THE UNIVERSITY
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Cardiovascular Disease, Type 2 Diabetes and Carotid Ultrasound

Christine Mary Robertson

Thesis presented for the degree of Doctor of Philosophy
University of Edinburgh
August 2014

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Declaration

I, Christine Robertson, declare that this thesis is of my own composition and that the work has not been submitted for any other degree or professional qualification. I contributed substantially to the data collection for the work presented and the contribution of others has been acknowledged within.

Any publications related to this thesis that are included as appendices have been done so with permission of the publisher.

Signed

Date

Publications relating to the work of this thesis

Published review article (Appendix F):

Robertson CM, Fowkes FG, Price JF. *Carotid intima-media thickness and the prediction of vascular events*. **Vascular Medicine** 2012;17(4):239-48

cIMT collaboration published papers contributed to:

The data provided for these publications was from the Edinburgh Artery Study (EAS) and NOT the ET2DS

1. den Ruijter HM, Peters SA, Groenewegen KA, Anderson TJ, Britton AR, Dekker JM, Engström G, Eijkemans MJ, Evans GW, de Graaf J, Grobbee DE, Hedblad B, Hofman A, Holewijn S, Ikeda A, Kavousi M, Kitagawa K, Kitamura A, Koffijberg H, Ikram MA, Lonn EM, Lorenz MW, Mathiesen EB, Nijpels G, Okazaki S, O'Leary DH, Polak JF, Price JF, **Robertson C**, Rembold CM, Rosvall M, Rundek T, Salonen JT, Sitzer M, Stehouwer CD, Witteman JC, Moons KG, Bots ML. *Common carotid intima-media thickness does not add to Framingham risk score in individuals with diabetes mellitus: the USE-IMT initiative*. **Diabetologia** 2013; 56(7): 1494-502
2. Den Ruijter HM, Peters SA, Anderson TJ, Britton AR, Dekker JM, Eijkemans MJ, Engström G, Evans GW, de Graaf J, Grobbee DE, Hedblad B, Hofman A, Holewijn S, Ikeda A, Kavousi M, Kitagawa K, Kitamura A, Koffijberg H, Lonn EM, Lorenz MW, Mathiesen EB, Nijpels G, Okazaki S, O'Leary DH, Polak JF, Price JF, **Robertson C**, Rembold CM, Rosvall M, Rundek T, Salonen JT, Sitzer M, Stehouwer CD, Witteman JC, Moons KG, Bots ML. *Common carotid intima-media thickness measurements in cardiovascular risk prediction: a meta-analysis*. **JAMA** 2012; 308(8):796-803
3. Lorenz MW, Polak JF, Kavousi M, Mathiesen EB, Völzke H, Tuomainen TP, Sander D, Plichart M, Catapano AL, **Robertson CM**, Kiechl S, Rundek T, Desvarieux M, Lind L, Schmid C, DasMahapatra P, Gao L, Ziegelbauer K, Bots ML, Thompson SG; PROG-IMT Study Group (inc Price J, Fowkes G). *Carotid intima-media thickness progression to predict cardiovascular events in the general population (the PROG-IMT collaborative project): a meta-analysis of individual participant data*. **Lancet** 2012;379(9831):2053-62

4. Bots ML, Groenewegen KA, Anderson TJ, Britton AR, Dekker JM, Engstrom G, et al (inc Price JF, **Robertson C**). Common carotid intima-media thickness measurements do not improve cardiovascular risk prediction in individuals with elevated blood pressure: the USE-IMT Collaboration. **Hypertension** 2014; published online 10 March

cIMT collaboration papers final draft pre-submission:

1. Lorenz MW, Price JF, **Robertson C**, Polak JF, Poppert H, Kavousi M, Dörr M, Stensland E, Ducimetiere P, Ronkainen K, Kiechl S, Sitzer M, Rundek T, Lind L, Liu J, Bergström G, Grigore L, Bokemark L, Friera A, Yanez D, Bickel H, Ikram A, Völzke H, Johnsen SH, Empana JP, Tuomainen TP, Steinmetz H, Desvarieux M, Xie W, Schmidt C, Norata GD, Suarez C, Sander D, Hofman A, Schminke U, Mathiesen M, Plichart M, Kauhanen J, Willeit J, Sacco RL, McLachlan S, Zhao D, Fagerberg B, Catapano AL, Gabriel R, Franco O, Bülbül A, Scheckenbach F, Pflug A, Gao L, Thompson S. *Carotid Intima Media Thickness progression and the risk of vascular events in people with diabetes - results from the PROG-IMT collaboration.* **Final draft, pre-submission**

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1. Jenks SJ, Conway BR, Hor TJ, Williamson RM, McLachlan S, **Robertson C**, Morling JR, Strachan MW, Price JF. *Hepatic steatosis and non-alcoholic fatty liver disease are not associated with decline in renal function in people with Type 2 diabetes*. **Diabetic Medicine** 2014; published online 31 March
2. Morling JR, Fallowfield JA, Guha IN, Nee LD, Glancy S, Williamson RM, **Robertson CM**, Strachan MWJ, Price JF, on behalf of the Edinburgh Type 2 Diabetes Study investigators. *Using non-invasive biomarkers to identify hepatic fibrosis in people with type 2 diabetes mellitus: The Edinburgh type 2 diabetes study*. **Journal of Hepatology** 2013; published online 26 October
3. Feinkohl I, Aung PP, Keller M, **Robertson CM**, Morling JR, McLachlan S, Deary IJ, Frier BM, Strachan MW, Price JF; on behalf of the Edinburgh Type 2 Diabetes Study (ET2DS) Investigators. *Severe hypoglycemia and cognitive decline in older people with type 2 diabetes: the Edinburgh Type 2 Diabetes Study*. **Diabetes Care** 2013; published online 8 October 2013
5. Feinkohl I, Keller M, **Robertson CM**, Morling JR, Williamson RM, Nee LD, McLachlan S, Sattar N, Welsh P, Reynolds RM, Russ TC, Deary IJ, Strachan MW, Price JF; Edinburgh Type 2 Diabetes Study (ET2DS) Investigators. *Clinical and subclinical macrovascular disease as predictors of cognitive decline*

in older patients with type 2 diabetes: the Edinburgh Type 2 Diabetes Study. **Diabetes Care** 2013; 36(9):2779-86

6. Anderssohn M, McLachlan S, Lüneburg N, **Robertson C**, Schwedhelm E, Williamson RM, Strachan MW, Ajjan R, Grant PJ, Böger RH, Price JF. *Genetic and environmental determinants of dimethylarginines and association with cardiovascular disease in patients with type 2 diabetes*. **Diabetes Care** 2013; published online 1 November 2013

Conference Presentations:

Diabetes UK 2012

Frequency and characteristics of carotid artery plaque in older people with Type 2 diabetes: the Edinburgh Type 2 Diabetes Study. **Robertson CM,** MWJ Strachan, L Nee, J Morling, S Masle and JF Price. **Diabetic Medicine** 29 (Supp. 1) 30-177 (Oral Presentation)

Diabetes UK 2011

Testosterone Levels and Cardiovascular Disease in Elderly Men with Type 2 Diabetes: the Edinburgh Type 2 Diabetes Study (ET2DS). **Robertson CM**, Strachan MWJ, Beckett G, Reynolds R, Cawood P, Murphy N, Price JF on behalf of the ET2DS Investigators. **Diabetic Medicine** 28 (supp.1) 1-214

Diabetes UK 2010

Carotid Intima Media Thickness and Cardiovascular Disease in Elderly People with Type 2 Diabetes: the Edinburgh Type 2 Diabetes Study (ET2DS) **Robertson CM**, Strachan MWJ, Nee L, Butcher I, Reynolds R, Fowkes FGR, Price JF on behalf of the ET2DS Investigators. **Diabetic Medicine** 27 (Supp. 1) 37-188) (Oral presentation)

Acknowledgements and my contribution to the work of the thesis

Firstly, I would like to take this opportunity to thank my supervisors Professor Jackie Price and Dr Mark Strachan. Particular thanks are due to Professor Price for her unerring belief in my ability to complete this thesis. Her helpful and insightful suggestions helped to shape the course of this thesis and kept me motivated over a challenging few years.

I would also like to thank all the staff and participants of the Edinburgh Type 2 Diabetes Study, without whom, this thesis would not have been possible. Particular thanks are due to all the (current and former) occupants of Room 666a. I would also like to acknowledge the PhD studentship funding that I received from the Economic and Social Research Council, without which I would not have been able to undertake this PhD.

Finally, I would like to thank all my friends and family, in particular my husband Hamish and my daughter Carolyn, for their continued patience with my work schedule and for supporting me in their many (unique!) ways over the course of this thesis.

Edinburgh Type 2 Diabetes Study and analysis

The Edinburgh Type 2 Diabetes study commenced in 2006 and has been conducted in 3 phases (baseline, year 1 and year 4). For this thesis, baseline and year 1 phases of the study provide the predictor variables for use in the analyses presented here. Baseline data collection, data linkage and CVD event determination were performed by the ET2DS baseline and year 1 study staff.

I became involved in the ET2DS in 2009, prior to the start of the year 4 phase of the study. I was involved in contacting patients prior to the follow up to ensure contact

details were correct and assisted Dr Joanne Morling in some aspects of planning the study clinics. I was heavily involved in the data collection clinics at year 4 along with Chris Martin, Marketa Keller and Insa Feinkohl, and undertook the following activities over the course of approximately 13 months of follow up:

- Venous blood sampling and preparation of blood samples for processing
- Organising follow up of abnormal blood test and ultrasound findings where necessary, in collaboration with Dr Mark Strachan and Dr Stephen Glancy, Western General Hospital
- ECGs and physical examinations
- Cognitive testing
- Problem solving throughout the clinics – eg administrative and technological problems
- Organisation of cIMT validation study and clinic measurement validation

Following completion of the data collection clinics, I facilitated the ISD data linkage for the individuals who had consented at the start of the study. I created the criteria for the incident event determination in collaboration with the Primary Investigator of the study, and identified individuals with potential events. In cases where a diagnosis could not be made using study data alone, I obtained permission to search clinical notes held by NHS Lothian and hand searched the paper records. If paper records were not available, Dr Joanne Morling (Clinical Research Fellow) accessed computerised records. I was also involved with the cleaning and preparation of the data, as well as double data entry, which was co-ordinated by Dr Stela MacLachlan.

