 0.08	0.59
IL6 ($pgml^{-1}$)	-0.41 ± 0.13	- 0.19 ± 0.15	- 0.62 ± 0.21	0.13
Adiponectin ($ugml^{-1}$)	+2.31 ± 0.47	+1.13 ± 0.74	+1.51 ± 0.61	0.69

Table 7.6 Stepwise linear regression analysis of the determinants of change in $_{cf}$PWV and CRP (Δ) after one year of multi-factorial intervention

Dependent Variable	Adjustment Parameter	R^2 Increment	B coefficient	p
Δ PWV	Baseline PWV	0.32	0.73 ± 0.02	<0.01
	Intensive treatment	<0.01	0.02 ± 0.10	0.48
	MAP change	0.05	0.18 ± 0.08	0.03
	Age	0.03	-0.29 ± 0.08	0.02
	CVD	-	-0.07 ± 0.54	0.82
	Variance explained R^2 = 0.40			
Δ CRP	Baseline CRP	0.42	0.64 ± 0.06	<0.01
	Intensive treatment	0.05	0.20 ± 0.55	0.03
	CVD	0.01	-0.18 ± 0.47	0.05
	Age	-	-0.08 ± 0.02	0.35
	Variance explained R^2 = 0.48			

7.5 Discussion

This study uniquely demonstrates that intensified cardiovascular risk management reduces inflammation more than standard care in people with type 2 diabetes identified through screening. Interestingly, this was not associated with similar improvements in arterial stiffness with multivariate modelling suggesting little between group effects in $_{cf}$PWV change over the study period. There were overall reductions in $_{cf}$PWV and CRP, important structural and inflammatory biomarkers predicting mortality in diabetes [124]. Changes were also observed for other selected biomarkers, probably reflecting a cumulative effect on the deleterious pro-inflammatory state believed to drive vascular complications in diabetes.

The management of newly diagnosed cases was intensive and impacted significantly upon established risk factors including dyslipidaemia, hypertension, hyperglycaemia and weight across the study. In fact we probably underestimated the effectiveness of "standard practice" in the routine care arm, which likely reflects a growing awareness of the importance of early, aggressive management of type 2 diabetes amongst general practitioners in the United Kingdom. This had the effect of attenuating biomarker differences between the groups and should be regarded as an unavoidable limitation of the study, as providing a true control population is not ethically possibile. Population age-related changes in vascular compliance suggest aortic pulse wave velocity increases by approximately 1.4 -1.6 ms^{-1} between the 5^{th} and 6^{th} decade of life [311, 312]. Extrapolating this estimate to provide a conservative annual incremental rise of approximately +0.14 ms^{-1} implies this particular multi-factorial approach, which lowered $_{cf}$PWV by +0.18 ms^{-1}, maybe

effective at reducing arterial stiffness in screen detected diabetes over a longer time frame.

The mechanisms mediating conduit vessel stiffening predominantly relate to structural alterations in the arterial wall as a consequence of exposure to distending stress cycles over many years. As an almost inevitable consequence of the ageing process it is intuitively unlikely that any short-term intervention would result in dramatic structural improvements in the artery wall. Nevertheless, a number of placebo controlled studies of similar or even shorter duration than 12 months have demonstrated improvements in measures of arterial stiffness using components of the multi-factorial approach used here [155,159,306,307]. It is therefore not unreasonable to have expected intensive multiple risk factor management to result in a significant reduction in pulse wave velocity. Published studies generally hypothesise that ACE inhibitors, statins and thiazolidinediones in particular possess pleiotropic anti-inflammatory properties which may “de-stiffen” elastic arteries via mechanisms independent of established roles in blood pressure, lipid and glucose lowering. The significant reduction in CRP in the intensive arm of the study does not support this “anti-inflammatory” theory as it was not accompanied by a statistically significant concomitant decrease in $_{cf}$PWV [313-315]. Independent association of $_{cf}$PWV change with mean arterial pressure rather than CRP suggests direct pressure lowering effects or mechanisms independent of this marker are involved in improving pulsatile function in people with diabetes.

Other studies have reported more dramatic improvements in markers of arterial stiffness in newly diagnosed type 1 diabetes or IGT compared with established type 2 diabetes groups [156,250]. This suggests that even earlier intervention than that

achieved here may be required to significantly ameliorate arteriosclerosis using existing therapies. The baseline $_{cf}$PWV of the diabetes group was 1.2 ms^{-1} higher than those with normal glucose tolerance (Chapter 5).

The study could have been strengthened by employing additional measures assessing local as well as regional vascular stiffness. Techniques determining vessel distensibilty and compliance are generally acknowledged to provide the most accurate estimates of elastic artery stiffness and such an approach may be particularly advantageous in intervention studies requiring repeated measurement of cutaneous distances to estimate arterial length. The intra-observer reproducibility study was small and by incorporating only seven people with diabetes, flawed in its design. This may have compromised our estimate of bias in the $_{cf}$PWV measurement method. The repeatability co-efficient and respective confidence interval obtained for both time points was small however and felt to be clinically acceptable for the expected intervention effect. The study may have also have been insufficiently powered to detect between group differences in some of its secondary outcomes. This was probably true of adiponectin, IL-6 and TNFα where anticipated directional but statistically non-significant changes were observed. Caution should therefore be exercised when interpreting these results in the context of intervention efficacy.

7.6 Conclusion

Intensive multi-factorial intervention in type 2 diabetes reduces circulating levels of C-reactive protein. One year of treatment does not reduce arterial stiffness, IL-6 or TNF-α more than conventional cardiovascular risk lowering in cases identified through screening.

Summary of findings and conclusion

Conceptually, the implementation of a national screening programme for diabetes could potentially improve the lives of many thousands of people by delaying the onset and/or preventing the complications of the disease. Type 2 diabetes fulfils many of the criteria for screening with one notable exception. It is unknown whether earlier detection ultimately improves cardiovascular outcomes by expediting interventions of proven efficacy in established disease. This thesis firstly tested the hypothesis that screening for glucose disorders within a United Kingdom multiethnic population identifies people at increased risk of modifiable cardiovascular disease and subsequent multi-factorial intervention in screen detected cases improves these measures of risk. Multi-factorial management in this context involves integrated life-style modification, education and pharmacological interventions via the prescription of existing cardio-protective therapies.

A major community based programme involving twenty general practices characterised glucose status with a standard tolerance test and enabled an initial detailed description of screen detected diabetes and Impaired Glucose Regulation (a composite of non-diabetes range hyperglycaemia). This was followed by a randomised controlled trial of protocol driven multi-factorial cardiovascular risk intervention in people identified with diabetes through the programme. The primary outcome of modelled Coronary Heart Disease risk was realistic within the timeframe of this three year project and provided novel data on achievable cardiovascular risk reduction in screened cases.

The results of the screening phase demonstrated that undiagnosed glucose disorders are associated with significant cardiovascular risk and remain surprisingly common in the United Kingdom, especially within ethnic minority south Asian groups. The prevalence of any glucose abnormality was twelve percent among white Europeans and nineteen percent among south Asians with adjusted ten year cardiovascular risk of fourteen and sixteen percent respectively. Importantly, cardiovascular risk was equally high in groups identified with diabetes or Impaired Glucose Regulation. After twelve months of intensive cardiovascular risk intervention, modelled five year cardiovascular risk in those identified with diabetes through screening was reduced by eight percent over standard care controls. These results support the primary hypothesis; screening for type 2 diabetes is feasible within a representative primary care setting and identifies populations at high cardiovascular risk which is subsequently improved by multi-factorial intervention.

As well as standard biomedical parameters, a validated assessment of arterial stiffness linked to vascular outcomes in diabetes served as a robust surrogate of arteriosclerosis for this study. Premature conduit vessel stiffening, measured via carotid-femoral pulse wave velocity, may be a key determinant of cardiovascular compromise in populations at high risk of metabolic disease. This important physiological measurement is not well characterised in glucose treatment naïve or ethnic minority populations and may be a useful marker of vascular disease in intervention studies. Measurement of pulse wave velocity in over six hundred volunteers revealed that any newly diagnosed glucose condition is associated with clinically significant premature arterial stiffening. In addition, fasting and post-challenge serum glucose estimations appear to contribute equally to arterial stiffness

in this population. Although arterial stiffness was not significantly ameliorated by twelve months of multi-factorial intervention, an indirect measure of inflammation (C-reactive protein) was reduced significantly by intensive treatment.

The magnitude of achievable cardiovascular risk reduction is determined not only by the efficacy of available treatments but also the cumulative baseline risk. Therefore populations known to be particularly susceptible to glucose disorders may benefit the most from diabetes screening programmes, especially if the sum of potentially modifiable cardiovascular risk is greater than background. Minority groups deriving first or second generation ancestry from the Indo-Asian sub-continent appear at increased risk of type 2 diabetes, coronary heart disease and stroke compared with indigenous white Europeans. An equally disparate recent decline in rates of cardiovascular disease suggests unidentified and therefore untreated risk factors contribute to disease susceptibility within south Asian migrant populations.

Earlier intensive intervention with existing cardiovascular preventative treatments may address some of the deleterious haemodynamic consequences of sustained risk factor exposure before irreversible vascular damage occurs. The metabolic memory hypothesis suggests there may be a timed threshold of risk factor exposure beyond which there is little benefit to be gained from aggressive glucose lowering intervention [160]. This theory is gaining popularity and convincingly explains the unexpected results of recent cardiovascular outcome trials in diabetes. It would also be consistent with the rationale for earlier identification and intervention of glucose disorders through screening, discussed at length in this thesis.

The concept of a threshold of risk factor exposure or “metabolic memory” appears particularly relevant when applied to arteriosclerotic disease. Prolonged exposure to

known and probably unknown factors results in an essentially irreversible burden of vascular disease well before the clinical diagnosis of diabetes is made. Compounding this, the biological actions of many existing cardioprotective drugs appears not to extend to reversing what is fundamentally a process of accelerated ageing rather than lipid plaque formation. A new therapeutic paradigm may be required to combat premature arterial stiffness in diabetes and IGR with future work exploring the potential of AGE cross-link breakers (eg. Alagebrium and Pyridoxamine), phosphodiasterase inhibitors (eg. Sildenafil) and novel anti-inflammatory agents. The baseline findings of both the ADDITION-Leicester and PACE studies confirm that undiagnosed type 2 diabetes carries a significant burden of cardiovascular risk which even at this early stage is driving potentially irreversible conduit vessel change. A comparison of the screen detected cohort with a group diagnosed “conventionally” revealed high levels of untreated risk in the undiagnosed group. Whilst intensive intervention rapidly reduces the modelled likelihood of future events through conventional risk factor control, it did not have such a dramatic effect on the recognised gold-standard measure of arterial stiffness. Recognising that a twelve month comparator study is probably not the ideal design for assessing outcome differences with this tool, the demonstrable lack of improvement does suggest alternative therapeutic approaches may be required to reduce premature arteriosclerosis. Through a variety of mechanisms, conduit vessel stiffening is a well recognised cause of myocardial disease and probably an understated contributor to large vessel complications in diabetes. Importantly, IGR and diabetes range categories identified through screening carry a similar risk of cardiovascular disease, yet in current clinical practice it is likely that the diabetes cases will be afforded the most attention in terms of complications prevention. This observation was supported

by comparable arterial stiffness measurements within these categories and suggests efforts to screen for asymptomatic glucose disorders should not be limited to diagnostic cut-offs based on the risk of micro and not macrovascular complications. Evidence of premature stiffening in IGR, which comprises approximately eighteen percent of this population-level assessment, suggests a large number of people are significantly at risk of the consequences of long-term or even irreversible arterial damage.

The results of the screening phase of ADDITION-Leicester are consistent with increasingly obsolete surveys conducted over fifteen years ago within other United Kingdom Asian (predominantly non-Indian) populations. The pattern of risk continues to be characterised by a major predisposition to dysglycaemia, with at least twice the odds of undiagnosed IGR and type 2 diabetes compared with age matched white Europeans. Conversely, dyslipidaemia and active smoking appear to be less frequent and blood pressure is similar. This implies screening for glucose disorders in this group may be a particularly effective strategy for capturing individuals at high risk of cardiovascular disease. Again cardiovascular risk was similar within IGR and diabetes populations independent of ethnic group, lending further weight to the argument to actively screen certain populations for glucose disorders below the diagnostic threshold for diabetes. The challenge is clearly going to be in engaging ethnic minority populations into programmes focused on the prevention rather than treatment of chronic disease, as the response rate in this group was less than twenty percent.

The final theme of this thesis relates to cardiovascular risk and arterial stiffness within well matched south Asian and white European groups attending the screening

programme. In particular, the aim was to test the hypothesis that 25-hydroxyvitamin D is an important factor contributing to arterial stiffness and possibly vascular risk in south Asians. Again, through sub-study pulse wave velocity measurements, over two hundred treatment naïve volunteers free of symptomatic cardiovascular disease were assessed. A novel finding of increased arterial stiffness within south Asians attending screening was observed, which independently associated with plasma 25-hydroxyvitamin D concentration. The finding that PWV is increased in Leicester south Asians compared with age-matched white Europeans is an important finding. Although entirely consistent with the increased prevalence of mainly coronary disease in this population, the modest contribution of hyperglycaemia to arterial stiffness demonstrated in PACE1 suggests this risk factor is not the only influence. As brachial arterial pressure indices are also similar we hypothesise this population has a lower set threshold for the deleterious effects of known risk factors such as glucose intolerance and hypertension. We tentatively suggest that vitamin D deficiency, which is almost universally present within this group may also play a role, either by lowering the exposure threshold or through other unknown mechanisms. The finding of a different relationship between PWV and MAP according to vitamin D status is supportive of this and requires complex non-linear statistical analyses to further define what may be an extremely important observation. These findings could readily provide pilot data supporting the rationale for a prospective trial of vitamin D intervention within this particularly deplete population with outcomes focusing upon haemodynamic and structural measures of vascular wall integrity.