The ultrasound measurements of cIMT and carotid plaque were performed by sonographer Lisa Nee at the Wellcome Trust Clinical Research Facility, Western General Hospital. Thanks are also due to Dr Calum Gray and Dr Tom McGillivray of the Clinical Research Imaging Centre who developed the measurement software used in this thesis in consultation with Professor Jackie Price and myself.

I designed and performed the statistical analysis reported in this thesis.

List of Abbreviations

ABI	Ankle Brachial Index
ACAPS	Asymptomatic Carotid Artery Progression Study
ACR	Albumin creatinine ratio
ARIC	Atherosclerosis Risk In Communities
BMI	Body Mass Index
BRHS	British Regional Heart Study
CCA	Common carotid artery
CHD	Coronary Heart Disease
CHS	Cardiovascular Health Study
cIMT	Carotid Intima Media Thickness
CT	Computer Tomography
CVD	Cardiovascular Disease
DBP	Diastolic Blood Pressure
ECA	External carotid artery
ECG	Electrocardiograph
eGFR	Estimated glomerular filtration rate
ET2DS	Edinburgh Type 2 Diabetes Study
FRS	Framingham Risk Score
GP	General Practitioner
HbA1c	Haemoglobin A1c
HDL	High Density Lipoprotein
IC	Intermittent Claudication
ICA	Internal carotid artery
ICC	Intraclass Correlation Coefficient
IL-6	Interleukin 6
IMT	Intima Media Thickness
ISD	Information Services Division
KIHD	Kuopio Ischaemic Heart Disease Risk Factor Study
LDL	Low Density Lipoprotein

Max cIMT	Maximum of the 6 readings of cIMT (both right and left)
Max Mean cIMT	Maximum of the mean right and mean left readings
Mean cIMT	Mean of the 6 readings of cIMT (3 right and 3 left)
Mean Max cIMT	Mean of the maximum right and maximum left readings
MI	Myocardial infarction
MRI	Magnetic Resonance Imaging
NHS	National Health Service
NRI	Net Reclassification Index
NTproBNP	Amino Terminal pro Brain Natriuretic Peptide
OPCS 4	Office of Population Censuses and Surveys: Tabular list of the classification of surgical operations and procedures Fourth Revision.
PAD	Peripheral Arterial Disease
SBP	Systolic Blood Pressure
SD	Standard Deviation
SIMD	Scottish Index of Multiple Deprivation
SOP	Standard Operating Procedure
SPECT	Single-Photon Emission Computerized Tomography
TIA	Transient Ischaemic Attack
UKPDS	United Kingdom Prospective Diabetes Study
USPSTF	United States Preventative Services Task Force

Abstract

Cardiovascular disease contributes significantly to global morbidity and mortality and is particularly prevalent among individuals with Type 2 diabetes, which is thought to in part be due to the association between diabetes and the metabolic syndrome. Traditional cardiovascular risk prediction scores perform well in the general population but their use in people with Type 2 diabetes is limited as they are thought to underperform in high risk groups. Indeed, the use of any risk prediction in people with Type 2 diabetes is a point of discussion among clinicians as people with diabetes are thought by some to be at immediate high risk of CVD, whereas others view them as having a degree of modifiable risk which can be addressed using risk prediction. In the general population, novel markers such as cIMT and carotid plaque, as well as other potential biomarkers of cardiovascular risk, have been explored as possible adjuncts to risk scores in the prediction of cardiovascular disease. The evidence for their use in general populations has been established, although there have been no firm conclusions with regard to recommendations for their use, which is partly due to the high degree of variability in cIMT measurement. However, the evidence for their use in people with Type 2 diabetes is sparse, despite the use of such markers as surrogate CV endpoints in clinical trials.

This thesis aimed to describe the frequency, distribution and change of cIMT and carotid plaque, as well as to explore the relationship of cIMT and carotid plaque with cardiovascular risk factors, prevalent cardiovascular disease and future cardiovascular events in older people with Type 2 diabetes. The association between cIMT, carotid plaque and other novel risk markers was also explored.

The analysis was performed using data from the Edinburgh Type 2 Diabetes Study (ET2DS). This study is a large, prospective cohort study of 1066 men and women with Type 2 diabetes, aged 60-75 years at recruitment, living in Edinburgh and the Lothians. cIMT and carotid plaque were measured at year 1 follow up of the study. Variables concerning cardiovascular risk factors used in this thesis were obtained

from the data collection performed at baseline and year 1. A mean of 3.5 years of follow up was available for analysis and is complete for the baseline cohort as data linkage was performed.

Mean values of cIMT in the ET2DS were comparable with other studies of cIMT in people with Type 2 diabetes and may indeed be higher than cIMT in the general population. Measurement of cIMT by the sonographer was comparable with computer aided measurements. Increasing cIMT was independently associated (although only modestly) with increasing age, male sex and raised systolic blood pressure. Mean cIMT was associated with prevalent vascular disease and was predictive of incident global cardiovascular events and coronary artery events (but not stroke) over and above UKPDS risk factors, although the clinical impact of this on the reclassification of vascular risk (as demonstrated by net reclassification index (NRI)) was limited.

There was a high prevalence of carotid plaque, and in particular "high risk" plaque, in the ET2DS. Different measures of carotid plaque were independently associated with several cardiovascular risk factors. Carotid plaque thickness was independently associated, albeit modestly, with increasing age, male sex, duration of diabetes and hypertension, plaque score with increasing age, hypertension, smoking and low BMI, and high risk plaque with hypertension and low BMI. All measures of carotid plaque were associated with prevalent vascular disease. However, despite these associations, carotid plaque did not have any additional predictive value for incident cardiovascular events over and above UKPDS risk factors.

Finally, measures of cIMT and carotid plaque in the ET2DS were associated with the biomarkers ankle brachial index (ABI) and NTproBNP. In addition these markers were significantly higher in those individuals with prevalent vascular disease, suggesting a more extensive exploration of the association of these markers in relation to cardiovascular disease in the ET2DS may be warranted.

Summary

cIMT and carotid plaque are modestly associated with traditional cardiovascular risk factors and prevalent cardiovascular disease in older adults with Type 2 diabetes. cIMT has been shown to be predictive of incident events while carotid plaque was not, in people with Type 2 diabetes, over and above traditional cardiovascular risk factors, although its impact on risk reclassification may only be small. Further evidence is required from the longer follow up of the ET2DS before firm conclusions can be drawn on the usefulness of cIMT and carotid plaque as risk markers in people with Type 2 diabetes. In addition, large collaborative studies could be used to further explore the relationship of carotid plaque, and change in cIMT with incident cardiovascular events, as well as exploring the additive effect of cIMT and plaque on risk prediction.

Chapter 1: Cardiovascular Disease, Diabetes and Carotid Ultrasound

Introduction

In 2008, the World Health Organisation (WHO) stated that cardiovascular disease (CVD) is the leading cause of morbidity and mortality worldwide, as well as the leading cause of death in people with Type 2 diabetes (WHO 2008). Although there is now considerably greater awareness and improved management of both modifiable vascular risk factors and acute vascular events, following decades of research, cardiovascular disease remains an extensive burden on health care systems. As is the case for many other major medical conditions, restrictions imposed upon health care funding in many countries means that there must be an element of patient selection when prescribing both pharmaceutical and non-pharmaceutical management plans for cardiovascular disease. Methods for predicting who might be at a greater cardiovascular risk, and therefore gain the most benefit from early intensive management (the most economically and medically beneficial strategy), range from highly invasive imaging procedures to non-invasive cardiovascular risk scores. However, none of these methods alone currently provide an accurate estimation of risk which is suitable for use in every patient and indeed, many events occur in people in whom risk was not identified as being raised (Taylor 2002). This has led to increasing interest in the use of 'novel' risk markers in the prediction of events. Non-invasive markers of vascular risk, including carotid intima media thickness and carotid plaque, have recently been cited as having the potential to improve risk prediction over and above traditional risk scoring (such as Framingham scores) in the general population (Calonge N, Petitti DB et al. 2009; Nambi V, Chambless L et al. 2010; Simon A, Megnien JL et al. 2010; Nambi V, Chambless L et al. 2012; Peters SAE, den Ruijter HM et al. 2012).

An additional growing burden upon health is Type 2 diabetes (T2DM). As well as the damage this chronic disease inflicts upon the renal, visual and neurological systems, Type 2 diabetes confers an increased cardiovascular risk over and above that of people without diabetes. Those with Type 2 diabetes more frequently present with vascular disease at an earlier age and often present with advanced disease at diagnosis due to the silent damage inflicted upon vessel walls by hyperglycaemia and the effects of established vascular risk factors that usually accompany a diagnosis of T2DM. However, there remains controversy as to whether Type 2 diabetes should be considered a risk equivalent for CVD (Haffner, Lehto et al. 1998) or a risk factor for CVD (Wong ND, Glovaci D et al. 2012; Sattar N 2013). If it is considered a risk equivalent, individuals should immediately be classed as high risk and treated as such. However, if it is to be considered as a risk factor for CVD, there is still a spectrum of risk and individuals could arguably be characterised in a similar way to the general population in terms of cardiovascular risk management.

Traditional risk scores that are used to predict incident cardiovascular events have largely been developed in people initially free of such events in the general population and as such, are thought to be less accurate for people with Type 2 diabetes. As in the general population, there is also an opportunity to use markers such as cIMT and carotid plaque in the evaluation of cardiovascular risk in people with T2DM. However the use of these markers in people with Type 2 diabetes is less well documented than in the general population. The United States Preventive Services Task Force (USPSTF) statement recently published (United States Preventative Services Task Force 2009), stated that whilst there was some evidence for the use of cIMT in risk prediction, more evidence for its use in people with diabetes was required.

This thesis aims to explore cIMT and carotid plaque in a large population based cohort of older people with Type 2 diabetes, assessing their relationship with traditional cardiovascular risk factors and prevalent cardiovascular disease, as well as other novel markers of vascular risk; and to assess the ability of both cIMT and

carotid plaque to predict future vascular events in this group. It will also address methodological issues around the measurement of cIMT in an epidemiological study. The introductory chapter provides a review of the current literature regarding cardiovascular disease, Type 2 diabetes and carotid ultrasound; the second chapter describes the specific aims and objectives of this thesis; Chapter 3 describes the methodology used in this thesis; Chapters 4,5 and 6 will describe descriptive, cross sectional and longitudinal analysis results; and Chapter 7 will be a discussion of the results in the context of current literature, with recommendations for further research.

1.1 Cardiovascular disease

1.1.1 Definition & frequency in the UK

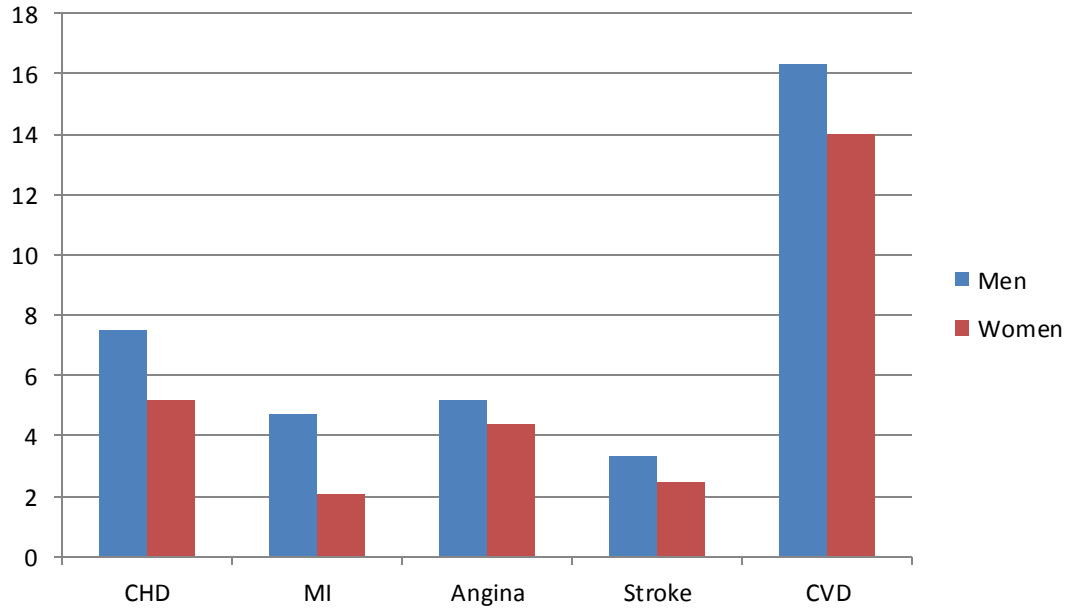
Cardiovascular disease is defined as a disease process which affects the structure and function of the heart, arteries and veins (WHO Regional Office for Europe 2012) . This includes outcomes such as myocardial infarction, angina, stroke, transient ischaemic attack (TIA) and intermittent claudication amongst many others. There are several terms that are used to describe different aspects of CVD, the most common of which are *coronary heart disease, coronary artery disease, cerebrovascular disease* and *peripheral arterial disease.* Whilst the common underlying pathology of these diseases is the process of atherosclerosis, each term describes a distinct anatomical location, reflecting the specific vessels and organs involved. *Coronary heart disease* and *coronary artery disease* commonly describe atherosclerotic disease of the coronary arteries and includes outcome events such as myocardial infarction and angina. *Cerebrovascular disease* refers more specifically to disease affecting the cerebral arteries, and includes stroke and transient ischaemic attacks (TIA) as outcomes, whilst *peripheral arterial disease* refers specifically to diseases of the arteries of the limbs, in particular, the lower limbs. Intermittent claudication is the chief symptom of peripheral vascular disease. The term *cardiovascular disease* is frequently used as an umbrella term to describe these

different groups, and there can be some confusion regarding the specific diseases being referred to.

For the purposes of this thesis, the terms *coronary artery disease* and *cerebrovascular disease* will be adopted to describe myocardial infarction (MI) & angina, and stroke & TIA respectively, while the term *cardiovascular disease* will refer to *coronary artery disease* and *cerebrovascular disease* as a group.

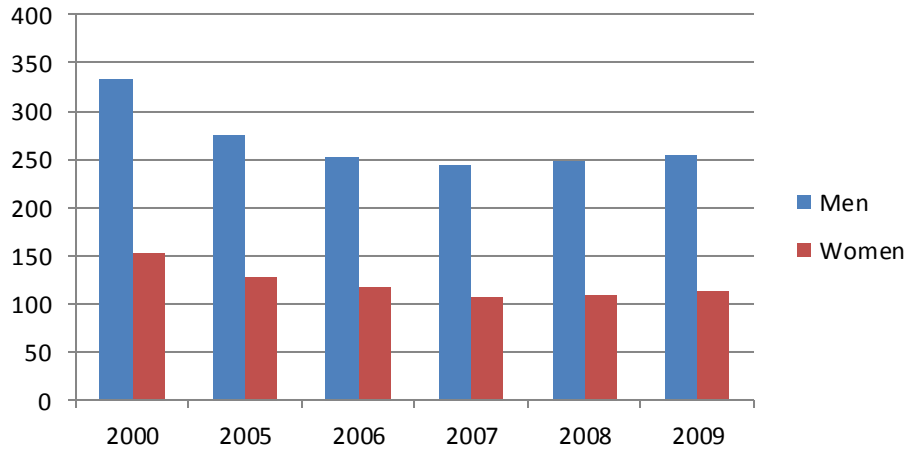
In 2010, the estimated prevalence of CVD in Scotland was 16.3% for men and 14.0% for women (of all ages), as reported in the British Heart Foundation Coronary Heart Disease Statistics 2012 (Townsend N, Wickramasinghe K et al. 2012), using data from the Scottish Health Survey 2010. Figures for men and women in England are reported as 13.6% and 13.0% respectively (using data from the Health Survey England 2006). Figure 1-1 describes the prevalence of CAD, MI, angina, and stroke that were estimated by the Scottish Health Survey 2010.

Figure 1-1 Prevalence of cardiovascular disease in Scotland 2010 (%) (Source: Scottish Health Survey 2010)



Due to general improvements in risk factor management, the incidence of myocardial infarction is reducing in the UK. In Scotland, there was a 25% reduction in the incidence of MI in the period 2000 – 2009 (figure 1-2). However, incidence remains higher in men than in women, although the difference between the sexes lessens with increasing age. In 2009, incidence of myocardial infarction was 255 per 100,000 per year in Scottish men and 113 per 100,000 in Scottish women. There are comparable data for England in the years 2005-2007, which also highlights the ongoing North-South divide in vascular disease, with the incidence of MI 20% higher in Scottish men and 35% higher in Scottish women than their English counterparts (Townsend N, Wickramasinghe K et al. 2012).

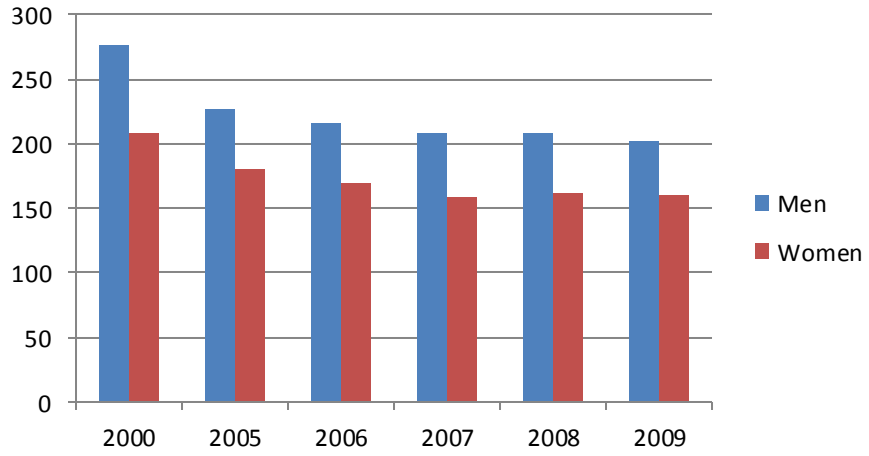
Figure 1-2 Age standardised incidence rate of myocardial infarction (per 100,000/year) in Scotland (*Source: BHF CHD statistics, 2012*)



The British Heart Foundation describes not only the incidence of MI but also angina and stroke (Townsend N, Wickramasinghe K et al. 2012). Stroke incidence has also decreased over the decades and in 2009, incidence in Scottish men was 202 per 100,000 per year and in Scottish women 160 per 100,000 per year (figure 1-3). Again, a comparison to figures from England in the period 2005-2007 highlight the North-South divide, with incidence of stroke in 2007 in Scottish men at 208 per 100,000 per year and in Scottish women 159 per 100,000 per year, whilst in English

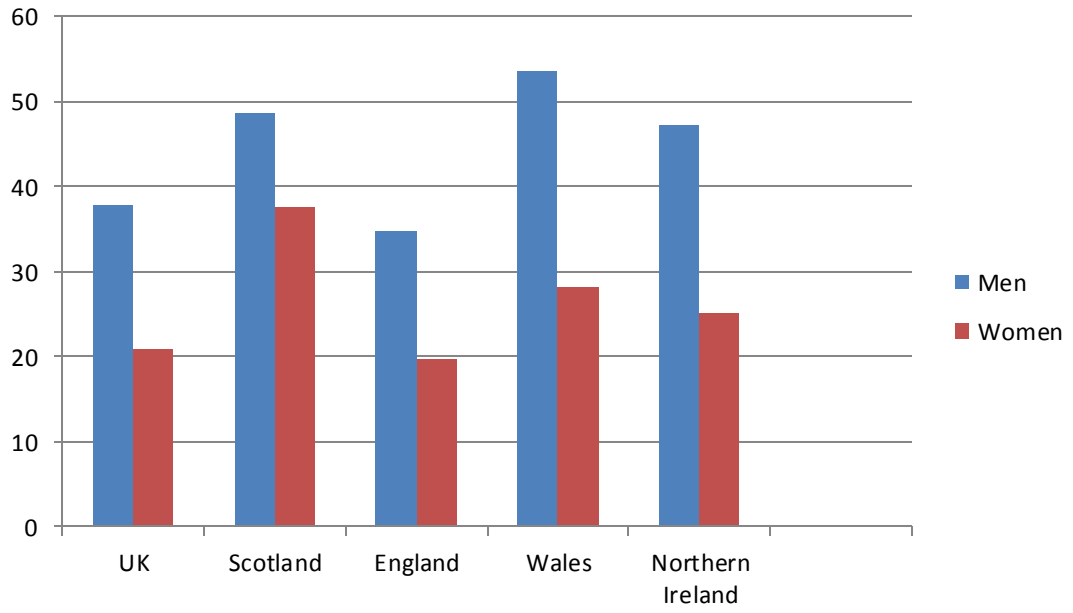
men, incidence was 178 per 100,000 per year and in English women it was 139 per 100,000 per year.