The novel findings described in this thesis provide non-selected, accurate information which will be of great relevance to primary care physicians and specialists charged with managing growing epidemics of type 2 diabetes. Of

particular importance are observations pertaining to both continued traditional and emerging novel cardiovascular risk factors in south Asians, a group that remains under researched and poorly understood. It is hoped these findings will make a difference to the future prospects of people with type 2 diabetes

In summary, fasting and post challenge glucose disorders were characterised in over six thousand south Asian and white European adults participating in a systematic diabetes screening programme. This permitted detailed description of cardiovascular risk and arterial stiffness within these groups and identified newly diagnosed diabetes cases for an intervention trial examining the effects of intensive cardiovascular risk intervention. A significant burden of cardiovascular risk is associated with early glucose disorders, coupled with deleterious arterial wall structural changes evident even at pre-symptomatic stages of metabolic disease. These effects are particularly profound in south Asian minorities and may be partially mediated by vitamin D dependent arteriosclerosis. Multi-factorial cardiovascular risk intervention reduces modelled vascular risk but does not significantly reduce arterial stiffness after one year. These results support the case for earlier identification of glucose disorders (possibly even below the diabetes diagnostic threshold) followed by intensive multi-factorial cardiovascular risk intervention in screen detected cases. This approach is likely to be effective in white Europeans, but additional risk factors may contribute to arterial disease in south Asians.

APPENDIX A

Standardised methodology for administering a 75g-OGTT
WHO definition, diagnosis and classification of Diabetes Mellitus and its complications, Part 1 Diagnosis and classification 1999 [21, 226].

- Test preceded by >3 days of normal unrestricted diet (>150g carbohydrate daily) with normal physical activity.
- Carbohydrate rich meal (30-50g) on the night before test
- Overnight fast of 8-14 hours, drink water only
- Record any factors that may affect interpretation of test, such as medication, inactivity, infection, acute psychological stress etc..
- Collect fasting samples in sodium fluoride tube
- Timing of test (0 hours) starts at beginning of glucose drink
- Adults ingest 75g glucose in 250 – 300 ml water over five minutes (296 ml standard Lucozade^{R})
- No smoking during test
- Take blood samples at 2 hours
- Ideally take sample from warmed vein on back of hand (antecubital fossa samples may be artificially lower)
- Glucose should be measured immediately after collection by near patient testing or if a blood sample for a laboratory is collected, plasma should be immediately separated or the sample should be collected into a container with glycolytic inhibitors and placed in ice-water until separated prior to analysis.

APPENDIX B

$_{cf}$PWV Preparation protocol for the PACE sub study of ADDITION-Leicester

- All subjects should be fasted from midnight and refrain from smoking, consuming caffeine containing drinks and engaging in vigorous physical activity on the morning of $_{cf}$PWV measurement.
- Subjects should be instructed to take prescribed medication as usual on the morning of $_{cf}$PWV measurement
- Record room temperature (Accept 10-20^{o}C)
- Explain procedure and record demographic details on PWV CRF and PT4000 device. Ensure consent is present in ADDITION master form
- Expose carotid and femoral areas and rest supine for 15 minutes
- Attach 3 lead Electrocardiogram and record brachial blood pressure and heart rate every five minutes
- Measure carotid – femoral distance in centimetres four times and record mean of three closest measurements
- Once blood pressure and heart rate is stable – defined as two readings within Systolic (+/-9mmHG), Diastolic(+/-6mmHG), and HR(+/-8beatsmin^{-1}) place lubricated probe over carotid pulse and obtain good waveform signal (subject may need to extend neck if soft tissues are lax)

- Record a ten second continuous cycle of carotid waveforms. PT4000 will only record acceptable traces with a Transit time standard deviation of <5%
- Repeat 8) and 9) over the femoral pulse and record calculated $_{cf}$PWV from transit time and distance measurements
- Repeat 8), 9) and 10) to obtain at least three concordant $_{cf}$PWV readings (+/- 0.3 ms^{-1})

APPENDIX C

Eligible, invited and screened populations of individual practices participating in the ADDITION-Leicester study

Practice	Practice size (n)	Eligible (n)	Invited (n)	Screened (n)
01	1857	707	302	16
02	9527	4581	3055	463
03	4444	1733	1031	160
04	8651	3517	2036	464
05	5081	2181	1289	295
06	4110	1922	1134	459
07	9000	3543	3543	1017
08	7276	3454	890	97
09	3935	1699	1699	381
10	3105	1268	759	125
11	4689	2201	1626	377
12	2870	1589	718	226
13	4200	1917	672	56
14	4111	1850	1051	225
15	13,444	5120	5120	669
16	3673	1445	1446	764
17	3155	2071	2071	297
18	34,375	14,895	913	338
19	4221	1095	1095	109
20	17,650	9540	501	211
Total	149,374	66,320	29,856	6749

APPENDIX D

Baseline and one year characteristics of the ADDITION-Leicester population by ethnic group
Data shown as mean (standard deviation) for continuous variables, count (percentage) for categorical variables. Pre-existing CVD includes MI, stroke and angina

WE: White European
SA: South Asian

	Routine Care						**Intensive Care**					
	Baseline			**1 year**			**Baseline**			**1 year**		
	Total	WE	SA	Total	WE	SA	Total	WE	SA	Total	WE	SA
Age, years	60.0 (10.0)	62.9 (8.4)	55.1 (10.6)	-	-	-	59.4 (10.0)	63.3 (8.7)	54.7 (9.3)	-	-	-
Male, n (%)	116 (58.3)	75 (60.5)	41 (55.4)	-	-	-	83 (56.9)	45 (58.4)	36 (56.3)	-	-	-
Ethnicity												
White European, n (%)	124 (62.3)	124 (100)	-	-	-	-	77 (52.7)	77 (100)	-	-	-	-
South Asian, n (%)	74 (37.2)	-	74 (100)	-	-	-	64 (43.8)	-	64 (100)	-	-	-
Other, n (%)	1 (0.5)	-	-	-	-	-	5 (3.4)	-	-	-	-	-
Haemoglobin A1c (%) (mmol/mol)	7.3 (1.8) 56 (20)	7.4 (2.0) 57 (22)	7.2 (1.5) 55 (17)	6.8 (1.1) 50 (12)	6.7 (1.1) 50 (12)	6.8 (1.2) 51 (13)	7.2 (1.5) 55 (16)	7.0 (1.4) 53 (15)	7.5 (1.6) 58 (17)	6.5 (0.5) 48 (6)	6.4 (0.5) 46 (5)	6.7 (0.6) 49 (6)
Weight (kg)	87.8 (18.7)	92.4 (17.9)	80.3 (17.8)	86.4 (20.0)	90.7 (19.5)	79.2 (18.9)	84.8 (18.6)	87.8 (19.0)	81.1 (17.8)	81.1 (18.5)	83.8 (18.0)	77.7 (19.0)

BMI (kg/m^2)	31.5 (5.7)	32.3 (5.9)	30.2 (5.3)	29.4 (9.1)	30.4 (8.6)	27.8 (9.7)	31.0 (5.9)	31.1 (5.8)	30.9 (6.0)	29.7 (5.8)	29.7 (5.5)	29.7 (6.1)
Waist circumference (cm)	104.4 (13.9)	106.8 (14.1)	100.6 (13.0)	102.3 (13.3)	104.2 (13.6)	98.8 (12.0)	102.9 (12.7)	103.9 (13.4)	101.7 (11.8)	100.3 (12.8)	100.2 (12.9)	100.4 (12.9)
Total Cholesterol (mmol/l)	5.6 (1.3)	5.9 (1.2)	5.2 (1.2)	4.7 (1.1)	4.8 (1.1)	4.5 (1.1)	5.3 (1.2)	5.4 (1.1)	5.2 (1.2)	4.0 (1.1)	4.0 (1.1)	4.0 (0.9)
LDL cholesterol (mmol/l)	3.5 (1.0)	3.7 (1.0)	3.2 (1.0)	2.6 (0.9)	2.6 (0.8)	2.6 (0.9)	3.2 (1.0)	3.3 (1.0)	3.1 (1.0)	2.1 (0.7)	2.0 (0.7)	2.1 (0.7)
HDL cholesterol (mmol/l)	1.2 (0.3)	1.2 (0.3)	1.1 (0.3)	1.2 (0.3)	1.2 (0.3)	1.2 (0.4)	1.2 (0.4)	1.2 (0.3)	1.2 (0.3)	1.2 (0.3)	1.2 (0.3)	1.1 (0.3)
Triglycerides (mmol/l)	2.1 (1.4)	2.2 (1.3)	2.0 (1.6)	1.9 (1.4)	2.0 (1.6)	1.6 (0.9)	2.1 (1.9)	2.0 (1.5)	2.2 (2.3)	1.7 (2.3)	1.7 (2.9)	1.7 (1.6)
Systolic blood pressure (mmHg)	148.4 (20.5)	152.7 (20.8)	141.5 (18.2)	140.1 (17.2)	143.9 (17.0)	133.8 (15.8)	145.7 (18.5)	147.6 (19.0)	143.0 (18.1)	129.3 (14.0)	128.8 (13.5)	130.0 (14.5)
Diastolic blood pressure (mmHg)	89.5 (10.7)	89.9 (11.6)	89.0 (9.1)	82.0 (9.5)	81.8 (9.9)	82.3 (8.9)	87.8 (10.4)	86.7 (11.0)	88.9 (9.7)	75.2 (9.9)	72.7 (10.2)	78.1 (8.8)
Current smoker, n (%)	20 (10.2)	15 (12.3)	5 (6.8)	20 (10.9)	17 (14.8)	3 (4.5)	22 (15.2)	14 (18.2)	7 (11.1)	18 (13.2)	12 (16.2)	5 (8.6)
Pre existing CVD, n (%)	21 (10.6)	14 (11.3)	7 (9.5)	-	-	-	23 (15.8)	14 (18.2)	8 (12.5)	-	-	-
Atrial fibrillation, n (%)	3 (1.5)	3 (2.4)	0 (0)	-	-	-	3 (2.1)	3 (3.9)	0 (0)	-	-	-
Medication use:												
Anti-hypertensives, n (%)	54 (27.1)	32 (25.8)	21 (28.4)	84 (42.2)	53 (42.7)	30 (40.5)	59 (40.4)	32 (41.6)	24 (37.5)	96 (65.8)	54 (70.1)	37 (57.8)
Lipid lowering, n (%)	5 (2.5)	4 (3.2)	1 (1.4)	96 (49.2)	68 (55.3)	24 (33.8)	7 (4.8)	4 (5.2)	3 (4.7)	118 (82.5)	64 (85.3)	49 (77.8)
Aspirin, n (%)	8 (4.0)	6 (4.8)	2 (2.7)	55 (28.2)	38 (30.9)	17 (23.9)	6 (4.1)	2 (2.6)	4 (6.3)	102 (71.3)	53 (70.7)	46 (73.0)
Metformin, n (%)	-	-	-	75 (38.5)	45 (36.6)	30 (42.3)	-	-	-	77 (53.9)	32 (42.7)	43 (60.3)

Sulfonylurea, n (%)	-	-	-	10 (5.1)	7 (5.7)	3 (4.2)	-	-	-	11 (7.7)	7 (9.3)	4 (6.4)
Insulin, n (%)	-	-	-	0 (0)	0 (0)	0 (0)	-	-	-	2 (1.4)	1 (0.5)	1 (0.8)
UKPDS 5 year risk CHD:												
Total (%)	9.3 (7.1)	10.8 (6.4)	6.7 (7.5)	7.0 (5.1)	8.2 (5.3)	5.0 (4.0)	8.5 (5.8)	10.0 (10.0)	6.5 (4.3)	5.1 (3.5)	5.9 (3.5)	4.2 (3.3)
Males (%)	11.9 (7.8)	13.6 (6.5)	9.0 (9.1)	9.1 (5.6)	10.5 (5.6)	6.3 (4.8)	11.1 (6.1)	13.4 (6.2)	8.0 (4.4)	6.6 (3.8)	7.4 (3.7)	5.6 (3.8)
Female (%)	5.4 (2.9)	6.4 (2.8)	3.7 (2.2)	4.3 (2.3)	4.9 (2.3)	3.3 (1.9)	4.9 (2.8)	5.2 (2.4)	4.4 (3.2)	3.2 (1.7)	3.8 (1.6)	2.6 (1.7)
UKPDS 5 year risk CVD:												
Total (%)	12.6 (10.7)	15.0 (10.9)	8.5 (9.0)	10.0 (8.5)	12.0 (9.2)	6.5 (5.5)	11.3 (8.0)	13.8 (8.8)	8.2 (5.6)	7.6 (5.7)	9.1 (6.1)	5.7 (4.5)
Males (%)	15.9 (12.1)	18.5 (11.9)	11.0 (11.0)	12.6 (9.7)	14.9 (10.3)	8.1 (6.7)	14.4 (8.7)	17.9 (9.0)	9.9 (5.9)	9.4 (6.5)	11.0 (7.0)	7.3 (5.1)
Female (%)	7.6 (5.1)	9.4 (5.4)	5.1 (3.3)	6.5 (4.5)	7.8 (4.9)	4.6 (2.8)	7.1 (4.2)	8.1 (3.9)	5.7 (4.1)	5.2 (3.1)	6.4 (3.1)	3.6 (2.5)

APPENDIX E

SPSS output demonstrating independent associations of glucose indices with PWV using a multivariate analysis adjusted for – Age, MAP, cardiovascular disease, sex, BMI, Heart rate and anti-hypertensive treatment (Model 1)

a) Fasting Glucose associations (FPG)

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.693(a)	**.480**	**.470**	1.3894

Predictors: (Constant), Fasting Glucose ($mmoll^{-1}$), Anti-hypertensive tx, MAP, Gender, Heart Rate (/min), Cardiovascular disease, Calculated BMI, Age (years)
a Dependent Variable: Pulse Wave Velocity (ms^{-1})