Figure 1-3 Age standardised incidence rate of stroke (per 100,000/year) in Scotland (Source: BHF CHD statistics, 2012)



Age standardised incidence rates for angina in 2009 in Scotland, England, Ireland and Wales are described in figure 1-4. Again, Scotland demonstrates a high incidence of angina in both men and women, with incidence of 48.6 per 100,000 per year in men and 37.7 per 100,000 per year in women, although overall, the highest incidence was noted in Welsh men and Scottish women (Townsend N, Wickramasinghe K et al. 2012).

Figure 1-4 Age standardised incidence of Angina (per 100,000 per year) in 2009 (Source: BHF CHD statistics, 2012)



1.1.2 Pathology & presentation

The global burden of cardiovascular disease worldwide is predominantly that of coronary artery disease and cerebrovascular disease, the underlying pathology of which is usually atherosclerosis.

Atherosclerosis

In most cases, events such as myocardial infarction are preceded by atherosclerotic changes of the vessel wall in which the major cardiovascular risk factors, predominantly smoking, hypertension and dyslipidaemia, play an important role. Atherosclerosis is a progressive disease, and there are many different stages of disease, from early, non-invasive changes of the vessel wall to the occlusive changes seen in advanced, symptomatic atherosclerosis (Lusis AJ 2000). Atherosclerotic changes can be seen in the vessel wall as early as childhood. Larger arteries such as the aorta, show these changes first, followed by the coronary arteries, and by the 4th decade of life, changes can be seen in the cerebral arteries (Lusis AJ 2000). Atherosclerosis commonly occurs at sites where endothelial injury has occurred.

Some of the main sources of endothelial injury are blood pressure associated shear stress, elevated low density lipoprotein (LDL) cholesterol, chemical toxins in cigarette smoke, reduced high density lipoprotein (HDL) cholesterol, insulin resistance and glycosylated end products produced in diabetes (Fuster 1994; Epstein and Ross 1999; Zaman, Helft et al. 2000).

The initial changes seen in artery walls are commonly known as "fatty streaks". These appear as white streaks in the artery wall and are due to the presence of cholesterol containing macrophages – foam cells – in the sub endothelial layer of the vessel wall. The driver behind the presence of foam cells in the artery wall is the deposition of LDL molecules within the endothelium. Endothelial cell morphology at sites of turbulent blood flood makes the endothelium more permeable to molecules such as LDL, leading to a higher propensity to lesion formation. Raised LDL levels also lead to increased uptake of LDL into the endothelium. The LDL molecules can become oxidised and cause direct damage to the surrounding wall tissue, promoting an inflammatory response as well as reducing the production of nitric oxide (a potent vasodilator). The production of pro-inflammatory molecules by the endothelial cells causes monocytes to be activated in response to the inflammation and invade the wall, where macrophage differentiation occurs. The macrophages then ingest the highly modified oxidised LDL molecules, and gradually enlarge, becoming known as foam cells as this process continues (Lusis AJ 2000).

The inflammatory process is further promoted by the death of the foam cells and so the cycle continues, with proliferation and migration of smooth muscle cells from the tunica media to the intima in response to cytokines released from damaged endothelial cells. The smooth muscles cells migrate to the intimal layer and proliferate, and generate excess extra-cellular matrix which produces a fibrous lesion in the artery wall (Lusis AJ 2000). Platelets are also attracted by the ongoing endothelial damage and they too release growth factors which can also result in the migration and proliferation of local smooth muscle cells (Willerson JT, Yao SK et al. 1991; Ross R 1993; Rosenfeld ME and Pestel E 1994). A low HDL cholesterol level

can exacerbate the deposition of LDL cholesterol in the endothelium. The main function of HDL cholesterol is to remove cholesterol from tissues and return it to the liver, where it is excreted in bile. If HDL cholesterol levels are reduced, LDL cholesterol can continue to promote uptake of cholesterol into tissues unopposed.

As the process of atherosclerosis progresses, the early arterial wall changes begin to extend. Further inflammatory changes lead to plaque development and the subsequent changes associated with the plaque can lead to complete occlusion of the artery, with infarction of the tissue supplied by the artery. The altered smooth muscle cell function leads to the formation of a fibrous cap over the lesion and it is at this point that the lesion can protrude into the lumen of the artery. Matrix metalloproteinase production is altered, leading to a remodelling of the artery wall, and compensatory dilatation of the artery, until plaque stenosis reaches 40-50%, at which point dilation can no longer compensate (Glagov S 1994; Godin D, Ivan E et al. 2000; Hall HA and Bassiouny HS 2012). Plaque stability is related to plaque structure. More stable plaques tend to have smaller lipid cores, thicker fibrous caps and fewer inflammatory cells. Those plaques that are prone to rupture and are less stable tend to have large lipid cores, thin fibrous caps, more inflammatory cells (Zaman, Helft et al. 2000; Davies, Rudd et al. 2004).

Angina and myocardial infarction

When an individual with coronary atherosclerosis exercises or is under emotional stress, the oxygen demand of the myocardium increases but the narrowed vascular lumen restricts the volume of oxygenated blood that can be carried to the tissues, resulting in ischaemia in the myocardial tissue (Fuchs RM and Becker LC 1982). As the individual rests, oxygen demand reduces and the available supply of oxygenated blood is again adequate and so chest pain resolves. The tissue is not usually damaged and can return to normal function. The manifests clinically as angina - the classical symptoms of which are exertional chest pain or chest tightness and in some cases, autonomic symptoms associated with the chest discomfort, such as nausea and light headedness. Recovery can be aided by the use of vasodilatory

drugs such as nitrates and in the case of recurrent severe episodes, coronary artery stenting or bypass grafting can be implemented to return blood flow to near normal. This predictable pattern of angina is typically referred to as stable angina. However, in some cases, chest pain can occur at rest or at a lesser level of exertion than usual. Angina symptoms may also worsen over a short period of time. This is known as unstable angina and is a higher risk entity than stable angina. It is usually the effect of an unstable plaque rupturing and further restricting the lumen. If left untreated, it is more likely to lead to myocardial infarction. Unstable angina can be identified as one sub classification of the acute coronary syndrome (Newby DE, Grubb NR et al. 2014) .

As an atherosclerotic plaque evolves, plaque rupture and clot formation are common. If the contents of the plaque are dispersed or a portion of thrombus becomes separated, embolisation to the coronary arteries can occur. If the embolus is of sufficient diameter, there can be complete occlusion of the coronary artery. The supply of oxygenated blood to the myocardium is completely restricted and this results in infarction of the tissue (Newby DE, Grubb NR et al. 2014). The infarcted tissue becomes necrotic and will release its contents e.g. cardiac enzymes such as troponin and creatinine kinase (Thygesen, Alpert et al. 2012). This damage to the tissue is irreversible and can be fatal if a large portion of the myocardium becomes dysfunctional. Thrombolysis or primary percutaneous interventions offer the best chance of recovery as they can restore blood flow to near normal (Newby DE, Grubb NR et al. 2014). Typical symptoms include a severe and intractable central crushing chest pain, often with radiation to the left arm or jaw. The pain does not resolve with rest and may be accompanied by a sense of impending death, nausea and vomiting.

TIA and stroke

Transient ischaemic attacks are characterized by temporary weakness or other neurological symptoms. They are commonly the result of small emboli from plaque in the carotid arteries or thrombus from the heart (as a result of atrial fibrillation) being carried to the distant cerebral circulation. In some cases, an almost complete

occlusion of the carotid arteries by plaque can cause such narrowing that the blood flow to the cerebral tissue is markedly reduced. There is usually spontaneous resolution of the interruption to blood flow and so there is usually only ischaemia of the cerebral tissue and not complete infarction. As a result, the neurological dysfunction normally resolves spontaneously. However, risk factor management will be necessary following an event to prevent further episodes (Langhorne P 2014).

Like TIAs, the pathology underlying a stroke is usually embolism of a thrombus from the heart or carotid arteries, or in some cases, massive haemorrhage from a cerebral aneurysm or other vascular abnormality. Cerebral blood flow is usually completely obscured and cerebral tissue will be damaged irreversibly after 3 hours of oxygen deprivation. As such, in the case of ischaemic stroke only (thrombolysis is contraindicated in haemorrhagic stroke), thrombolysis is not given after 4.5 hours as it cannot reverse any damage that has occurred (Langhorne P 2014).

1.1.3 Diagnosis

Angina

During periods of chest pain, angina can be diagnosed on a 12 lead ECG as ST segment depression in any lead. Exertional chest pain can be elicited during exercise stress testing. Patients undergo ECG whilst performing progressive exercise (walking) on a treadmill and the appearance of ischaemic changes (ST segment depression) during exercise can be diagnostic of angina. This test can also be used to demonstrate the severity of disease in people with known angina. If stress testing is not conclusive, more invasive diagnostic testing may be required (Newby DE, Grubb NR et al. 2014). Coronary artery angiography can reveal any narrowing of the artery lumen and can be used to diagnose the site and extent of obstruction, allowing stenting to be undertaken (Newby DE, Grubb NR et al. 2014).

Myocardial infarction

Myocardial infarction usually presents with a history of acute, unremitting and unresolving central, crushing chest pain that may or may not radiate to the left arm

and/or jaw. There may also be complete circulatory collapse associated with cardiac arrest (Newby DE, Grubb NR et al. 2014). The ECG during myocardial infarction typically displays ST segment elevation, representing infarction of the myocardial tissue. Current diagnostic standards include the measurement of troponin-I or troponin-T - subunits of the cardiac enzyme troponin. Any elevation of troponin (according to individual lab standards) indicates damage to the myocardium and can be diagnostic of an MI (SIGN 93 2013). Angiography may be necessary to identify occlusions and is usually undertaken as part of the primary treatment of myocardial infarction. Primary percutaneous intervention is used as a first line treatment in many health boards, including NHS Lothian. Angiography will demonstrate a complete occlusion of one or more of the coronary arteries. Angioplasty and/or stenting may be carried out depending on the severity of disease (Newby DE, Grubb NR et al. 2014).

In epidemiological studies and clinical trials, systematic coding of 12 lead ECGs, such as Minnesota coding (Prineas RJ, Crowe RS et al. 1982) are often used to identify changes specific to angina and MI. In addition, tools such as the Rose chest pain questionnaire can be used to elicit further information regarding coronary artery disease (Rose G, McCartney P et al. 1977).

TIA

Transient ischaemic attacks usually present with a history of short lived, self-limiting rapid onset neurological deficits, which can result in temporary numbness, weakness or paralysis of the limbs or face. In addition, if the retinal vessels, optic radiation or visual cortex are affected, there may be a temporary visual defect. Following a diagnosis of suspected TIA, a brain CT can rule out any cerebral abnormalities and carotid ultrasound is performed looking for evidence of carotid stenosis or plaque (NICE CG68 2008). However, CT is often normal and carotid ultrasound tends to provide more useful information.

Stroke

Like a transient ischaemic attack, acute stroke presents with rapid onset of numbness, weakness or paralysis of the limbs and/or face. Depending on the location of the cerebral ischaemia, there may be paralysis of an entire side of the body or it may be limited just to the face. Symptoms tend not to resolve spontaneously as there is infarction of the cerebral tissue, and as a result, the neurological deficit may be long term. CT imaging of the brain is important for management of an evolving stroke. Ischaemic and haemorrhagic strokes have differing appearances on CT (NICE CG68 2008)

1.1.4 Traditional cardiovascular risk factors

As well as the accepted non-modifiable risk factors of age, gender and ethnicity, the major modifiable environmental cardiovascular risk factors for CVD include hypertension, dyslipidaemia, obesity, smoking, and physical inactivity, in addition to diabetes.

Age, gender & ethnicity

Age is one of the major non-modifiable risk factors for cardiovascular disease with rate of disease increasing steadily across increasing age groups in both men and women (Castelli WP 1984; Tunstall-Pedoe H, Kuulasmaa K et al. 1994; Rich-Edwards JW, Manson JAE et al. 1995). The additive effect of vascular stress and other cardiovascular risk factors on the vessel wall is thought to contribute to the effect of age on vascular risk. Men are more likely to develop coronary artery disease than women (Jousilahti P, Vartiainen E et al. 1999), although once women reach the menopause, the difference between the sexes attenuates (Pappa T and Alevizaki M 2012). Several theories have been postulated for this, including that either oestrogen is a protective factor for CAD, or that testosterone increases the risk for CAD (Pappa T and Alevizaki M 2012), however, there is still no clear answer to this question. Those from certain ethnic backgrounds are also known to be at higher risk of CVD than those from other backgrounds, including an increased prevalence of CAD in people of Indian and Pakistani backgrounds (6% and 8% respectively) compared to

other people living in the UK, who were not born in the UK (Townsend N, Wickramasinghe K et al. 2012). In the UK, both men and women of South Asian origin had a higher risk of CAD and stroke than European people (Chaturvedi 2003) and people of Afro-Caribbean origin had a reduced risk of CAD but an increased risk of stroke when compared to European residents. This may be due in part to higher levels of glucose intolerance, central obesity, fasting triglycerides and insulin levels in South Asians when compared with Europeans (McKeigue PM, Shah B et al. 1991). This is also seen in people of Afro Caribbean descent; however, their levels of LDL cholesterol and triglycerides are lower than that of Europeans. Black Caribbean, South Asian, Pakistani and Bangladeshi men have a higher prevalence of diabetes (Townsend N, Wickramasinghe K et al. 2012), supporting the theory that ethnic differences may be influenced by differences in insulin resistance and endothelial dysfunction (Chaturvedi 2003).

Hypertension, dyslipidaemia and smoking

The three most "traditional" cardiovascular risk factors are hypertension, smoking and dyslipidaemia. Hypertension is prevalent throughout the UK. The Scottish Health Survey (2010) revealed that 35% of men and 30% of women in Scotland had hypertension (defined as systolic blood pressure >140mmHg or diastolic blood pressure >90mmHg), comparable with rates in England (Townsend N, Wickramasinghe K et al. 2012). On a meta-analysis of data from over 1 million subjects, the Prospective Studies Collaboration in 2002 revealed that each 20mmHg rise in systolic blood pressure (equivalent to 10mmHg increase in diastolic blood pressure) CAD risk doubles for adults aged 40-69 years (Prospective Studies Collaboration 2002). Essential hypertension is a complex condition and the pathophysiology is not fully understood. Exposure of the vessel wall to raised arterial pressure promotes the formation of atheromatous lesions which in turn increase the risk of vascular events such as MI or stroke. Therefore, the aim of management is to reduce blood pressure. NICE guidelines recommend a target blood pressure of 140/90mm (NICE CG127 2011) while in those with Type 2 diabetes,

target blood pressure is tighter, with a target systolic pressure of <130 and target diastolic pressure of <80 (SIGN 116 2010).

Dyslipidaemia is a term that is usually used when referring to hyperlipidaemia (raised total cholesterol, LDL cholesterol or triglycerides), with the exception of HDL cholesterol which is abnormal when reduced. There are several factors which are known to disrupt lipid levels. Cholesterol production occurs primarily in the liver, but dietary intake and lifestyle factors can also contribute. Increased consumption of lipids such as saturated fats, and reduced levels of physical activity and genetic factors can lead to raised plasma lipid levels. Additionally, insulin resistance has also been linked with altered blood lipid levels, including raised triglyceride levels and low HDL levels (Garg A 1996). In terms of the association between dyslipidaemia and cardiovascular disease risk, the Prospective Studies Collaboration identified an independent positive association between total cholesterol and ischaemic heart disease, but not stroke (Prospective Studies Collaboration 2007). This relationship persisted at all levels of blood pressure. There is also a strong body of evidence that statin use can reduce the occurrence of vascular outcomes (Taylor F, Huffman MD et al. 2013), supporting a role of dyslipidaemia in the development of cardiovascular disease.

The association between smoking and cardiovascular disease is well described. The effect of smoking on the endothelium encourages the development of atherosclerotic plaque. A 50 year study of the relationship between smoking and cause specific mortality (commenced in 1951 following case control studies that highlighted a possible link between cigarette smoking and lung cancer) highlighted the increased mortality rate for ischaemic heart disease associated with smoking (Doll, Peto et al. 2004). Men who continued to smoke had an age standardized mortality rate of 10.01 per 1000men/year for IHD, compared with 6.19 per 1000 men/year for lifelong non-smokers, whilst former smokers were in between with a mortality rate of 7.61 per 1000 men per year. A similar trend was seen for current smokers versus never smokers for cerebrovascular disease (4.32vs2.75 per 1000men/year respectively).

An increase in mortality rate was also seen for both IHD and CVD as the number of cigarettes smoked increased (Doll, Peto et al. 2004). The prevalence of cigarette smoking in Great Britain has decreased substantially over the decades. From 1972-1994, prevalence in men decreased from 52% to 28%, and in women, from 41% to 26%, and indeed has continued to fall, albeit at a slower rate. More recent figures from 2010 estimate the prevalence of cigarette smoking in the UK to be 21% for men and 20% for women (Townsend N, Wickramasinghe K et al. 2012).

Lifestyle (diet and activity) and obesity

Lifestyle factors also play an important role in the development of cardiovascular disease, including diet and physical activity. A diet high in saturated fat and salt, and low in fibre, fruit and vegetables can lead to hypercholesterolaemia, hypertension and a greater risk of cardiovascular disease (Townsend N, Wickramasinghe K et al. 2012). Excessive calorie intake leads to obesity, whereas expending excess calories through physical exercise prevents the development of obesity and overweight. The first study which demonstrated a link between level of activity and cardiovascular risk, published over 5 decades ago by Morris et al, demonstrated that those people with more physically demanding jobs such as bus conductors or postmen, had a lower rates of cardiovascular disease (Morris, Heady et al. 1953). Initial limitations of the study, such as confounding by obesity and other vascular risk factors, were later accounted for and Morris et al were able to demonstrate the cardioprotective nature of physical activity (Morris, Chave et al. 1973). More recently, Myers et al demonstrated that exercise capacity was predictive of all-cause mortality (Myers, Kaykha et al. 2004).

Obesity is an independent risk factor for cardiovascular disease (Poirier and Eckel 2002; Poirier, Giles et al. 2006), as well as being a major factor in the development of Type 2 diabetes and other vascular risk factors such as hypercholesterolaemia and hypertension (Townsend N, Wickramasinghe K et al. 2012). Mean body mass index (BMI) has increased over the past 30-40 years, a combination of both reduced physical activity and change in diet (Butland, Jebb et al. 2007). In the 1960s and

early 1970s, both men and women largely had a BMI within the 'normal range' of 20-25 but by the early 1990s, the mean BMI was above 25 and has continued to increase, to such an extent that by the 2050, it is estimated that 60% of UK men and 50% of UK women will be obese (Butland, Jebb et al. 2007). Childhood obesity is also a growing problem, bringing with it the increased burden of diabetes and its associated risks. Prevalence of both overweight and obese children has increased in English children from 1974 to 2003 (Stamatakis, Primatesta et al. 2005).

1.1.5 Cardiovascular risk management & assessment

Primary and secondary prevention

Despite the many manifestations of cardiovascular disease, the overall management of these conditions has a common theme. The primary aim is to reduce exposure to cardiovascular risk factors and prevent events. Blood lipids, blood pressure and smoking are the major risk factors to target and these can be tackled with a combination of lifestyle adaptation and medication. Primary prevention is the management of cardiovascular risk factors in someone with no prior history of cardiovascular disease (SIGN 97 2007). The aim of primary prevention is to educate patients with regards to diet, lifestyle and potential medical intervention, in order to stop them having a first event. In Scotland, SIGN Guideline 97 provides information and advice for clinicians initiating primary prevention (SIGN 97 2007). Control of blood pressure, blood lipids, platelet function and lifestyle factors such as smoking cessation are the main stay of primary prevention of CAD. Statins are the mainstay of the management of elevated plasma cholesterol, and anti-hypertensive agents such as beta blockers and ACE-inhibitors among many others are used to reduce blood pressure. Lifestyle interventions to reduce smoking are available, including the use of nicotine replacement therapy and newer pharmacological agents such as bupropione. Aspirin had also been used as an anti-platelet therapy although the usefulness of this in primary prevention has been called into question recently following publication of data regarding the risk/benefit of aspirin (Antithrombotic Trialists' (ATT) Collaboration, Baigent C et al. 2009; Fowkes FR, Price JF et al. 2010). Another important aspect of primary prevention is the assessment of

cardiovascular risk with a view to optimising cardiovascular risk factor management. Risk scores, family history and current medical history are taken into account when assessing an individual's risk of having a cardiovascular event. Other methods of risk evaluation involving direct measurement of disease, for example coronary angiography and coronary artery calcium (CAC) determined by CT involve higher doses of radiation than for example ultrasound, and in the case of angiography is more invasive, and therefore may not be suitable investigations for risk prediction in general healthcare settings. Interest has therefore increased in the use non-invasive measures of atherosclerosis, including the carotid intima media thickness.