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	-3.897	.924		-4.216	.000	-5.714	-2.081
	Age (years)	.113	.007	.568	15.118	.000	.098	.127
	MAP	.040	.007	.219	6.042	.000	.027	.053
	CVD Y/N	.64	.061	0.27	-.746	.000	0.016	0.74
	Anti-hypertensive tx	.318	.157	.078	2.030	.043	.010	.625
	BMI	.034	.014	.086	2.330	.020	.005	.062
	Gender	-.592	.137	-.154	-4.319	.000	-.861	-.322
	Heart Rate (/min)	.029	.007	.154	4.298	.000	.016	.043
	Fasting Glucose (mmol/l)	**.068**	**.036**	**0.09**	**1.882**	**.001**	**0.01**	**.140**

a) 2 hour post glucose associations (2HPG)

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.699(a)	**.51**	**.499**	**1.3785**

Model		Unstandardized Coefficients		Standardized Coeffi	t	Sig.	95% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	-3.686	.915		-4.028	.000	-5.485	-1.888
	Age (years)	.114	.007	.574	15.395	.000	.099	.128
	MAP	.039	.007	.215	5.991	.000	.026	.052
	CVD Y/N	.68	.061	0.24	-.746	.000	.016	0.79
	Anti-hypertensive tx	.280	.156	.068	1.791	.074	-.027	.588
	MIcroalb	.191	.251	.027	.761	.447	-.303	.685
	Calculated BMI	.032	.014	.083	2.290	.022	.005	.060
	Gender	-.600	.134	-.156	-4.473	.000	-.864	-.336
	Heart Rate (/min)	.028	.007	.147	4.139	.000	.015	.041
	Post-challenge Glucose (mmol/l)	**.054**	**.016**	**.14**	**3.335**	**.001**	**.022**	**.162**

c) HbA1c% associations

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.694(a)	**.481**	**.471**	1.3888

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	-4.304	.962		-4.473	.000	-6.195	-2.413
	Age (years)	.114	.007	.574	15.196	.000	.099	.128
	MAP	.039	.007	.216	5.966	.000	.026	.052
	Anti-hypertensive tx	.316	.157	.077	2.017	.044	.008	.624
	CVD Y/N	.72	.061	.22	-.746	.000	.021	0.99
	Calculated BMI	.034	.014	.087	2.375	.018	.006	.062
	Gender	-.596	.136	-.155	-4.390	.000	-.863	-.329
	Heart Rate (/min)	.029	.007	.155	4.335	.000	.016	.043
	HBa1c%	**.125**	**.061**	**.081**	**2.055**	**.007**	**.054**	**.245**

d) log HOMA-IR associations

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.685(a)	.512	.499	1.4004

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	-3.238	1.064		-3.042	.003	-5.331	-1.144
	Age (years)	.114	.009	.567	13.016	.000	.096	.131
	MAP	.040	.007	.228	5.361	.000	.025	.055
	Anti-hypertensive tx	.114	.187	.027	.607	.544	-.255	.482
	CVD Y/N	.72	.061	.22	-.746	.000	.021	0.99
	Calculated BMI	.023	.018	.058	1.233	.219	-.013	.058
	Gender	-.519	.161	-.136	-3.216	.001	-.836	-.202
	Heart Rate (/min)	.025	.008	.132	3.149	.002	.010	.041
	log_HOMA_IR	**.647**	**.266**	**.148**	**2.428**	**.000**	**.123**	**1.171**

APPENDIX F

SPSS output summary for multivariate vitamin D linear regression

Model Summary

Ethniciy	Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
WE	1	.706[a]	.499	.473	1.14946
SA	1	.781[b]	.610	.584	1.01214

Coefficients[a]

Ethnic	Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
			B	Std. Error	Beta		
WE	1	(Constant)	-.544	1.262		-.431	.667
		MAP	.042	.010	.313	4.243	.000
		Gender	-.720	.257	-.181	-2.586	.035
		HR	-.006	.005	-.093	-1.256	.212
		Age	.104	.013	.597	8.051	.000
		HOMA_IR	.198	.053	.277	3.757	.000
		Log vitD	-.016	.010	-.111	-1.498	.137
SA	1	(Constant)	-3.396	1.431		-2.374	.020
		MAP	.044	.012	.281	3.823	.000
		Gender	-.032	.388	-.005	-.0389	0.88
		HR	-.015	.009	-.122	-1.628	.108
		Age	.116	.013	.702	9.056	.000
		HOMA_IR	.102	.042	.180	2.434	.017
		Log vitD	.030	.012	-.181	2.472	.016

a. Dependent Variable: PWV

Model Summary

Model Summary

Ethnicity	Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
WE	2	.684[a]	.467	.431	1.21054
SA	2	.731[b]	.535	.503	1.20494

Coefficients[a]

Ethnicity	Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
			B	Std. Error	Beta		
WE	2	(Constant)	1.904	2.059		.925	.358
		Age	.120	.015	.672	8.097	.000
		Anti-hypertensive tx	1.164	1.742	.075	.668	.506
		waist	.000	.001	-.042	-.378	.707
		fasting_glu	.059	.057	.086	1.032	.305
		Log vitd	-.008	.005	-.131	-1.572	.120
		gender	-.615	.260	-.191	-2.365	.020
SA	2	(Constant)	1.146	1.071		1.071	.287
		Age	.128	.014	.726	9.336	.000
		Anti-hypertensive tx	.116	.425	.022	.272	.786
		waist	.000	.001	.029	.377	.707
		fasting_glu	.234	.090	.193	2.613	.011
		Log vitd	-.022	.010	-.157	-2.091	.039
		gender	-.010	.257	-.003	-.037	.970

a. Dependent Variable: PWV

Ethnicity	Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
WE	3	.744[a]	.553	.522	1.11131
SA	3	.825[b]	.680	.652	1.03848

Coefficients[a]

Ethnicity	Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
			B	Std. Error	Beta		
WE	3	(Constant)	-1.322	1.537		-.860	.392
		Age	.114	.014	.623	8.154	.000
		gender	-.595	.275	-.185	-2.168	.033
		MAP	.043	.010	.316	4.287	.000
		120_glu	.022	.025	.063	.855	.395
		WHR	.743	1.029	.062	.722	.472
		Log vitd	-.010	.005	-.162	-2.141	.035
SA	3	(Constant)	-3.555	1.624		-2.189	.032
		Age	.114	.014	.626	7.983	.000
		gender	.204	.283	.057	.719	.475
		MAP	.045	.013	.247	3.411	.001
		120_glu	.084	.031	.193	2.697	.009
		WHR	1.681	.956	.140	1.758	.083
		Log vitd	-.021	.010	-.152	-2.113	.028

a. Dependent Variable: PWV

Model Summary

Ethnicity	Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
WE	4	.724[a]	.454	.403	1.19775
SA	4	.687[b]	.553	.577	1.28340

a. Predictors: (Constant), vitd, Anti-hypertensive tx, log_IPAQ, gender, Age, HOMA_IR

b. Predictors: (Constant), vitd, gender, HOMA_IR, log_IPAQ, Anti-hypertensive tx, Age

Coefficients[a]

Ethnicity	Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
			B	Std. Error	Beta		
WE	4	(Constant)	3.506	1.857		1.888	.063
		Age	.118	.016	.643	7.334	.000
		gender	-.453	.301	-.136	-1.504	.137
		Anti-hypertensive tx	-1.095	1.225	-.075	-.893	.375
		HOMA_IR	.247	.067	.345	3.702	.000
		log_IPAQ	-.024	.287	-.008	-.084	.933
		Log vitd	-.002	.006	-.035	-.392	.697
SA	4	(Constant)	3.064	2.438		1.257	.219
		Age	.125	.029	.692	4.341	.000
		gender	.009	.470	.003	.019	.985
		Anti-hypertensive tx	.396	.686	.092	.577	.568
		HOMA_IR	.088	.068	.192	1.285	.209
		log_IPAQ	-.213	.452	-.067	-.472	.640
		Log vitd	-.029	.021	-.193	-1.383	.023

a. Dependent Variable: PWV

Presentations and publications relating to this thesis

Papers

Webb DR, Gray LJ, Khunti K, Davies MJ, et al. (2011) Intensive multi-factorial intervention improves modelled coronary heart disease outcomes in screen-detected type 2 diabetes mellitus: a cluster randomised controlled trial. *Diabetic Med* doi: 10.1111/j.1464-5491.2011.03441.x

Webb DR, Gray LJ, Khunti K, Davies MJ, et al. (2011) Screening for Diabetes using an Oral Glucose Tolerance Test within a Western multiethnic population identifies modifiable cardiovascular risk. The ADDITION-Leicester study. *Diabetologia* 54: 2237-2246

Webb DR, Gray LJ, Khunti K, Campbell S, Dallosso H, Davies MJ (2011) Contrasting cardiovascular risk profiles and prescribed cardio-protective therapies in newly diagnosed type 2 diabetes identified through screening and standard practice. *Diabetes Research & Clinical Practice* 91:280-285

Webb DR, Khunti K, Davies MJ et al. (2010) Rationale and design of the ADDITION-Leicester study, a systematic screening programme and Randomised Controlled Trial of multi-factorial cardiovascular risk intervention in people with Type 2 Diabetes Mellitus detected by screening *BMC Trials* 11:16 doi:10.1186/1745-6215-11-16

Webb DR, Khunti K, Silverman R et al. (2010) Impact of metabolic indices on central artery stiffness: independent associations of insulin resistance and glucose with aortic pulse wave velocity. *Diabetologia* 53:1190-1194

Webb DR, Davies MJ, Gray LJ et al. (2010) Searching for the right outcome? A systematic review and meta analysis of controlled trials using Carotid Intima-Media Thickness or Pulse Wave Velocity to infer anti-atherogenic properties of Thiazolidinediones. *Diabetes Obesity & Metabolism* 12:124-132

Yates T, Davies MJ, **Webb DR** et al. (2010) Levels of physical activity and relationship with markers of diabetes and cardiovascular disease risk in 5474 white European and South Asian adults screened for type 2 diabetes. *Preventative Medicine* 51:290-294

Gray LJ, Khunti K, **Webb DR**, et al. (2010) The Leicester Risk Assessment Score for detecting undiagnosed Type 2 Diabetes and Impaired Glucose Regulation for use in a multiethnic UK setting. *Diabetic Med* 27**:**887-895

Mostafa SA, Davies MJ, **Webb DR**, et al. (2010)The potential impact of using glycated haemoglobin (HbA1c) as the preferred diagnostic tool for Type 2 Diabetes Mellitus *Diabetic Med* 27:762-769

Gray LJ, Davies MJ, **Webb DR** et al. (2010) Screening for type 2 diabetes in a multiethnic setting using known risk factors to identify those at high risk: a cross sectional study. *Vascular Health and Risk Management* 6:837-842

Mostafa SA, Khunti K, **Webb DR**, Gray LJ, Davies MJ (2010) The potential impact and optimal cut-points of using glycated haemoglobin, HbA1c, to detect people with impaired glucose regulation in a multi-ethnic cohort. *Diabetes Research & Clinical Practice* 90:100-108

Aujla N, Davies MJ, Skinner TC, **Webb DR**, Gray LJ, Khunti K (2011) The association between anxiety and measures of glycaemia in a population based diabetes screening programme. *Diabetic Med* 28:785-788

Platform presentations
* Indicates presenting author

European Association for Study of Diabetes (EASD), Rome, Italy, 2008
***Webb DR**, Khunti K, Gray LJ, Srinivasan BT, Farooqi A, Wareham N, Griffin SC, Davies MJ. Does screening work for the common good? ADDITION-Leicester: design of a multi factorial intervention trial in an ethnically diverse population with screen-detected type 2 diabetes

American Diabetes Association (ADA), San Francisco, US, 2008
***Webb DR**, Taub NT, Khunti K, Davies MJ. Fasting, post challenge and HbA1c associate with a surrogate of atherosclerosis independently of other cardiovascular risk factors. *Diabetes* 57(s1) A182

Diabetes UK Annual professional conference, Glasgow UK, 2009
***Webb DR**, Gray LJ, Khunti K, Srinivasan B, Davies MJ., et al
ADDITION-Leicester: prevalence of IGR and screen detected Type 2 diabetes in a mixed ethnic UK population. *Diabetic Med* 26(s1) 3

***Webb DR**, Silverman R, Lacy P, Khunti K, Davies MJ. Digital photoplethysmography in IGR: is peripheral; waveform analysis an index of vascular function in type 2 diabetes? *Diabetic Med* 26(s1) 7

***Webb DR**, Srinivasan BT, Das S, Khunti K, Davies MJ. Beware of surrogate markers: a systematic review of controlled trials using Carotid Intima-Media Thickness or Pulse Wave Velocity to infer anti-atherogenic properties of Thiazolidinediones**.** *Diabetic Med* 26(s1) 30

Diabetes UK Annual professional conference, Liverpool UK, 2010
*Mostafa SA, **Webb DR**, Gray LJ, Davies MJ, Khunti K The potential impact of utilising Hba1c >6.0 and <6.5 as the preferred diagnostic tool for detecting IGR A65. *Diabetic Med* 27(s1) 24.