Secondary prevention is the management of cardiovascular risk factors, initiated after someone has a vascular event, with the aim of preventing a further event occurring. Additional medication may be added on top of current treatments and the importance of lifestyle modification is reinforced (SIGN 93 2013).

Cardiovascular risk assessment scores

Primary prevention describes managing cardiovascular risk factors before any cardiovascular events occur. Therefore, information regarding an individual's future risk of events must be taken into consideration when considering how aggressive a strategy should be implemented. This is particularly desirable in a general population because included in that population are individuals who are at low risk, intermediate risk and high risk of vascular disease. Subsequently, not all members require active risk factor management, whereas some require rather more intensive risk factor management. In addition to guiding the correct management of individual patients, risk prediction also allows health systems to target expensive treatments to those who will gain the most benefit.

Because the symptoms of cardiovascular disease usually only manifest once disease is significantly advanced, there is a need for cardiovascular risk prediction tools to use early markers of disease risk that are easily measurable, for example hypertension or dyslipidaemia. Well established methods for predicting vascular risk

include the combination of known cardiovascular risk factors into risk prediction models or algorithms. A variety of such risk scores have been developed and proposed for use in the general population, including the Framingham Risk Score (Anderson KM et al., 1991), SCORE (Conroy RM et al., 2003), ASSIGN (Woodward M et al., 2007) and QRISK2 (Hippisley-Cox J et al., 2007, Collins GS and Altman DG, 2010), among others. These algorithms are based upon known cardiovascular risk factors, including age, sex, smoking status, diabetes status, total & HDL cholesterol and systolic blood pressure, and differ from each other primarily in the exact risk factors included in the model. Although their non-invasive nature means they are often used in clinical practice, cardiovascular risk algorithms have been shown to be inaccurate in low risk populations and high risk populations, such as those people with diabetes (Brindle P et al., 2006). Until recently, in the United Kingdom, the National Institute for Health and Clinical Excellence (NICE) recommended the use of the Framingham risk equation when evaluating individual patient risk in the general population, and following an update to their guidance did not recommend any particular score and left the selection of the appropriate tool to the clinician (Collins GS and Altman DG 2010). However, the most recent NICE guideline for managing cardiovascular disease recommends the use of the QRISK2 risk score when evaluating cardiovascular risk (NICE CG 181 2014) .

Many of the traditional risk factors for cardiovascular disease discussed previously, as well as history of cardiovascular disease and diabetes are common features of many risk prediction algorithms. As an example to illustrate the differences in choice of included predictors, listed in table 1-1 are the key factors considered in the four well known and commonly used risk scores: Framingham, QRISK2, ASSIGN and SCORE (Anderson KM, Odell PM et al. 1991; Conroy RM, Pyörälä K et al. 2003; Hippisley-Cox J, Coupland C et al. 2007; Woodward M, Brindle P et al. 2007; Collins GS and Altman DG 2010).

Table 1-1 Example of predictor variables included in cardiovascular risk scores developed and used in the general population

OUTCOME	Framingham CHD	QRISK2	ASSIGN	SCORE
Age	✓	✓	✓	✓
Sex	✓	✓	✓	✓
Diabetes	✓	✓	✓	
Smoking	✓	✓		✓
Number of cigarettes			✓	
Systolic Blood Pressure	✓	✓	✓	✓
Total Cholesterol	✓		✓	✓
HDL Cholesterol	✓		✓	
Cholesterol/HDL Ratio		✓		
BMI	✓	✓		
Postcode		✓		
Scottish Postcode			✓	
Previous CVD		✓		
History of angina/MI		✓		
CKD		✓		
AF		✓		
Blood pressure treatment		✓		
RA		✓		
Family history of CHD/Stroke			✓	

HDL=high density lipoprotein, BMI=body mass index, CVD=cardiovascular disease, MI=myocardial infarction, CKD=chronic kidney disease, AF=atrial fibrillation, RA=rheumatoid arthritis, CHD=coronary heart disease

Alternative methods of risk prediction

Although cardiovascular risk scores are effective in clinical practice, they do not capture the full extent of an individual's risk, especially in lower or higher risk groups (Brindle P, Beswick A et al. 2006). Therefore, researchers have turned to other methods to inform and improve current methods. The most prominent of these are non-invasive markers of cardiovascular risk. Included in this category are physical markers such as carotid intima media thickness, carotid plaque, coronary artery calcium score and ankle brachial index (ABI). Other potential additions to existing risk scores being considered include biochemical markers such as ABI, eGFR, albuminuria, IL-6, CRP, NTproBNP (Ridker PM, Rifai N et al. 2002; Weir MR 2007; Fowkes FG, Murray GD et al. 2008; Lee JK, Bettencourt R et al. 2012; Donfrancesco C, Palleschi S et al. 2013). cIMT and plaque are easily assessed using carotid B mode ultrasound, and ankle brachial index can be measured using a hand held Doppler. Coronary artery calcium score can be assessed using thoracic x-ray or

thoracic computed tomography (CT). However, compared with other non-invasive markers, this carries a slightly greater risk to individuals due to the use of radiation.

Because the main focus of this thesis is cIMT and carotid plaque, they will be discussed in more detail in section 1.3 of this chapter. Novel biomarkers are discussed in the following section.

1.1.6 Novel cardiovascular biomarkers

Several other potential markers of vascular risk are being considered for use in vascular risk prediction. These include estimated glomerular filtration rate (eGFR), microalbuminuria, interleukin-6 (IL-6), C-reactive protein (CRP) and N-terminal pro-brain natriuretic peptide (NTProBNP). Ultrasound markers such as cIMT and carotid plaque will be discussed in detail in section 1.3 and 1.4 of this chapter.

Ankle brachial index

The ankle brachial index is a measure of peripheral arterial disease. It is the ratio of the systolic pressure in the ankle to the systolic pressure in the arm. As well as being an indicator of peripheral arterial disease, there is evidence that it is a marker of generalized atherosclerosis. There is evidence of a relationship between ABI and vascular risk factors and prevalent vascular disease, as well as a prospective relationship between ABI and future vascular risk. Newman et al found that in the older adult participants of the Cardiovascular Health Study (CHS) there was an in inverse relationship between ABI and vascular risk factors, with those with a lower ABI having higher levels of vascular risk factors (Newman, Siscovick et al. 1993). There was a similar pattern seen between ABI and prevalent subclinical and clinical CVD. Thus, those with a lower ABI had a greater cardiovascular risk, even those with only a modest reduction in ABI. More recent data from the Edinburgh Artery Study demonstrated that ABI was predictive of cardiovascular events and mortality, independently of conventional cardiovascular risk factors (Leng, Fowkes et al. 1996; Wild, Byrne et al. 2006). These findings have been replicated by other European studies (Ögren, Hedblad et al. 1993; Kornitzer, Dramaix et al. 1995; Hooi, Kester et

al. 2004; van der Meer, Bots et al. 2004). More recently, a large meta-analysis by Fowkes et al was published and included data from over 48000 individuals. It demonstrated that ABI may provide additional information over and above the Framingham risk score in prediction of cardiovascular events ((Fowkes FG, Murray GD et al. 2008). Ankle brachial index has also been found to be similar to cIMT in terms of ability to predict future vascular risk and in fact combination of the two measures improve prediction (Price JF, Tzoulaki I et al. 2007).

eGFR and microalbuminuria

It has been established that individuals with chronic kidney disease (CKD) are more likely to die than develop renal failure, especially from cardiovascular disease (Keith DS, Nichols GA et al. 2004) and several recent studies also point to the estimated glomerular filtration rate (eGFR) as a marker of cardiovascular risk. One study by Donfrancesco et al suggests that even a modest reduction of eGFR in a low risk general population can significantly increase the risk of incident cardiovascular disease and all-cause mortality (Donfrancesco C, Palleschi S et al. 2013). A recent meta-analysis of 1 234 182 individuals from 21 different studies identified eGFR as being an independent predictor of all cause and cardiovascular mortality, with increasing hazard ratios as eGFR falls below 75mg/ml/1.73m^2 (Chronic Kidney Disease Consortium 2010).

Microalbuminuria, the excretion of albumin into the urine, has also been increasingly linked with cardiovascular disease. Early work by Keen et al first described microalbuminuria in diabetic patients in 1969 (Keen H, Chlouverakis C et al. 1969). It was first linked to essential hypertension in 1974 by Parving et al, and in 1984, Mogensen et al published data demonstrating that microalbuminuria could predict early mortality in maturity onset diabetes (Parving H-H, Mogensen CE et al. 1974; Mogensen CE 1984). A 2007 review highlighted that there is evidence that microalbuminuria can risk prediction for CVD over and above traditional vascular risk factors (Weir MR 2007). The Chronic Kidney Disease Consortium (2010) also

identified microalbuminuria as an independent predictor of both all cause and cardiovascular mortality (Chronic Kidney Disease Consortium 2010).

Interleukin-6

Interleukin-6 is a pro-inflammatory cytokine secreted by T cells and macrophages in response to infection and trauma. Its potential as a marker of vascular risk has been discussed in recent studies. Several published studies have described the relationship between circulating levels of IL-6 and risk of cardiovascular disease (Ridker PM, Rifai N et al. 2000; Danesh J, Kaptoge S et al. 2008; Sattar, Murray et al. 2009). The results of the Rancho Bernardo Study, published in 2012, highlight a strong association between circulating IL-6 and all-cause, CVD, cancer and liver related mortality (Lee JK, Bettencourt R et al. 2012). They demonstrated a CVD risk factor adjusted HR of 1.38 (95% CI, 1.16–1.65) for CVD mortality. Adjustment for CRP levels attenuated the effects somewhat but the association between IL-6 and CVD mortality remained statistically significant. A study of 121 Japanese participants identified that IL-6 was a strong independent predictor of future cardiovascular events (HR 2.80 (1.34–5.83) for highest tertile of IL-6) (Nishida H, Horio T et al. 2011).

C reactive protein

CRP is a general marker of inflammation. Measurement of high sensitivity CRP (hsCRP) has been incorporated into several guidelines for the assessment and management of those suspected of having CVD. The relationship between hsCRP and CVD in men and women has been assessed in several studies (Kuller LH, Tracy RP et al. 1996; Ridker PM, Cushman M et al. 1997). Its use over and above traditional risk factors has also been assessed (Ridker PM, Rifai N et al. 2002). However, more recent evidence suggests that the role of CRP in the prediction of cardiovascular risk may not be clear cut. In 2006, Sattar and Lowe highlight that while early studies may have demonstrated that hs-CRP was predictive of vascular events over traditional risk factors, more recent studies show a far less strong association (Sattar and Lowe 2006). This, along with the short term variability in

CRP as well as the remaining questions regarding the causal role CRP plays lead them to conclude that the current focus should remain on traditional risk factors.

NTproBNP

Several studies have established the potential for NTproBNP (the pro-hormone fragment of brain-type natriuretic peptide secreted by the ventricular myocardium during ventricular stretch (Heart Protection Study Collaborative Group 2007) in the prediction of cardiovascular events. A large study of 3199 individuals in the Heart Outcomes Prevention Evaluation (HOPE) Study (a secondary prevention population) assessed the incremental predictive value of a range of plasma biomarkers and found that while various inflammatory markers were related to future cardiovascular risk, the model containing NTproBNP as well as traditional cardiovascular risk factors was more predictive of future vascular events (HR1.72 per 1-SD change in NTproBNP) (Blankenberg, McQueen et al. 2006). In a further study of 20536 individuals in the MRC/BHF heart protection study, high NTproBNP levels were highly predictive of major cardiovascular events including MI, stroke and revascularisation (adjusted RR 2.26), coronary events (RR 3.09) and stroke (RR 1.80) (Heart Protection Study Collaborative Group 2007).

1.2 Type 2 diabetes and vascular disease

Type 2 diabetes is a chronic condition characterised by hyperglycaemia, insulin resistance and relative insulin deficiency. It occurs primarily in individuals aged 40 years and above, in particular those who are overweight and obese. As insulin resistance progresses, insulin production by the endocrine pancreas increases, to counter the effect. Continual over-production of insulin leads to a failure of the pancreatic islet cells and plasma glucose levels begin to rise (Pearson ER and McCrimmon RJ 2014). This is the stage at which Type 2 diabetes becomes clinically apparent, or detectable by laboratory tests, as blood glucose levels are no longer maintained in the normal range. This can also be referred to as a relative insulin

deficiency as the balance between available insulin and the effect of insulin resistance is disrupted. A clinical diagnosis of Type 2 diabetes most often occurs after years of subclinical hyperglycaemia and consequently, at the point of diagnosis, the long term damage to end organs has already been initiated. Long term complications include diabetic nephropathy, neuropathy, retinopathy (microvascular complications) and an increased risk of cardiovascular disease (macrovascular disease), and due to the long subclinical phase of Type 2 diabetes, these complications may be somewhat advanced at the point of diagnosis.

1.2.1 Pathology of Type 2 diabetes

Type 1 and Type 2 diabetes demonstrate differing pathologies. Type 1 diabetes is thought to result from autoimmune destruction of beta cells, which once reaching a critical mass loss, results in a symptomatic lack of endogenous insulin production, requiring exogenous insulin treatment (Atkinson, Eisenbarth et al. 2014). Previously, it was believed that the beta cell loss in Type 1 diabetes was complete but more recent work has demonstrated persistent residual C peptide secretion, suggesting that in some individuals, there are remaining beta cells that may retain some function (Keenan, Sun et al. 2010). In contrast, Type 2 diabetes displays a spectrum of insulin resistance and relative insulin deficiency. In the early stages of disease, the pancreas continues to produce insulin, and as such, the initial management can range from simple diet and lifestyle modification, to a variety of pharmacological interventions, including metformin, sulphonylureas and insulin, as well as non-pharmacological management. However, once the disease has progressed to levels of insulin resistance that outweigh the physiological effect of the available endogenous insulin, despite lifestyle modification and oral hypoglycaemic agents, exogenous insulin therapy and insulin sensitising medications are often the only remaining option.

Insulin resistance

Insulin resistance is defined as the resistance of target tissues to the action of insulin. As food is digested and carbohydrates are broken down into smaller sugar molecules,

islet cells in the pancreas are stimulated to release insulin in response to both the elevated plasma glucose and the effect of gut hormones. In normal glucose tolerant people, insulin then acts upon tissues such through insulin receptors, activating a complex signalling cascade which promotes the transport of the glucose receptor GLUT4 to the cell surface, which in turn allows the uptake of glucose into the cells via these receptors (Pearson ER and McCrimmon RJ 2014). This reduces the plasma glucose concentration and as it continues to fall, insulin production in the pancreas also falls. This, along with regulation of hepatic glucose production, maintains plasma blood glucose at normal levels (Pearson ER and McCrimmon RJ 2014). In people with Type 2 diabetes, the same level of insulin is unable to exert the same effect on glucose levels and so the pancreas increases insulin production to compensate. The pathology underlying insulin resistance is complex. It is currently thought that there may be a genetic basis to insulin resistance and there is strong evidence in favour of this, with a 58% concordance of development of Type 2 diabetes in twins (Newman B, Selby JV et al. 1987).

Insulin resistance itself is related to a number of cardiovascular risk factors, including hypertension, central obesity and dyslipidaemia. This constellation of conditions is often referred to as the Metabolic Syndrome (Pearson ER and McCrimmon RJ 2014). Visceral obesity in particular seems to be associated with insulin resistance. Intra-abdominal adipose tissue is metabolically active and produces cytokines such as TNF-α and IL-6, which disrupt the action of insulin in adipose tissue. Other conditions in which insulin resistance is affected include infection, in which insulin resistance is also mediated by TNF-α. Visceral adiposity is also associated with the development of non-alcoholic fatty liver disease (NAFLD) and leads to the production of excess free fatty acids which increase hepatic glucose production and can lead to insulin resistance.

Insulin deficiency and pancreatic beta cell dysfunction

The second component thought to underpin the pathology of Type 2 diabetes is a failure of the pancreatic beta cells in the islets of the pancreas. As has been

previously discussed, the pancreatic pathology in Type 2 diabetes is not the same as that of Type 1 Diabetes, and whilst there is a failing of the pancreatic beta cells, there is rarely such a complete failure and most people with Type 2 diabetes continue to produce insulin in some quantity. The resulting effect is a *relative deficiency* of insulin, as resistance to insulin causes a requirement for the pancreas to overproduce insulin to compensate for the increased demands to produce the same effect as in normal glucose tolerant individuals. However, many people with insulin resistance can continue to produce enough insulin to counteract the resistance without developing diabetes (Weyer C, Bogardus C et al. 1999) . An example of this which is often used is that of an obese person with insulin resistance who does not go on to develop diabetes (Gerlich JE 1999). In the broadest terms, those who do go on to develop diabetes, the ability to produce insulin in response to resistance decreases with time due to a progressive loss of beta cell function. At this point, insulin resistance and demand exceeds production, which results in hyperglycaemia and the development of diabetes (Buchanan TA 2001).

The pathology underlying pancreatic beta cell insufficiency is not well understood. Several hypotheses have been put forward, including the effects of glucose toxicity and lipotoxicity on reduced beta cell mass. One theory suggests that there is a failure of receptors in the pancreas that respond to rising glucose levels and so pancreatic beta cell mass does not increase in response (Kasuga M 2006). However, there is believed to be more than just loss of beta cell mass. The role of beta cell dysfunction in Type 2 diabetes has recently been reviewed by Ferrannini and Mari (Ferrannini and Mari 2014). They highlight that in Type 2 diabetes, insulin resistance is associated with several beta cell function abnormalities. Firstly, they suggest that there is increased fasting insulin secretion and total stimulated insulin output (as a result of hyperglycaemia) and that with time, there is a reduction in the total insulin output. Further to this, reduction in glucose sensitivity of the beta cell is thought to be a *key* part of beta cell defect, and in addition, they highlight that a reduction in the incretin effect (increased insulin production in response to oral glucose loading) is another key facet of the pathology of Type 2 diabetes. Other key deficits include a

reduction in the ability of the beta cells to respond rapidly to changes in glucose levels (rate sensitivity). Ferrannini describe these changes as *"predominantly functional and potentially reversible"*,

Resulting hyperglycaemia

Hyperglycaemia is the resultant end product of insulin resistance and insulin deficiency. As insulin resistance worsens and eventually, pancreatic insulin production reduces, blood glucose levels start to rise. The effect of hyperglycaemia on body physiology is wide and varied and is responsible for many symptoms of diabetes, including polydipsia & polyuria, visual problems, fatigue, weight loss; poor wound healing or recurrent infections, dry mouth & skin; tingling in feet or heels, erectile dysfunction, cardiac arrhythmia, stupor, coma and seizures. The classical triad of polydipsia, polyuria and polyphagia are usually the key symptoms of hyperglycaemia and are a common presentation of Type 1 diabetes, along with weight loss. While acidosis is common at presentation in Type 1 (diabetic ketoacidosis), it is becoming increasingly so for those with Type 2 diabetes (hyper-osmolar non-ketotic acidosis (HONK)). Ketosis is usually absent in Type 2 diabetes as there is still some residual insulin production, which prevents lipolysis from occurring, leading to production of ketones (Pearson ER and McCrimmon RJ 2014).

1.2.2 Risk factors and frequency of Type 2 diabetes in the UK

There is no single identified trigger point for the development of Type 2 diabetes. Most commonly, there is a complex multifactorial pathway that leads to an eventual diagnosis. A variety of lifestyle factors can precede the development of diabetes (table 1-2). These risk factors are very similar to those of cardiovascular disease and the metabolic syndrome, a cluster of risk factors that put an individual at increased risk of cardiovascular disease, diabetes and liver disease.