European Association for Study of Diabetes, Stockholm, Sweden, 2010
Srinivasan BT, ***Webb DR**, Khunti K, Gray LJ, , Farooqi A, Wareham N, Griffin SC, Davies MJ. Screening for type 2 diabetes identifies a majpr burden of modifiable cardiovascular risk. *Diabetologia* 53(s1) s19

***Webb DR**, Srinivasan BT, Gray LJ, Mostafa SA, Talbot D, Khunti K, Davies MJ. Vitamin D is associated with progression from impaired glucose regulation to type 2 diabetes in a UK multiethnic population. *Diabetologia* 53(s1) s81

*Mostafa SA, **Webb DR**, Gray LJ, Davies MJ, Khunti K, Srinivasan BT. A comparison of performance from using two HbA1c cut-points (a rule in, rule out) and one $HBA_{1c\%}$ cut point to detect type 2 diabetes in a multi-ethnic cohort. *Diabetologia* 53(s1) s193

Conference abstracts (2010)

Webb DR, Davies MJ, Khunti K Vitamin D status relates to premature aortic sclerosis in south Asians – a novel risk factor for cardiovascular disease? *Endocrine abstracts* 21: 184

Webb DR, Khunti K, Lacy P, Gray LJ, Williams B, Davies MJ 25-Hydroxyvitamin D concentration is independently associated with accelerated conduit vessel arteriosclerosis in south Asians. *Journal of Hum Hypertension* 24(s1) s14

Mostafa SA, Khunti K, Srinivasan BT, **Webb DR**, Gray LJ, Davies MJ. The potential impact and optimal cut-points of using glycated haemoglobin, HbA1c, as the preferred diagnostic tool for Type 2 diabetes in comparison to an OGTT in a UK multi-ethnic population. *Diabetic Med* 27(s) 1

Khunti K, Taub NT, Gillies CL, Gray LJ, Hiles SL, **Webb DR**, Davies MJ et al. A comparison of screening strategies for type 2 diabetes and impaired glucose tolerance in a UK community setting a cost per case analysis. *Diabetic Med* 27(s) 28

Brady EM, Gray LJ, Yates T, Khunti K, **Webb DR**, Srinivasan B, Sattar N, Davies MJ. Using markers of inflammation to redefine obesity in people of south Asian ethnicity in the UK. *Diabetic Med* 27(s) 48

Yates T, Gray LJ, Brady E, Davies MJ, **Webb DR**, Sattar N, Khunti K. Defining BMI cut-off points for south Asians in the UK. *Diabetic Med* 27(s) 107

Gray LJ, Yates T, Brady EM, Davies MJ, **Webb DR**, Sattar N, Khunti K. Defining waist circumference cut points for south Asians in the UK using measures of glycaemia. *Diabetologia* 53 (s1) s368

Aujla N, Davies MJ, Skinner TC, **Webb DR**, Gray LJ, Khunti K, et al. The association of screening anxiety with fasting and 2-hour plasma glucose. *Diabetologia* 53 (s1) s395

Key to Tables

Key to Figures

Bibliography

[1] Stumvoll M, Goldstein BJ, van Haeften TW (2005) Type 2 diabetes: principles of pathogenesis and therapy. *Lancet* 365:1333-1346

[2] Yach D, Stuckler D, Brownell KD (2006) Epidemiologic and economic consequences of the global epidemics of obesity and diabetes. *Nat Med* 12: 62-66

[3] Franco OH, Steyerberg EW, Hu FB, Mackenbach J, Nusselder W (2007) Associations of diabetes mellitus with total life expectancy and life expectancy with and without cardiovascular disease. *Arch Intern Med* 167:1145-1151

[4] Dall TM, Zhang Y, Chen YJ, Quick WG, Fogli Y, Fogli J. (2010) The economic burden of diabetes. *Health Affairs* 29:297-303

[5] Huang ES, Basu A, O'Grady M, Capretta JC (2009) Projecting the future diabetes population size and related costs for the U.S. *Diabetes Care* 32: 2225-2229

[6] IDF (2010) Diabetes Atlas: www.diabetesatlas.org/content

[7] Mainous AG, Baker R, Koopman RJ, et al. (2007) Impact of the population at risk of diabetes on projections of diabetes burden in the United States: an epidemic on the way. *Diabetologia* 50:934-940

[8] Narayan KM, Boyle JP, Geiss LS, Saaddine JB, Thompson TJ (2006) Impact of recent increase in incidence on future diabetes burden: U.S. 2005-2050. *Diabetes Care* 29:2114-2116

[9] King H, Aubert RE, Herman WH (1998) Global burden of diabetes, 1995-2025: prevalence, numerical estimates and projections. *Diabetes Care* 21:1414-1431

[10] Wild S, Roglic G, Green A, Sicree R, King H (2004) Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 27:1047-1053

[11] APHO Diabetes prevalence model. Yorkshire and Humber public health obsevatory (YHPHO) (2010) http://www.yhpho.org.uk/default.aspx?RID=8467

[12] Ryan R, Newnham A, Khunti K, Majeed A (2005) New cases of diabetes mellitus in England and Wales, 1994-1998: a database study. *Public Health* 119:892-899

[13] Masso Gonzalez EL, Johansson S, Wallander M, Garcia Rodriguez LA (2009) Trends in the prevalence and incidence of diabetes in the UK: 1996-2005. *J Epidemiol Community Health* 63:332-336

[14] Shaw JE, Sicree RA, Zimmet PZ (2010) Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Research & Clinical Practice* 87:4-14

[15] IDF (2009) Diabetes Atlas: Prevalence. http://www.eatlas.idf.org/indexcc67.html

[16] Kirchhof M, Popat N, Malowany J (2008) A historical perspective of the diagnosis of Diabetes. *University of Western Ontario Medical Journal* 78:7-10

[17] Slein MW (1963) D-glucose determination with hexokinase and glucose-6-phosphate dehydrogenase. *Methods of Enzymatic Analysis* 1:117-123

[18] National Diabetes and Data Group (NDDG) (1979) Classification and diagnosis of Diabetes Mellitus and other categories of glucose intolerance. *Diabetes* 28:1039 - 1057

[19] World Health Organisation (1980) World Health Organisation Expert Committee on Diabetes Mellitus: Technical report 646, second report. Geneva, Switzerland.

[20] Colagiuri S, Lee CM, Wong TY, et al. (2011) Glycaemic thresholds for diabetes-specific retinopathy: implications for diagnostic criteria for diabetes. *Diabetes Care* 34:145-150

[21] World Health Organisation (1999) Definition, Diagnosis, and Classification of Diabetes Mellitrus and its Complications. Report of a WHO consultation. Part 1: Diagnosis and Classification of Diabetes Mellitus.

[22] World Health Organisation (2011) Use of glycated haemoglobin (HbA1c) in the diagnosis of Diabetes Mellitus. Expert committee consultation.

[23] Expert Committee on the diagnosis and classification of diabetes mellitus (1997) Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes care* 20:1183 - 1197

[24] Expert Committee on the diagnosis and classification of diabetes mellitus (2003) Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care* 26:S5-20

[25] American Diabetes Association (2010) Diagnosis and classification of diabetes mellitus. *Diabetes Care* 33:S62-9

[26] World Health Organisation (2006) Definition, Diagnosis of Diabetes Mellitus and intermediate hyperglycaemia: Report of a WHO/IDF consultation, Geneva, Switzerland

[27] International expert committee (2009) International expert committee report on the role of the A1C assay in the diagnosis of diabetes. *Diabetes care* 32:1327-1334

[28] Abdul-Ghani MA, Tripathy D, DeFronzo RA (2006) Contributions of beta-cell dysfunction and insulin resistance to the pathogenesis of impaired glucose tolerance and impaired fasting glucose. *Diabetes Care* 29:1130-1139

[29] Davies MJ, Raymond NT, Day JL, Hales CN, Burden AC (2000) Impaired glucose tolerance and fasting hyperglycaemia have different characteristics. *Diabetic Med* 17:433-440

[30] DREAM (Diabetes REduction Assessment with ramipril and rosiglitazone Medication) Trial Investigators. Gerstein HC. Yusuf S. Bosch J. et al. (2006) Effect of rosiglitazone on the frequency of diabetes in patients with impaired glucose tolerance or impaired fasting glucose: a randomised controlled trial. *Lancet* 368:1096-1105

[31] Knowler WC, Barrett-Connor E, Fowler SE, et al. (2002) Reduction in the incidence of type 2 diabetes with life-style intervention or metformin. *N Engl J Med* 346:393-403

[32] Torgerson JS, Hauptman J, Boldrin MN, Sjostrom L (2004) XENical in the Prevention of Diabetes in Obese Subjects (XENDOS) Study: A randomized study of orlistat as an adjunct to life-style changes for the prevention of type 2 diabetes in obese patients. *Diabetes Care* 27:155-161

[33] DECODE study group on behalf of the European Diabetes Epidemiology Group (2001) Glucose Tolerance and Cardiovascular Mortality: Comparison of Fasting and 2-Hour Diagnostic Criteria. *Arch Intern Med* 161:397-405

[34] Ford ES, Zhao G, Li C (2010) Pre-diabetes and the risk for cardiovascular disease: a systematic review of the evidence. *J Am Coll Cardiol* 55:1310-1317

[35] Lowe LP, Liu K, Greenland P, Metzger BE, Dyer AR, Stamler J (1997) Diabetes, asymptomatic hyperglycemia, and 22-year mortality in black and white men. The Chicago Heart Association Detection Project in Industry Study. *Diabetes Care* 20:163-169

[36] Gossain VV, Aldasouqi S (2010) The challenge of undiagnosed pre-diabetes, diabetes and associated cardiovascular disease. *International Journal of Diabetes Mellitus* 2:43-46

[37] Chamnan P, Simmons RK, Jackson R, Khaw KT, Wareham NJ, Griffin SJ (2011) Non-diabetic hyperglycaemia and cardiovascular risk: moving beyond categorisation to individual interpretation of absolute risk. *Diabetologia* 54:291-299

[38] DeFronzo RA (2004) International Textbook of Diabetes Mellitus. John Wiley, Chichester, Hoboken, New Jersey

[39] Wingard DL, Barrett-Connor E (1995) Heart Disease and Diabetes. In: Harris MI, Cowie CC, Stern MP, Boyko EJ, Bennett PH (eds) Diabetes in

America. Bethesda, MD, National Institute of Health, National Institute of diabetes, digestive and kidney disease pp. 429-448

[40] United Kingdom Prospective Diabetes Study Group (1990) Complications in newly diagnosed type 2 diabetic patients and their association with different clinical and biochemical risk factors. UKPDS 6. *Diabetes Research* 13:1-11

[41] Chowdhury TA, Lasker SS (2002) Complications and cardiovascular risk factors in South Asians and Europeans with early-onset type 2 diabetes. *QJM* 95:241-246

[42] Hu FB, Stampfer MJ, Haffner SM, Solomon CG, Willett WC, Manson JE (2002) Elevated risk of cardiovascular disease prior to clinical diagnosis of type 2 diabetes. *Diabetes Care* 25:1129-1134

[43] Jensen LO, Maeng M, Thayssen P, et al. (2010) Long-term outcomes after percutaneous coronary intervention in patients with and without diabetes mellitus in Western Denmark. *Am J Cardiol* 105:1513-1519

[44] Yusuf S, Zucker D, Peduzzi P, et al. (1994) Effect of coronary artery bypass graft surgery on survival: overview of 10-year results from randomised trials by the Coronary Artery Bypass Graft Surgery Trialists Collaboration. *Lancet* 344:563-570

[45] The BARI 2D Study group (2009) A randomised trial of therapies for type 2 diabetes and coronary heart disease. *N Engl J Med* 360:2503-2515

[46] Colhoun HM, Betteridge DJ, Durrington PN, et al. (2004) Primary prevention of cardiovascular disease with atorvastatin in type 2 diabetes in the Collaborative Atorvastatin Diabetes Study (CARDS): Multicentre randomised placebo-controlled trial. *Lancet* 364:685-696

[47] Heart Protection Study Collaborative Group (2003) MRC/BHF Heart Protection Study of cholesterol-lowering with simvastatin in 5963 people with diabetes: randomised placebo-controlled trial. *Lancet* 361:2005-16

[48] Knopp RH, d'Emden M, Smilde JG, Pocock SJ (2006) Efficacy and safety of atorvastatin in the prevention of cardiovascular end points in subjects with type 2 diabetes: the Atorvastatin Study for Prevention of Coronary Heart Disease Endpoints in non-insulin-dependent diabetes mellitus (ASPEN). *Diabetes Care* 29:1478-1485

[49] ALLHAT Officers and Coordinators for the ALLHAT Collaborative Research Group, The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (2002) Major outcomes in moderately hypercholesterolemic, hypertensive patients randomized to pravastatin vs usual care: The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT-LLT). *JAMA* 288:2998-3007

[50] Sever PS, Poulter NR, Dahlöf B, et al. (2005) Reduction in cardiovascular events with atorvastatin in 2,532 patients with type 2 diabetes: Anglo-Scandinavian Cardiac Outcomes Trial - lipid-lowering arm (ASCOT-LLA). *Diabetes Care* 28:1151-1157

[51] Frick MH, Elo O, Haapa K, et al. (1987) Helsinki Heart Study: primary-prevention trial with gemfibrozil in middle-aged men with dyslipidemia. Safety of treatment, changes in risk factors, and incidence of coronary heart disease. *N Engl J Med* 317:1237-1245

[52] Keech A, Simes RJ, Barter P, et al. (2005) Effects of long-term fenofibrate therapy on cardiovascular events in 9,795 people with type 2 diabetes mellitus (the FIELD study): randomised controlled trial. *Lancet* 366:1849-1861

[53] Hansson L, Zanchetti A, Carruthers SG, et al. (1998) Effects of intensive blood-pressure lowering and low-dose aspirin in patients with hypertension: principal results of the Hypertension Optimal Treatment (HOT) randomised trial. HOT Study Group. *Lancet* 351:1755-1762

[54] UK Prospective Diabetes Study Group (1998) Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38. *BMJ* 317:703-713

[55] Schrier RW, Estacio RO, Esler A, Mehler P (2002) Effects of aggressive blood pressure control in normotensive type 2 diabetic patients on albuminuria, retinopathy and strokes. *Kidney Int* 61:1086-1097

[56] Yusuf S, Dagenais G, Pogue J, Bosch J, Sleight P (2000) Vitamin E supplementation and cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators. *N Engl J Med* 342:154-160

[57] ALLHAT Officers and Coordinators for the ALLHAT Collaborative Research Group, The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (2002) Major outcomes in high-risk hypertensive patients randomized to angiotensin-converting enzyme inhibitor or calcium channel blocker vs diuretic: The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). *JAMA* 288:2981-2997

[58] Poulter NR, Wedel H, Dahlöf B, et al.(2005) Role of blood pressure and other variables in the differential cardiovascular event rates noted in the Anglo-Scandinavian Cardiac Outcomes Trial-Blood Pressure Lowering Arm (ASCOT-BPLA). *Lancet* 366:907-913

[59] ACCORD Study Group, Cushman WC, Evans GW, et al. (2010) Effects of intensive blood-pressure control in type 2 diabetes mellitus. *N Engl J Med* 362:1575-1585

[60] Costa J, Borges M, David C, Vaz Carneiro A (2006) Efficacy of lipid lowering drug treatment for diabetic and non-diabetic patients: meta-analysis of randomised controlled trials. *BMJ* 332:1115-1124

[61] British Cardiac Society, British Hypertension Society, Diabetes UK, HEART UK, Primary Care Cardiovascular Society, Stroke Association (2005) JBS-2: Joint British Societies' guidelines on prevention of cardiovascular disease in clinical practice. *Heart* S5:v1-52

[62] Action to Control Cardiovascular Risk in Diabetes Study Group, Gerstein HC, Miller ME, et al. (2008) Effects of intensive glucose lowering in type 2 diabetes. *N Engl J Med* 358:2545-2559

[63] Duckworth W, Abraira C, Moritz T, et al. (2009) Glucose control and vascular complications in veterans with type 2 diabetes. *N Engl J Med* 360:129-139

[64] The ADVANCE Collaborative Group (2008) Intensive Blood Glucose Control and Vascular Outcomes in Patients with Type 2 Diabetes. *N Engl J Med* 358:2560-2572

[65] Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HAW (2008) 10-Year follow-up of intensive glucose control in type 2 diabetes. *N Engl J Med* 359:1577-1589

[66] Gaede P, Vedel P, Larsen N, Jensen GVH, Parving H, Pedersen O (2003) Multi-factorial intervention and cardiovascular disease in patients with type 2 diabetes. *N Engl J Med* 348:383-393

[67] Gaede P, Lund-Andersen H, Parving H, Pedersen O (2008) Effect of a multi-factorial intervention on mortality in type 2 diabetes. *N Engl J Med* 358:580-591

[68] Zoungas S, de Galan BE, Ninomiya T, et al. (2009) Combined effects of routine blood pressure lowering and intensive glucose control on macrovascular and microvascular outcomes in patients with type 2 diabetes: New results from the ADVANCE trial. *Diabetes Care* 32:2068-2074

[69] Bellary S, O'Hare JP, Raymond NT, et al. (2008) Enhanced diabetes care to patients of south Asian ethnic origin (the United Kingdom Asian Diabetes Study): a cluster randomised controlled trial. *Lancet* 371:1769-1776

[70] Cederholm J, Zethelius B, Nilsson PM, Eeg-Olofsson K, Eliasson B, Gudbjörnsdottir S. Swedish National Diabetes Register (2009) Effect of tight control of HbA1c and blood pressure on cardiovascular diseases in type 2 diabetes: an observational study from the Swedish National Diabetes Register (NDR). *Diabetes Research & Clinical Practice* 86:74-81

[71] Zimmet P, Alberti KG, Shaw J (2001) Global and societal implications of the diabetes epidemic. *Nature* 414:782-787

[72] Yusuf S, Hawken S, Ounpuu S, et al. (2004) Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): Case-control study. *Lancet* 364:937-952

[73] Wilson JMG, Junger G (1968) Principles and Practice of screening for disease. WHO Chronicle 22:473

[74] Department of Health (2009) NHS Health Check Programme - Vascular Programme, putting prevention first NHS Health Check: Vascular risk assessment and management. Best Practice Guidance. Reference 11473. Last accessed 01/12/11.

[75] Diabetes UK (2009) Position Statement: Early identification of people with Type 2 Diabetes.

[76] American Diabetes Association (2009) Standards of medical care in diabetes 2009. *Diabetes Care* 32:S13-61

[77] Norris SL, Kansagara D, Bougatsos C, Fu R, U.S. Preventive Services Task Force (2008) Screening adults for type 2 diabetes: a review of the evidence for the U.S. Preventive Services Task Force. *Ann Intern Med* 148:855-868

[78] Feig DS, Palda VA, Lipscombe L, Canadian Task Force on Preventive Health Care (2005) Screening for type 2 diabetes mellitus to prevent vascular complications: updated recommendations from the Canadian Task Force on Preventive Health Care. *Canadian Medical Association Journal* 172:177-180

[79] Waugh N, Scotland G, McNamee P, et al. (2007) Screening for type 2 diabetes: literature review and economic modelling. Health Technology Assessment 11

[80] Yorkshire and Humber Public Observatory (2008) Phase 3 PBS Diabetes prevalence model. www.yhpho.org.uk/viewResource. Last accessed 01/12/11.

[81] Roglic G, Unwin N, Bennett PH, et al. (2005) The burden of mortality attributable to diabetes: realistic estimates for the year 2000. *Diabetes Care* 28:2130-2135

[82] National diabetes support team (2008) Improving emergency and inpatient care for people with diabetes. NHS Evidence Diabetes. www.diabetes.nhs.uk/document.php. Last accessed 01/12/11.

[83] Sampson MJ, Dozio N, Ferguson B, Dhatariya K (2007) Total and excess bed occupancy by age, specialty and insulin use for nearly one million diabetes patients discharged from all English Acute Hospitals. *Diabetes Research & Clinical Practice* 77:92-98

[84] Department of Health (2006) Turning the corner: improving diabetes care. 6421. Last accessed 01/12/11.

[85] Currie CJ, Peters JR, Evans M (2010) Dispensing patterns and financial cost of glucose lowering therapies in the UK from 2000-2008. *Diabetic Med* 27:744-752

[86] Harris MI, Klein R, Welborn TA, Knuiman MW (1992) Onset of NIDDM occurs at least 4-7 years before clinical diagnosis. *Diabetes Care* 15:815-819

[87] Cowie CC, Rust KF, Ford ES, et al. (2009) Full accounting of diabetes and pre-diabetes in the U.S. population in 1988-1994 and 2005-2006. *Diabetes Care* 32:287-294

[88] Weyer C, Bogardus C, Mott DM, Pratley RE (1999) The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus. *J Clin Invest* 104:787-794

[89] Ealovega MW, Tabaei BP, Brandle M, Burke R, Herman WH (2004) Opportunistic screening for diabetes in routine clinical practice. *Diabetes Care* 27:9-12

[90] Davies MJ, Williams DRR, Metcalfe J, Day JL. (1993) Community screening for non insulin dependent diabetes mellitus: self-testing for post-prandial glycosuria. *QJM* 86:677-684

[91] Rolka DB, Narayan KM, Thompson TJ, et al. (2001) Performance of recommended screening tests for undiagnosed diabetes and dysglycemia. *Diabetes Care* 24:1899-1903

[92] Qiao Q, Keinänen-Kiukaanniemi S, Rajala U, Uusimäki A, Kivelä SL (1995) Random capillary whole blood glucose test as a screening test for diabetes mellitus in a middle-aged population. *Scandinavian Journal of Clinical & Laboratory Investigation* 55:3-8

[93] Somannavar S, Ganesan A, Deepa M, Datta M, Mohan V (2009) Random capillary blood glucose cut points for diabetes and pre-diabetes derived from community-based opportunistic screening in India. *Diabetes Care* 32:641-643

[94] Buell C, Kermah D, Davidson MB (2007) Utility of A1C for diabetes screening in the 1999-2004 NHANES population. *Diabetes Care* 30:2233-2235

[95] van't Riet E, Alssema M, Rijkelijkhuizen JM, Kostense PJ, Nijpels G, Dekker JM (2010) Relationship between A1C and glucose levels in the general Dutch population: the new Hoorn study. *Diabetes Care* 33:61-66

[96] Christensen DL, Witte DR, Kaduka L, et al. (2010) Moving to an A1C-based diagnosis of diabetes has a different impact on prevalence in different ethnic groups. *Diabetes Care* 33:580-582

[97] Lindström J, Tuomilehto J (2003) The diabetes risk score: a practical tool to predict type 2 diabetes risk. *Diabetes Care* 26:725-731

[98] Rahman M, Simmons RK, Harding AH, Wareham NJ, Griffin SJ (2008) A simple risk score identifies individuals at high risk of developing Type 2 diabetes: a prospective cohort study. *Fam Pract* 25:191-196

[99] Hoerger TJ, Hicks KA, Sorensen SW, et al. (2007) Cost-effectiveness of screening for pre-diabetes among overweight and obese U.S. adults. *Diabetes Care* 30:2874-2879

[100] Goyder E, Wild S, Fischbacher C, Carlisle J, Peters J (2008) Evaluating the impact of a national pilot screening programme for type 2 diabetes in deprived areas of England. *Fam Pract* 25:370-375

[101] Gillies CL, Lambert PC, Abrams KR, et al. (2008) Different strategies for screening and prevention of type 2 diabetes in adults: Cost effectiveness analysis. *BMJ* 336:1180-1184

[102] Chatterjee R, Narayan KM, Lipscomb J, Phillips LS (2010) Screening adults for pre-diabetes and diabetes may be cost-saving. *Diabetes Care* 33:1484-1490

[103] Kahn R, Alperin P, Eddy D, et al. (2010) Age at initiation and frequency of screening to detect type 2 diabetes: a cost-effectiveness analysis. *Lancet* 375:1365-1374

[104] Davies MJ, Heller S, Skinner TC, et al. (2008) Effectiveness of the diabetes education and self-management for ongoing and newly diagnosed (DESMOND) programme for people with newly diagnosed type 2 diabetes: Cluster randomised controlled trial. *BMJ* 336:491-495

[105] Stewart-Brown S, Farmer A (1997) Screening could seriously damage your health. *BMJ* 314:533-534

[106] Paddison CAM, Eborall HC, Sutton S, et al. (2010) Are people with negative diabetes screening tests falsely reassured? Parallel group cohort study embedded in the ADDITION (Cambridge) randomised controlled trial. *BMJ* 340:84

[107] Skinner TC, Davies MJ, Farooqi AM, Jarvis J, Tringham JR, Khunti K (2005) Diabetes screening anxiety and beliefs. *Diabetic Med* 22:1497-1502

[108] Adriaanse MC, Dekker JM, Spijkerman AM, et al. (2004) Health-related quality of life in the first year following diagnosis of Type 2 diabetes: newly diagnosed patients in general practice compared with screening-detected patients. The Hoorn Screening Study. *Diabetic Med* 21:1075-1081

[109] Fox CS, Coady S, Sorlie PD, et al. (2007) Increasing cardiovascular disease burden due to diabetes mellitus: the Framingham Heart Study. *Circulation* 115:1544-1550

[110] Fox CS, Coady S, Sorlie PD, et al. (2004) Trends in cardiovascular complications of diabetes. *JAMA* 292:2495-2499

[111] Kannel WB, McGee DL (1979) Diabetes and cardiovascular disease, the Framingham study. *JAMA* 241:2035-2038

[112] Haffner SM, Lehto S, Ronnemaa T, Pyorala K, Laakso M (1998) Mortality from coronary heart disease in subjects with type 2 diabetes and in non-diabetic subjects with and without prior myocardial infarction. *N Engl J Med* 339:229-234

[113] Gerstein HC (2010) Prevention of the consequences of diabetes - a commentary. In: Herman WH, Kinmouth AL, Wareham NJ, Williams R (eds) The Evidence Base for Diabetes Care. John Wiley and Sons, Ltd, UK, pp. 266 -271

[114] Danesh J, Wheeler JG, Hirschfield GM, et al. (2004) C-Reactive Protein and Other Circulating Markers of Inflammation in the Prediction of Coronary Heart Disease. *N Engl J Med* 350:1387-1397

[115] Rutter MK, Meigs JB, Sullivan LM, D'Agostino RB, Wilson PW (2004) C-reactive protein, the metabolic syndrome, and prediction of cardiovascular events in the Framingham Offspring Study. *Circulation* 110:380-385

[116] Kern PA, Ranganathan S, Li C, Wood L, Ranganathan G (2001) Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. *American Journal of Physiology, Endocrinology & Metabolism* 280:E745-51

[117] Stern MP (1995) Diabetes and cardiovascular disease: 'The common soil' hypothesis. *Diabetes* 44:369-374

[118] Aronson D, Rayfield EJ (2002) How hyperglycaemia promotes atherosclerosis: molecular mechanisms. *Cardiovascular Diabetology* 1:1-10

[119] Safar ME (2007) Atherosclerosis, Large Arteries and Cardiovascular Risk. *Adv Cardiol* 44:1-18

[120] Cameron JD, Cruickshank JK (2007) Glucose, insulin, diabetes and mechanisms of arterial dysfunction. *Clinical & Experimental Pharmacology & Physiology* 34:677-682

[121] Franklin SS (2007) Arterial stiffness: is it ready for prime time? *Curr Cardiol Rep* 9:462-469

[122] Zieman SJ, Melenovsky V, Kass DA (2005) Mechanisms, pathophysiology, and therapy of arterial stiffness. *Arterioscler Thromb Vasc Biol* 25:932-943

[123] Blacher J, Guerin AP, Pannier B, Marchais SJ, Safar ME, London GM (1999) Impact of aortic stiffness on survival in end-stage renal disease. *Circulation* 99:2434-2439

[124] Cruickshank K, Riste L, Anderson SG, Wright JS, Dunn G, Gosling RG (2002) Aortic pulse-wave velocity and its relationship to mortality in diabetes and glucose intolerance: an integrated index of vascular function. *Circulation* 106:2085-2090

[125] Nicholls SJ, Tuzcu EM, Kalidindi S, et al. (2008) Effect of diabetes on progression of coronary atherosclerosis and arterial remodeling: a pooled analysis of five intravascular ultrasound trials. *J Am Coll Cardiol* 52:255-262

[126] Singh DK, Winocour P, Farrington K (2010) Endothelial cell dysfunction, medial arterial calcification and osteoprotegerin in diabetes. *Br J Diabetes Vasc Dis* 10:71-77

[127] Shanahan CM, Cary NR, Salisbury JR, Proudfoot D, Weissberg PL, Edmonds ME (1999) Medial localization of mineralization-regulating proteins in association with Mönckeberg's sclerosis: evidence for smooth muscle cell-mediated vascular calcification. *Circulation* 100:2168-2176

[128] Secchiero P, Corallini F, Pandolfi A, et al. (2006) An increased osteoprotegerin serum release characterizes the early onset of diabetes mellitus and may contribute to endothelial cell dysfunction. *Am J Pathol* 169:2236-2244

[129] London GM, Blacher J, Pannier B, Guerin AP, Marchais SJ, Safar ME (2001) Arterial wave reflections and survival in end-stage renal failure. *Hypertension* 38:434-438

[130] Schram MT, Chaturvedi N, Fuller JH, Stehouwer CD, EURODIAB Prospective Complications Study G (2003) Pulse pressure is associated with age and cardiovascular disease in type 1 diabetes: the Eurodiab Prospective Complications Study. *J Hypertension* 21:2035-2044

[131] Rönnback M, Fagerudd J, Forsblom C, Pettersson-Fernholm K, Reunanen A, Groop PH (2004) Finnish Diabetic Nephropathy (FinnDiane) Study Group. Altered age-related blood pressure pattern in type 1 diabetes. *Circulation* 110:1076-1082

[132] Ahlgren A, Astrand H, Sundkvist G, Länne T (2005) Increased aortic stiffness is persistent in type 1 diabetic women: a follow-up study. *Diabetologia* 48:780-783

[133] Brooks BA, Molyneaux LM, Yue DK (2001) Augmentation of central arterial pressure in Type 2 diabetes. *Diabetic Med* 18:374-380

[134] Ravikumar R, Deepa R, Shanthirani C, Mohan V (2002) Comparison of carotid intima-media thickness, arterial stiffness, and brachial artery flow

mediated dilatation in diabetic and nondiabetic subjects (The Chennai Urban Population Study (CUPS-9). *Am J Cardiol* 90:702-707

[135] Kimoto E, Shoji T, Shinohara K, et al. (2003) Preferential stiffening of central over peripheral arteries in type 2 diabetes. *Diabetes* 52:448-452

[136] Rahman S, Ismail AA, Ismail SB, Naing NN, Rahman ARA (2008) Early manifestation of macrovasculopathy in newly diagnosed never treated type II diabetic patients with no traditional CVD risk factors. *Diabetes Research & Clinical Practice* 80:253-258

[137] Henry RM, Kostense PJ, Spijkerman AM, et al. (2003) Arterial stiffness increases with deteriorating glucose tolerance status: the Hoorn Study. *Circulation* 107:2089-2095

[138] Ohnishi H, Saitoh S, Takagi S, et al. (2003) Pulse wave velocity as an indicator of atherosclerosis in impaired fasting glucose: The Tanno and Sobetsu study. *Diabetes Care* 26:437-440

[139] Schram MT, Henry RM, van Dijk RA, et al. (2004) Increased central artery stiffness in impaired glucose metabolism and type 2 diabetes: the Hoorn Study. *Hypertension* 43:176-181

[140] van Popele NM, Hak EA, Mattace-Raso FU, et al. (2006) Impaired fasting glucose is associated with increased arterial stiffness in elderly people without diabetes mellitus: the Rotterdam Study. *J Am Geriatr Soc* 54:397-404

[141] Safar ME, Thomas F, Blacher J, et al. (2006) Metabolic syndrome and age-related progression of aortic stiffness. *J Am Coll Cardiol* 47:72-75

[142] Ferreira I, Boreham CA, Twisk JWR, et al. (2007) Clustering of metabolic syndrome risk factors and arterial stiffness in young adults: The Northern Ireland Young Hearts Project. *J Hypertension* 25:1009-1020

[143] Henry RM, Ferreira I, Dekker JM, Nijpels G, Scheffer PG, Stehouwer CD (2009) The metabolic syndrome in elderly individuals is associated with greater muscular, but not elastic arterial stiffness, independent of low-grade inflammation, endothelial dysfunction or insulin resistance - The Hoorn Study. *J Hum Hypertension* 23:718-727

[144] Laurent S, Cockcroft J, Van Bortel L, et al. (2006) Expert consensus document on arterial stiffness: methodological issues and clinical applications. *Eur Heart J* 27:2588-2605

[145] Karamanoglu M, Gallagher DE, Avolio AP, O'Rourke MF (1995) Pressure wave propagation in a multi-branched model of the human upper limb. *Am J Physiol* 269:1363-1369

[146] Lehmann ED, Parker JR, Hopkins KD, Taylor MG, Gosling RG (1993) Validation and reproducibility of pressure-corrected aortic distensibility

measurements using pulse wave velocity doppler ultrasound. *J Biomed Eng* 15:221-228

[147] Asmar R, Benetos A, Topouchian J, et al. (1995) Assessment of arterial distensibility by automatic pulse wave velocity measurement. Validation and clinical application studies. *Hypertension* 26:485-490

[148] Kool MJ, van Merode T, Reneman RS, Hoeks AP, Struyker Boudier HA, Van Bortel LM (1994) Evaluation of reproducibility of a vessel wall movement detector system for assessment of large artery properties. *Cardiovasc Res* 28:610-614

[149] Hoeks AP, Brands PJ, Smeets FA, Reneman RS (1990) Assessment of the distensibility of superficial arteries. *Ultrasound Med Biol* 16:121-128

[150] Pannier BM, Avolio AP, Hoeks A, Mancia G, Takazawa K (2002) Methods and devices for measuring arterial compliance in humans. *Am J Hypertension* 15:743-753

[151] Pauca AL, O'Rourke MF, Kon ND (2001) Prospective evaluation of a method for estimating ascending aortic pressure from the radial artery pressure waveform. *Hypertension* 38:932-937

[152] Finkelstein SM, Collins VR, Cohn JN (1988)Arterial vascular compliance response to vasodilators by Fourier and pulse contour analysis. *Hypertension* 12:380-387

[153] Millasseau SC, Guigui FG, Kelly RP, et al. (2000) Non-invasive assessment of the digital volume pulse. Comparison with the peripheral pressure pulse. *Hypertension* 36:952-956

[154] Grundy SM, Bazzarre T, Cleeman J, et al. (2000) Prevention Conference V. Beyond secondary prevention: identifying the high-risk patient for primary prevention: medical office assessment: Writing Group I. *Circulation* 101:E3-E11

[155] Tropeano AI, Boutouyrie P, Pannier B, et al. (2006) Brachial pressure-independent reduction in carotid stiffness after long-term angiotensin-converting enzyme inhibition in diabetic hypertensives. *Hypertension* 48:80-86

[156] Manolis AJ, Iraklianou S, Pittaras A, et al. (2005) Arterial compliance changes in diabetic normotensive patients after angiotensin-converting enzyme inhibition therapy. *Am J Hypertension* 18:18-22

[157] Williams B, Lacy PS, Cruickshank JK, et al. (2009) Impact of statin therapy on central aortic pressures and hemodynamics: principal results of the Conduit Artery Function Evaluation-Lipid-Lowering Arm (CAFE-LLA) Study. *Circulation* 119:53-61

[158] Orr JS, Dengo AL, Rivero JM, Davy KP (2009) Arterial destiffening with atorvastatin in overweight and obese middle-aged and older adults. *Hypertension* 54:763-768

[159] Efrati S, Averbukh M, Dishy V, Faygenzo M, Friedensohn L, Golik A (2007) The effect of simvastatin, ezetimibe and their combination on the lipid profile, arterial stiffness and inflammatory markers. *Eur J Clin Pharmacol* 63:113-121

[160] Meyer C, McGrath BP, Teede HJ(2007) Effects of medical therapy on insulin resistance and the cardiovascular system in polycystic ovary syndrome. *Diabetes Care* 30:471-478

[161] Agarwal N, Rice SP, Bolusani H, et al. (2010) Metformin reduces arterial stiffness and improves endothelial function in young women with polycystic ovary syndrome: a randomized, placebo-controlled, crossover trial. *Journal of Clinical Endocrinology & Metabolism* 95:722-730

[162] Stakos DA, Schuster DP, Sparks EA, Wooley CF, Osei K, Boudoulas H (2005) Long term cardiovascular effects of oral antidiabetic agents in non-diabetic patients with insulin resistance: double blind, prospective, randomised study. *Heart* 91:589-594

[163] Koshiba K, Nomura M, Nakaya Y, Ito S (2006) Efficacy of glimepiride on insulin resistance, adipocytokines, and atherosclerosis. *Journal of Medical Investigation* 53:87-94

[164] Rahman S, Ismail AA, Ismail SB, Naing NN, Adbul Rahman AR (2007) Effect of rosiglitazone / ramipril on preclinical vasculopathy in newly diagnosed, untreated diabetes and IGT patients. one-year randomised, double blind, placebo-controlled study. *Eur J Clin Pharmacol* 63: 733-741

[165] Yu J, Jin N, Wang G, Zhang F, Mao J, Wang X (2007) Peroxisome proliferator-activated receptor gamma agonist improves arterial stiffness in patients with Type 2 Diabetes mellitus and coronary heart disease. *Metabolism* 56:1396-1401

[166] Schneider R, Golzman B, Turkot S, Kogan J, Oren S (2005) Effect of weight loss on blood pressure, arterial compliance, and insulin resistance in normotensive obese subjects. *Am J Med Sci* 330:157-160

[167] Ceriello A(2009) Hypothesis: the "metabolic memory" the new challenge of diabetes. *Diabetes Research & Clinical Practice* 86:S2-6

[168] Webb DR, Davies MJ, Gray LJ, et al. (2010) Searching for the right outcome? A systematic review and meta-analysis of controlled trials using carotid intima-media thickness or pulse wave velocity to infer antiatherogenic properties of thiazolidinediones. *Diabetes Obes Metab* 12:124-132

[169] Lee JH, O'Keefe JH, Bell D, Hensrud DD, Holick MF (2008) Vitamin D deficiency an important, common, and easily treatable cardiovascular risk factor? *J Am Coll Cardiol* 52:1949-1956

[170] Martins D, Wolf M, Pan D, et al. (2007) Prevalence of cardiovascular risk factors and serum levels of 25-hydroxyvitamin D in the United States: data from the Third National Health and Nutrition Examination Survey. *Arch Intern Med* 167:1159-1165

[171] Ford ES, Ajani UA, McGuire LC, Liu S (2005) Concentrations of serum vitamin D and the metabolic syndrome among U.S. adults. *Diabetes Care* 28:1228-1230

[172] Scragg R, Sowers M, Bell C (2007) Serum 25-hydroxyvitamin D, ethnicity, and blood pressure in the Third National Health and Nutrition Examination Survey. *Am J Hypertension* 20:713-719

[173] Autier P, Gandini S (2007) Vitamin D supplementation and total mortality: a meta-analysis of randomized controlled trials. *Arch Intern Med* 167:1730-1737

[174] Forouhi NG, Luan J, Cooper A, Boucher BJ, Wareham NJ (2008) Baseline serum 25-hydroxy vitamin D is predictive of future glycaemic status and insulin resistance: the Medical Research Council Ely Prospective Study 1990-2000. *Diabetes* 57:2619-2625

[175] Giovannucci E, Liu Y, Hollis BW, Rimm EB (2008) 25-hydroxyvitamin D and risk of myocardial infarction in men: a prospective study. *Arch Intern Med* 168:1174-1180

[176] Chiu KC, Chu A, Go VL, Saad MF (2004) Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. *Am J Clin Nutr* 79:820-825

[177] Watson KE, Abrolat ML, Malone LL, et al. (1997) Active serum vitamin D levels are inversely correlated with coronary calcification. *Circulation* 96:1755-1760

[178] Sugden JA, Davies JI, Witham MD, Morris AD, Struthers AD (2008) Vitamin D improves endothelial function in patients with Type 2 diabetes mellitus and low vitamin D levels. *Diabetic Med* 25:320-325

[179] Suzuki A, Kotake M, Ono Y, et al. (2006) Hypovitaminosis D in type 2 diabetes mellitus: Association with microvascular complications and type of treatment. *Endocr J* 53:503-510

[180] Holick MF (2008) The vitamin D deficiency pandemic and consequences for nonskeletal health: Mechanisms of action. *Mol Aspects Med* 29:361-368

[181] Anand DV, Lahiri A, Lim E, Hopkins D, Corder R (2006) The relationship between plasma osteoprotegerin levels and coronary artery calcification in uncomplicated type 2 diabetic subjects. *J Am Coll Cardiol* 47:1850-1857

[182] Price PA, June HH, Buckley JR, Williamson MK (2001) Osteoprotegerin inhibits artery calcification induced by warfarin and by vitamin D. *Arterioscler Thromb Vasc Biol* 21:1610-1616

[183] Coussens A, Timms PM, Boucher BJ, et al. (2009) 1alpha, 25-dihydroxyvitamin D3 inhibits matrix metalloproteinases induced by Mycobacterium tuberculosis infection. *Immunology* 127:539-548

[184] London GM, Guérin AP, Verbeke FH, et al. (2007) Mineral metabolism and arterial functions in end-stage renal disease: potential role of 25-hydroxyvitamin D deficiency. *Journal of the American Society of Nephrology* 18:613-620

[185] Andrade J, Er L, Ignaszewski A, Levin A (2008) Exploration of association of 1,25-OH2D3 with augmentation index, a composite measure of arterial stiffness. *Clinical Journal of the American Society of Nephrology* 3:1800-1806

[186] Michos ED, Melamed ML (2008) Vitamin D and cardiovascular disease risk. *Curr Opin Clin Nutr Metab Care* 11:7-12

[187] Pilz S, Henry RM, Snijder MB, et al. (2009) 25-hydroxyvitamin D is not associated with carotid intima-media thickness in older men and women. *Calcif Tissue Int* 84:423-424

[188] Targher G, Bertolini L, Padovani R, et al. (2006) Serum 25-hydroxyvitamin D3 concentrations and carotid artery intima-media thickness among type 2 diabetic patients. *Clin Endocrinol* 65:593-597

[189] Freedman BI, Wagenknecht LE, Hairston KG, et al. (2010) Vitamin D, adiposity, and calcified atherosclerotic plaque in African-Americans. J*ournal of Clinical Endocrinology & Metabolism* 95:1076-1083

[190] Dong Y, Stallmann-Jorgensen IS, Pollock NK, et al. (2010) A 16-week randomised clinical trial of 2000IU D3 supplementation in black youth: 25 hydroxyvitaminD, adiposity and arterial stiffness. *Journal of Clinical Endocrinology & Metabolism* 95:4584-4591

[191] United Nations (2006) Standard country or area codes for statistical use. UN publication series M, No49, Rev4. http://unstats.un.org/unsd/pubs. Last accessed 01/12/11

[192] Joshi R, Cardona M, Iyengar S, et al. (2006) Chronic diseases now a leading cause of death in rural India - mortality data from the Andhra Pradesh Rural Health Initiative. *Int J Epidemiol* 35:1522-1529

[193] Enas EA (2000) Coronary artery disease epidemic in Indians: a cause for alarm and call for action. *J Indian Med Assoc* 98:694-695

[194] Khunti K, Kumar S, Brodie J (2009) Diabetes UK and South Asian Health Foundation recommendations on diabetes research priorities for British South Asians. Diabetes UK. Last accessed 01/12/2011

[195] Wild S, McKeigue P (1997) Cross-sectional analysis of mortality by country of birth in England and Wales, 1970-92. *BMJ* 314:705-710

[196] Wild SH, Fischbacher C, Brock A, Griffiths C, Bhopal R (2007) Mortality from all causes and circulatory disease by country of birth in England and Wales 2001-2003. *Journal of Public Health* 29:191-198

[197] Enas EA, Garg A, Davidson MA, Nair VM, Huet BA, Yusuf S (1996) Coronary heart disease and its risk factors in first-generation immigrant Asian Indians to the United States of America. *Indian Heart J* 48:343-353

[198] Harding S, Rosato M, Teyhan A (2008) Trends for coronary heart disease and stroke mortality among migrants in England and Wales, 1979-2003: slow declines notable for some groups. *Heart* 94:463-470

[199] Forouhi NG, Sattar N, Tillin T, McKeigue PM, Chaturvedi N (2006) Do known risk factors explain the higher coronary heart disease mortality in South Asian compared with European men? Prospective follow-up of the Southall and Brent studies, UK. *Diabetologia* 49:2580-2588

[200] Pickup JC, Crook MA (1998) Is type II diabetes mellitus a disease of the innate immune system? *Diabetologia* 41:1241-1248

[201] Neel JV (1962) Diabetes Mellitus: A "thrifty" genotype rendered detrimental by "progress"? *Am J Human Genetics* 14:353-362

[202] Hales CH, Barker DJ (2001) The thrifty phenotype hypothesis - Type 2 Diabetes. *Br Med Bulletin* 60:5-20

[203] Health Survey for England (2004) Health of ethnic minorities. www.ic.nhs.uk/pubs/hse04ethnic.Last accessed 01/12/11.

[204] Hippisley-Cox J, Coupland C, Robson J, Sheikh A, Brindle P (2009) Predicting risk of type 2 diabetes in England and Wales: Prospective derivation and validation of QDScore. *BMJ* 338:811-816

[205] Simmons D, Williams DR, Powell MJ (1991) The Coventry Diabetes study: prevalence of diabetes and impaired glucose tolerance in Europids and Asians. *QJM* 81:1021-1030

[206] Unwin N, Alberti KGMM, Bhopal R, Harland J, Watson W, White M (1998) Comparison of the current WHO and new ADA criteria for the

diagnosis of diabetes mellitus in three ethnic groups in the UK. *Diabetic Med* 15:554-557

[207] Riste L, Khan F, Cruickshank K (2001) High prevalence of type 2 diabetes in all ethnic groups, including Europeans, in a British inner city: relative poverty, history, inactivity or 21st century Europe?. *Diabetes Care* 24:1377-1383

[208] Forouhi NG, Merrick D, Goyder E, et al. (2006) Diabetes prevalence in England, 2001--estimates from an epidemiological model. *Diabetic Med* 23:189-197

[209] McKeigue PM, Shah B, Marmot MG (1991) Relation of central obesity and insulin resistance with high diabetes prevalence and cardiovascular risk in South Asians. *Lancet* 337:382-386

[210] Gill PS, Kai J, Bhopal S, Wild S (2004) Health care needs assessments: Black and minority ethnic groups. In: Raferty J (ed) Health Care Needs Assessments. The epidemiologically based needs assessment review. Radcliffe Medical Press Ltd., Abingdon, UK

[211] Petersen S, Peto V, Rayner M (2004) Coronary heart disease statistics. British Heart Foundation. http://www.heartstats.org/datapage.asp?id=1652. Last accessed 01/12/11.

[212] Anand SS, Yusuf S, Vuksan V, et al. (2000) Differences in risk factors, atherosclerosis, and cardiovascular disease between ethnic groups in Canada: the Study of Health Assessment and Risk in Ethnic groups (SHARE). *Lancet* 356:279-284

[213] Bhatnagar D, Anand IS, Durrington PN, et al. (1995) Coronary risk factors in people from the Indian subcontinent living in West London and their siblings in India. *Lancet* 345:982-983

[214] Chambers JC, Eda S, Bassett P, et al. (2001) C-reactive protein, insulin resistance, central obesity, and coronary heart disease risk in Indian Asians from the United Kingdom compared with European whites. *Circulation* 104:145-150

[215] Bhopal R, Unwin N, White M, et al. (1999) Heterogeneity of coronary heart disease risk factors in Indian, Pakistani, Bangladeshi, and European origin populations: cross sectional study. *BMJ* 319:215-220

[216] Lauritzen T, Griffin S, Borch-Johnsen K, Wareham NJ, Wolffenbuttel BHR, Rutten G (2000) The ADDITION study: Proposed trial of the cost-effectiveness of an intensive multi-factorial intervention on morbidity and mortality among people Type 2 diabetes detected by screening. *Int J Obes* 24:S6-S11

[217] Cummins C, Winter H, Cheng KK, Maric R, Silcocks P, Varghese C (1999) An assessment of the Nam Pehchan computer program for the identification of names of south Asian ethnic origin. *J Public Health Med* 21:401-406

[218] Office of the Deputy Prime Minister (2004) The English Indices of Deprivationhttp://www.communities.gov.uk/pub/446/indiciesofdeprivation2004. Last accessed 01/12/2011

[219] Index of Multiple Deprivation (IMD) http://www.communities.gov.uk/publications/communities/englishindices. Last accessed 01/12/2011

[220] Prineas R, Crow R, Blackburn H (1982) The Minnesota Code Classification for electrocardiographic findings

[221] Craig CL, Marshall AL, Sjostrom M, et al. (2003) International physical activity questionnaire: 12-Country reliability and validity. *Med Sci Sports Exerc* 35:1381-1395

[222] Ware JE, Kosinski M, Keller SD (1995) SF12-How to score the SF-12 physical and mental health summary scales. Health Institute of New England, Boston, Mass, USA

[223] Bradley C (1994) The Diabetes Treatment Satisfaction Questionnaire: DTSQ. In: Handbook of psychology and diabetes: a guide to psychology measurement in Diabetes research and practice. Harwood academic press, pp. 111-132

[224] John OP, Donahue EM, Kentle RL (1988) The "Big five" inventory - version 4a and 5b. Berkeley Institute of personality and social research, University of California, USA

[225] Kind P, Dolan P, Gudex C, Williams A (1998) Variations in population health status: results from a United Kingdom national questionnaire survey. *BMJ* 316: 736-741

[226] Alberti KGMM, Zimmet PZ (1998) Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: Diagnosis and classification of diabetes mellitus. Provisional report of a WHO consultation. *Diabetic Med* 15:539-553

[227] Sandbaek A, Griffin SJ, Rutten G, et al. (2008) Stepwise screening for diabetes identifies people with high but modifiable coronary heart disease risk. The ADDITION study. *Diabetologia* 51:1127-1134

[228] Department of Health (1998) National Screening Committee; first report of the National Screening Committee.Last accessed 01/12/11.

[229] Khunti K, Skinner TC, Heller S, Carey ME, Dallosso HM, Davies MJ (2008) Biomedical, lifestyle and psychosocial characteristics of people newly diagnosed with type 2 diabetes: Baseline data from the DESMOND randomized controlled trial. *Diabetic Med* 25:1454-1461

[230] Brindle P, May M, Gill P, et al. (2006) Primary prevention of cardiovascular disease: a web-based risk score for seven British black and minority ethnic groups. *Heart* 92:1595-1602

[231] Lawrence JM, Bennett P, Young A, Robinson AM (2001) Screening for diabetes in general practice: Cross sectional population study. *BMJ* 323:548-551

[232] Christensen JO, Sandbaek A, Lauritzen T, Borch-Johnsen K (2004) Population-based stepwise screening for unrecognised Type 2 diabetes is ineffective in general practice despite reliable algorithms. *Diabetologia* 47:1566-1573

[233] Janssen PG, Gorter KJ, Stolk RP, Akarsubasi M, Rutten GE (2009) Three years follow-up of screen-detected diabetic and non-diabetic subjects: who is better off? The ADDITION Netherlands study. *BMC family practice* 9:67-70

[234] Spijkerman AM, Adriaanse MC, Dekker JM, et al. (2002) Diabetic patients detected by population-based stepwise screening already have a diabetic cardiovascular risk profile. *Diabetes Care* 25:1784-1789

[235] Sargent LA, Simmons RK, Barling RS, et al. (2010) Who attends a UK diabetes screening programme? Findings from the ADDITION-Cambridge study. *Diabetic Med* 27:995-1003

[236] Bullimore SP, Keyworth C (1997) Finding diabetics - A method of screening in general practice. *Br J General Practice* 47:371-373

[237] Joshi P, Islam S, Pais P, et al. (2007) Risk factors for early myocardial infarction in South Asians compared with individuals in other countries. *JAMA* 297:286-294

[238] Mostafa SA, Davies MJ, Webb DR, et al. (2010) The potential impact of using glycated haemoglobin as the preferred diagnostic tool for detecting Type 2 diabetes mellitus. *Diabetic Med* 27:762-769

[239] Samanta A, Burden AC, Fent B (1987) Comparative prevalence of non-insulin-dependent diabetes mellitus in Asian and white Caucasian adults. *Diabetes Research & Clinical Practice* 4:1-6

[240] Department of Health (2004) Quality and Outcomes Framework (QoF): guidance. Department of Health, London. Last accessed 01/12/11.

[241] De Vegt F, Dekker JM, Ruhe HG, et al. (1999) Hyperglycaemia is associated with all-cause and cardiovascular mortality in the Hoorn population: The Hoorn study. *Diabetologia* 42:926-931

[242] Jorgensen T, Borch-Johnsen K, Thomsen TF, Ibsen H, Glumer C, Pisinger C (2003) A randomized non-pharmacological intervention study for prevention of ischaemic heart disease: Baseline results of Inter99. *European Journal of Cardiovascular Prevention and Rehabilitation* 10:377-386

[243] McKeigue PM, Ferrie JE, Pierpoint T, Marmot MG (1993) Association of early-onset coronary heart disease in South Asian men with glucose intolerance and hyperinsulinemia. *Circulation* 87:152-161

[244] Bhopal R, Fischbacher CM (2002) Many South Asian people probably need pre-diabetes care *BMJ* 325:965-966

[245] Turner RC, Cull CA, Frighi V, Holman RR (1999) Glycemic control with diet, sulfonylurea, metformin, or insulin in patients with type 2 diabetes mellitus. Progressive requirement for multiple therapies (UKPDS 49). *JAMA* 281:2005-2012

[246] Stevens RJ, Kothari V, Adler AI, Stratton IM, Holman RR (2001) The UKPDS risk engine: A model for the risk of coronary heart disease in type II diabetes (UKPDS 56). *Clin Sci* 101:671-679

[247] Clarke M (2000) The QUORUM statement. *Lancet* 355:756-757

[248] Janessen PG, Gorter KJ, Stolk RP, Rutten GE (2009) Randomised controlled trial of intensive multi-factorial treatment for cardiovascular risk in patients with screen-detected Type 2 Diabetes: 1-year data from the ADDITION-Netherlands study. *Br J General Practice* 59:43-48

[249] Coutinho M, Gerstein HC, Wang Y, Yusuf S (1999) The relationship between glucose and incident cardiovascular events. A meta-regression analysis of published data from 20 studies of 95,783 individuals followed for 12.4 years. *Diabetes Care* 22:233-240

[250] Satoh N, Ogawa Y, Usui T, et al. (2003) Antiatherogenic effect of pioglitazone in type 2 diabetic patients irrespective of the responsiveness to its antidiabetic effect. *Diabetes Care* 26:2493-2499

[251] Dilaveris P, Giannopoulos G, Riga M, Synetos A, Stefanadis C (2007) Beneficial effects of statins on endothelial dysfunction and vascular stiffness. *Current Vascular Pharmacology* 5:227-237

[252] Nakamura T, Matsuda T, Kawagoe Y, et al. (2004) Effect of pioglitazone on carotid intima-media thickness and arterial stiffness in type 2 diabetic nephropathy patients. *Metabolism* 53:1382-1386

[253] van Dijk RA, Nijpels G, Twisk JW, et al. (2000) Change in common carotid artery diameter, distensibility and compliance in subjects with a recent history of impaired glucose tolerance: a 3-year follow-up study. *J Hypertens* 18:293-300

[254] Ahluwalia N, Drouet L, Ruidavets J, et al. (2006) Metabolic syndrome is associated with markers of subclinical atherosclerosis in a French population-based sample. *Atherosclerosis* 186:345-353

[255] Czernichow S, Bertrais S, Blacher J, et al. (2005) Metabolic syndrome in relation to structure and function of large arteries: A predominant effect of blood pressure - A report from the SU.VI.MAX. vascular study. *Am J Hypertension* 18:1154-1160

[256] Nakanishi N, Suzuki K, Tatara K (2003) Clustered features of the metabolic syndrome and the risk for increased aortic pulse wave velocity in middle-aged Japanese men. *Angiology* 54:551-559

[257] Schillaci G, Pirro M, Vaudo G, et al. (2005) Metabolic syndrome is associated with aortic stiffness in untreated essential hypertension. *Hypertension* 45:1078-1082

[258] Kim YJ, Kim YJ, Cho BM, Lee S (2010) Metabolic syndrome and arterial pulse wave velocity. *Acta Cardiol* 65:315-321

[259] Goodarzi MO, Psaty BM (2008) Glucose lowering to control macrovascular disease in type 2 diabetes: Treating the wrong surrogate end point? *JAMA* 300:2051-2053

[260] Yki-Jarvinen H, Westerbacka J (2007) Insulin resistance, arterial stiffness and wave reflection. *Adv Cardiol* 44:252-260

[261] Sengstock DM, Vaitkevicius PV, Supiano MA (2005) Arterial stiffness is related to insulin resistance in nondiabetic hypertensive older adults. *Journal of Clinical Endocrinology & Metabolism* 90:2823-2827

[262] Salomaa V, Riley W, Kark JD, Nardo C, Folsom AR (1995) Non-insulin-dependent diabetes mellitus and fasting glucose and insulin concentrations are associated with arterial stiffness indexes: The ARIC study. *Circulation* 91:1432-1443

[263] Van Dijk RAJM, Bakker SJL, Scheffer PG, Heine RJ, Stehouwer CDA (2003) Associations of metabolic variables with arterial stiffness in type 2 diabetes mellitus: Focus on insulin sensitivity and postprandial triglyceridaemia. *Eur J Clin Invest* 33:307-315

[264] De Angelis L, Millasseau SC, Smith A, et al.(2004) Gender differences in age-related stiffening of the aorta in subjects with type 2 diabetes. *Hypertension* 44:67-71

[265] Protogerou AD, Blacher J, Aslangul E, et al. (2007) Gender influence on metabolic syndrome's effects on arterial stiffness and pressure wave reflections in treated hypertensive subjects. *Atherosclerosis* 193:151-158

[266] Matthews DR, Hosker JP, Rudenski AS (1985) Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28:412-419

[267] Morita S, Kasayama S, Otsuki M, et al. (2006) Atherosclerotic risk factors in Japanese subjects with isolated impaired fasting glucose and those with isolated impaired glucose tolerance according to 1997 and 2003 American Diabetes Association criteria. *Diabetes Care* 29:2123-2126

[268] Stehouwer CD, Henry RM, Ferreira I (2008) Arterial stiffness in diabetes and the metabolic syndrome: a pathway to cardiovascular disease *Diabetologia* 51:527-539

[269] Williams ED, Stamatakis E, Chandola T, Hamer M (2011) Physical activity behaviour and coronary heart disease mortality among South Asian people in the UK: an observational longitudinal study. *Heart* 97:655-659

[270] Forouhi NG, Sattar N (2006) CVD risk factors and ethnicity--a homogeneous relationship? *Atherosclerosis* S7:11-19

[271] Kayaniyil S, Vieth R, Retnakaran R, et al. (2010) Association of vitamin D with insulin resistance and beta-cell dysfunction in subjects at risk for type 2 diabetes. *Diabetes Care* 33:1379-1381

[272] Ganji V, Zhang X, Shaikh N, Tangpricha V (2011) Serum 25-hydroxyvitamin D concentrations are associated with prevalence of metabolic syndrome and various cardiometabolic risk factors in US children and adolescents based on assay-adjusted serum 25-hydroxyvitamin D data from NHANES 2001-2006. *Am J Clin Nutr* 94:225-233

[273] Forman JP, Giovannucci E, Holmes MD, et al. (2007) Plasma 25-hydroxyvitamin D levels and risk of incident hypertension. *Hypertension* 49:1063-1069

[274] Burgaz A, Orsini N, Larsson SC, Wolk A (2011) Blood 25-hydroxyvitamin D concentration and hypertension: a meta-analysis. *J Hypertension* 29:636-645

[275] Robinson JG, Manson JE, Larson J, et al. (2011) Lack of association between 25(OH)D levels and incident type 2 diabetes in older women. *Diabetes Care* 34:628-634

[276] Pittas AG, Chung M, Trikalinos T, et al. (2010) Systematic review: Vitamin D and cardiometabolic outcomes. *Ann Intern Med* 152:307-314

[277] Wang L, Manson JE, Song Y, Sesso HD (2010) Systematic review: Vitamin D and calcium supplementation in prevention of cardiovascular events. *Ann Intern Med* 152:315-323

[278] Witham MD, Nadir MA, Struthers AD (2009) Effect of vitamin D on blood pressure: a systematic review and meta-analysis. *J Hypertension* 27:1948-1954

[279] Al Mheid I, Patel R, Murrow J, et al. (2011) Vitamin D status is associated with arterial stiffness and vascular dysfunction in healthy humans. *J Am Coll Cardiol* 58:186-192

[280] McEniery CM, McDonnell BJ, So A, et al. (2009) Aortic calcification is associated with aortic stiffness and isolated systolic hypertension in healthy individuals. *Hypertension* 53:524-531

[281] Judd SE, Nanes MS, Ziegler TR, Wilson PW, Tangpricha V (2008) Optimal vitamin D status attenuates the age-associated increase in systolic blood pressure in white Americans: results from the third National Health and Nutrition Examination Survey. *Am J Clin Nutr* 87:136-141

[282] Chaturvedi N, Bulpitt CJ, Leggetter S, et al. (2004) Ethnic differences in vascular stiffness and relations to hypertensive target organ damage. *J Hypertension* 22:1731-1737

[283] Ferreira AV, Viana MC, Mill JG, Asmar RG, Cunha RS (1999) Racial differences in aortic stiffness in normotensive and hypertensive adults. *J Hypertension* 17:631-637

[284] Kalra L, Iveson E, Rambaran C, et al. (2008) An international matched cohort study of the contribution of metabolic impairments to subclinical atherosclerosis in United Kingdom and Jamaican African-Caribbeans. *Atherosclerosis* 199:95-101

[285] Pinto ES, Mensah R, Meeran K, et al. (2005) Peripheral arterial compliance differs between races: comparison among Asian, Afro-Caribbeans, and white Caucasians with type 2 diabetes. *Diabetes Care* 28:496

[286] Strain WD, Chaturvedi N, Dockery F, et al. (2006) Increased arterial stiffness in Europeans and African Caribbeans with type 2 diabetes cannot be accounted for by conventional cardiovascular risk factors. *Am J Hypertension* 19:889-896

[287] Nguyen QM, Srinivasan SR, Xu JH, Chen W, Berenson GS (2008) Racial (Black-White) Divergence in the association between Adiponectin and Arterial Stiffness in Asymptomatic Young Adults: The Bogalusa Heart Study. *Am J Hypertension* 21:553-557

[288] Din JN, Ashman OA, Aftab SM, Jubb AW, Newby DE, Flapan AD (2006) Increased arterial stiffness in healthy young South Asian men. *J Hum Hypertension* 20:163-165

[289] Gunarathne A, Patel JV, Gammon B, Hughes EA, Lip GY (2008) Impact of mean arterial blood pressure on higher arterial stiffness indices in South Asians compared to white Europeans. *J Hypertension* 26:1420-1426

[290] Rezai M, Wallace AM, Sattar N, Finn JD, Wu FCW, Cruickshank JK (2011) Ethnic differences in aortic pulse wave velocity occur in the descending aorta and may be related to Vitamin D. *Hypertension* 58:247-253

[291] Institute of medicine, food and nutrition board. Dietary reference intakes for calcium and Vitamin D (2010) National Academy press Washigton DC, USA

[292] Kalra L, Rambaran C, Chowienczyk P, et al. (2005) Ethnic differences in arterial responses and inflammatory markers in Afro-Caribbean and Caucasian subjects. *Arterioscler Thromb Vasc Biol* 25:2362-2367

[293] Lind L, Wengle B, Wide L, Ljunghall S (1989) Reduction of blood pressure during long-term treatment with active vitamin D (alphacalcidol) is dependent on plasma renin activity and calcium status. A double-blind, placebo-controlled study. *Am J Hypertension* 2:20-25

[294] Klar J, Sigl M, Obermayer B, Schweda F, Kramer BK, Kurtz A (2005) Calcium inhibits renin gene expression by transcriptional and post-transcriptional mechanisms. *Hypertension* 46:1340-1346

[295] Cheng Q, Li YC, Boucher BJ, Leung PS (2011) A novel role for vitamin D: Modulation of expression and function of the local renin-angiotensin system in mouse pancreatic islets. *Diabetologia* 54:2077-2081

[296] Xiang W, Kong J, Chen S, et al. (2005) Cardiac hypertrophy in vitamin D receptor knockout mice: role of the systemic and cardiac renin-angiotensin systems. *American Journal of Physiology Endocrinology & Metabolism* 288:E125-32

[297] Hayden MR, Tyagi SC, Kolb L, Sowers JR, Khanna R (2005) Vascular ossification-calcification in metabolic syndrome, type 2 diabetes mellitus, chronic kidney disease, and calciphylaxis-calcific uremic arteriolopathy: the emerging role of sodium thiosulfate. *Cardiovascular Diabetology* 4:4-10

[298] Timms PM, Mannan N, Hitman GA, et al. (2002) Circulating MMP9, vitamin D and variation in the TIMP-1 response with VDR genotype: mechanisms for inflammatory damage in chronic disorders? *QJM* 95:787-796

[299] Bahar-Shany K, Ravid A, Koren R (2010) Up-regulation of MMP-9 production by TNFalpha in keratinocytes and its attenuation by vitamin D. *J Cell Physiol* 222:729-737

[300] Jablonski KL, Chonchol M, Pierce GL, Walker AE, Seals DR (2011) 25-Hydroxyvitamin D deficiency is associated with inflammation-linked vascular endothelial dysfunction in middle-aged and older adults. *Hypertension* 57:63-69

[301] Wakasugi M, Noguchi T, Inoue M, et al. (1991) Vitamin D3 stimulates the production of prostacyclin by vascular smooth muscle cells. *Prostaglandins* 42:127-136

[302] Tarcin O, Yavuz DG, Ozben B, et al. (2009) Effect of vitamin D deficiency and replacement on endothelial function in asymptomatic subjects. *Journal of Clinical Endocrinology & Metabolism* 94:4023-4030

[303] Rubin MR, Maurer MS, McMahon DJ, Bilezikian JP, Silverberg SJ (2005) Arterial stiffness in mild primary hyperparathyroidism. *Journal of Clinical Endocrinology & Metabolism* 90:3326-3330

[304] Laurent S, Boutouyrie P, Asmar R, et al. (2001) Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. *Hypertension* 37:1236-1241

[305] Zieman SJ, Melenovsky V, Kass DA (2005) Mechanisms pathophysiology, and therapy of arterial stiffness. *Arterioscler Thromb Vasc Biol* 25:932-943

[306] Hodis HN, Mack WJ, Zheng L, et al. (2006) Effect of peroxisome proliferator-activated receptor gamma agonist treatment on subclinical atherosclerosis in patients with insulin-requiring type 2 diabetes. *Diabetes Care* 29:1545-1553

[307] Stocker DJ, Taylor AJ, Langley RW, Jezior MR, Vigersky RA (2007) A randomized trial of the effects of rosiglitazone and metformin on inflammation and subclinical atherosclerosis in patients with type 2 diabetes. *Am Heart J* 153:445-450

[308] Lonn EM, Yusuf S, Jha P, et al. (1994) Emerging role of angiotensin-converting enzyme inhibitors in cardiac and vascular protection. *Circulation* 90:2056-2069

[309] Ray KK, Cannon CP (2005) Atorvastatin and cardiovascular protection: a review and comparison of recent clinical trials. *Expert Opin Pharmacother* 6:915-927

[310] Yki-Jarvinen H (2004) Thiazolidinediones. *N Engl J Med* 351:1106-1118

[311] Franklin SS, Gustin IV W, Wong ND, et al. (1997) Hemodynamic patterns of age-related changes in blood pressure: The Framingham heart study. *Circulation* 96:308-315

[312] McEniery CM, Yasmin, Maki-Petaja KM, et al. (2010) The impact of cardiovascular risk factors on aortic stiffness and wave reflections depends on age: the Anglo-Cardiff Collaborative Trial (ACCT III). *Hypertension* 56:591-597

[313] Beckman J, Raji A, Plutzky J (2003) Peroxisome proliferator activated receptor gamma and its activation in the treatment of insulin resistance and atherosclerosis: issues and opportunities. *Curr Opin Cardiol* 18:479-485

[314] Di Napoli M, Papa F (2003) Angiotensin-converting enzyme inhibitor use is associated with reduced plasma concentration of C-reactive protein in patients with first-ever ischemic stroke. *Stroke* 34:2922-2929

[315] Ridker PM, JUPITER Study Group (2003) Rosuvastatin in the primary prevention of cardiovascular disease among patients with low levels of low-density lipoprotein cholesterol and elevated high-sensitivity C-reactive protein: rationale and design of the JUPITER trial. *Circulation* 108:2292-2297