Table 1-2 Risk Factors for Type 2 Diabetes

Age > 45 years
Obesity (especially central obesity)
Impaired glucose tolerance
Previous gestational diabetes
Family history of diabetes
Given birth to a baby weighing more than 9 pounds
HDL cholesterol < 35mg/Dl
Plasma triglycerides > 250 mg/Dl
High blood pressure ≥140/90 mmHg
Low activity level (exercising less than 3 times a week)
Metabolic syndrome
Polycystic ovarian syndrome
Ethnicity

In October 2011, the Quality and Outcomes Framework (QOF) provided statistics on the prevalence of diabetes (both Type 1 and Type 2) in the UK and these were reported by Diabetes UK (table 1-3). In Scotland, the estimated prevalence of diabetes in adults over 17 years of age was 4.3% (n=223,494), with a UK average of 4.45% for the period April 2010-March 2011. NHS Lothian had a prevalence of 3.67%, just below the Scottish and national averages (Quality & Outcomes Framework 2010-2011). The prevalence of diabetes has increased in recent years, with prevalence in the UK in 2006 estimated at 3.54% (Diabetes UK), with a year on year increase evident from QOF data. In 2004, the WHO estimated worldwide prevalence of diabetes for all ages in 2000 was 2.8% but they project that by 2030, this will have risen to 4.4% (Wild, Roglic et al. 2004). Thus, it is evident that diabetes poses, and will continue to pose, a significant challenge for health care provision.

Table 1-3 Prevalence of Diabetes in the UK (Source: Diabetes UK) (Diabetes UK 2011)

Country	Prevalence	Number of people
England	5.5 %	2,455,937
Northern Ireland	3.8 %	72, 693
Scotland	4.3 %	223,494
Wales	5.0 %	160, 533

1.2.3 Diagnosing Type 2 diabetes

The development of Type 2 diabetes occurs over a considerable period of time. As insulin resistance worsens and pancreatic function begins to decline, patients often remain asymptomatic. It is because of this that the diagnosis of Type 2 diabetes is most commonly made at a point of opportunistic screening, such as that done in GP practices, or when patients present to a hospital clinic with other medical problems. The presence of a raised glucose leads to follow up testing with an oral glucose tolerance test. Diagnosis in the UK is made following a fasting blood glucose of >7 mmol/l or an oral glucose tolerance test OGTT of >11mmol/l 2 hours after glucose loading, in addition to symptoms of diabetes (World Health Organization 2006). If fasting glucose is <7mmol/l but OGTT is between 7.8 and 11.1mmol/l, a diagnosis of impaired glucose tolerance is made. In 2011, the WHO produced updated guidelines for the diagnosis of diabetes, stating that HbA1c can be used to diagnose type 2 diabetes in certain patient groups (World Health Organization 2011). However there are constraints on the patients in which this testing can be used and additional guidance regarding management in cases where HbA1c is not raised and yet there is still a clinical suspicion of diabetes.

Although detection of Type 2 diabetes in this way can lead to diagnosis before symptoms appear, there can already be damage to the retina, kidneys and coronary arteries. In some cases, an abnormality in these organs is what prompts testing for diabetes, for example the occurrence of myocardial infarction or in those with loss of

vision. Occasionally, Type 2 diabetes presents acutely with the uncontrolled symptoms of diabetes. These are: excessive thirst, polyuria, polydipsia, nocturia, fatigue, weight loss and non-healing wounds. This is similar to the presentation of Type 1 Diabetes, but as discussed earlier, often there is no acidosis at diagnosis, although patients with Type 2 diabetes can present with similarly raised glucose levels.

1.2.4 Microvascular complications of Type 2 diabetes

The complications of Type 2 diabetes are manifest in many different anatomical beds and can be broadly categorised as microvascular, macrovascular and neuropathic. In terms of cardiovascular disease, both microvascular disease and macrovascular disease are of importance. The microvascular complications of Type 2 diabetes are most commonly manifest in the renal, retinal and nervous systems (Fowler MJ 2008).

Diabetic retinopathy

Diabetic retinopathy is the most common microvascular complication of diabetes. Duration and severity of hyperglycaemia are the main factors underlying the development of retinopathy. In addition, the UKPDS study published data suggesting that hypertension may also play a role in some patients with Type 2 diabetes. In some cases, the development of retinopathy starts before a diagnosis of diabetes has been made (Fong DS, Aiello LP et al. 2004). Aldose reductase, glycoproteins, oxidative stress and growth factors have all been implicated in the development of diabetic retinopathy (Fowler MJ 2008).

Diabetic neuropathy

Diabetic nephropathy is the leading cause of renal failure in the USA (Fowler MJ 2008). Similarly to diabetic retinopathy, almost 7% of people with Type 2 diabetes may have microalbuminuria at the point of diagnosis of diabetes (Gross JL, De Azevedo MJ et al. 2005). Glucose control has strong associations with the development of nephropathy and good glycaemic control is an important part of

disease prevention. In addition treatment with ACE inhibitors has been shown to reduce development of nephropathy in people with Type 2 diabetes but not those with Type 1 diabetes (Gerstein HC 2000; Gross JL, De Azevedo MJ et al. 2005).

Diabetic neuropathy is another complication that is related to both the magnitude and duration of hyperglycaemia. The American Diabetes Association describes peripheral neuropathy in diabetes as a diagnosis of exclusion (American Diabetes Association - Standards of Medical Care in Diabetes 2011). Diabetic neuropathies can take a variety of forms, including sensory and autonomic neuropathy, and many people with neuropathy go on to undergo amputation as a result of the increased risk of foot ulceration and injury in cases of neuropathy. They cause significant morbidity and affect many difference body systems, including the digestive tract and cardiac function in addition to sensory dysfunction (Fowler MJ 2008).

1.2.5 Macrovascular complications of Type 2 diabetes

The Task Force on Diabetes and Cardiovascular Diseases of the European Society of Cardiology (ESC) and of the European Association for the Study of Diabetes (EASD) published a guideline on diabetes and cardiovascular disease. In this, they highlight that people with diabetes have a 2 to 3 times higher risk of developed coronary artery disease than those people without diabetes (Rydén, Standl et al. 2007). They highlight that there is a difference in cardiovascular risk between people with Type 1 and Type 2 diabetes, noting that CVD caused 44% of mortality in people with Type 1 diabetes, compared with 52% in people with Type 2 diabetes (Morrish, Wang et al. 2001).

Macrovascular complications of Type 2 Diabetes include coronary artery disease, stroke and peripheral vascular disease. The pathology that underlies macrovascular disease in diabetes is atherosclerosis. In those people with diabetes, development of atherosclerosis seems to be accelerated and several major factors have been postulated as possible mechanisms, including hyperglycaemia, dyslipidaemia and subclinical inflammation (Beckman, Creager et al. 2002; Mazzone T, Chait A et al.

2008). Endothelial cells, smooth muscle cells and platelets can all be affected by these factors through a variety of mechanisms, resulting in endovascular injury.

Endothelial production of nitric oxide is one of the key factors involved in vascular health. Nitric oxide has a vital role in the control of vascular relaxation, through its promotion of vessel dilation (Moncada S, Palmer RM et al. 1991; Moncada S 1999). Additionally, nitric oxide plays a role in several other steps that are protective of the vessel wall. It inhibits platelet activation, reduces leucocyte adhesion to the endothelium and subsequent migration into the vessel wall (which reduces inflammation) and lessens smooth muscle cell proliferation and migration (Moncada S, Palmer RM et al. 1991; Moncada S 1999; Verma S and Anderson TJ 2001). In diabetes, nitric-oxide mediated vasodilation is impaired and nitric oxide mediated vasodilation is limited while platelet aggregation is increased (Fowler MJ 2008).

Chronic subclinical inflammation has also been implicated in the development of both atherosclerosis and Type 2 diabetes. The Hoorn Study investigated the link between endothelial dysfunction and low grade inflammation and cardiovascular mortality in people with Type 2 diabetes (de Jager J, Dekker JM et al. 2006). The basis for the work they undertook was the commonly held belief that endothelial dysfunction and low grade inflammation can explain why deteriorating glucose tolerance is associated with cardiovascular events in those with Type 2 diabetes.

The association between diabetes and the metabolic syndrome also plays a key role in the development of cardiovascular disease, as the major risk factors for Type 2 diabetes are the same as those for cardiovascular disease. People with Type 2 diabetes have an increased risk of cardiovascular disease, such that people with diabetes and no previous history of myocardial infarction have as a high a risk of future myocardial infarction as people without diabetes who have a previous myocardial infarction (Haffner, Lehto et al. 1998). This has led to Type 2 diabetes being considered by some to be a cardiovascular risk equivalent.

Metabolic syndrome

Typically, individuals with Type 2 diabetes display a poorer cardiovascular risk factor profile than those in the general population. The complex interplay between Type 2 diabetes, cardiovascular disease and the metabolic syndrome goes some way to explaining this phenomenon. The metabolic syndrome is a group of risk factors that, collectively, lead to an increased risk of developing cardiovascular disease, Type 2 diabetes and liver disease. The components necessary for a diagnosis of metabolic syndrome include: dyslipidaemia, increased body fat (in particular central obesity), insulin resistance and hyperglycaemia, fatty liver disease, hypertension, raised inflammatory and pro-thrombotic markers and endothelial dysfunction (Kalofoutis C, Piperi C et al. 2007). Following initial work done in the late 1980s by Reaven (Reaven 1988) where he described "syndrome X", the original theory has been extended to suggest that the underlying pathology of the metabolic syndrome lies in the presence of obesity, which leads to insulin resistance, however, the exact mechanism underlying the syndrome is still unidentified (Ferrannini, Natali et al. 1997).

The metabolic syndrome is commonly diagnosed by the International Diabetes Federation (IDF) and American Heart Association (AHA) criteria (Alberti, Eckel et al. 2009), which are demonstrated in table 1-4. These criteria are very similar to the criteria for the diagnosis of Type 2 diabetes, reflecting the close relationship between the two.

Table 1-4 IDF/AHA criteria for diagnosis of metabolic syndrome

3 or more of the following:
– Increased waist ethnicity specific waist circumference
– Body mass index (BMI) above 30 kg/m2,
– Blood pressure above 130/85 or treatment for hypertension
– Triglycerides above 1.7 mmol/L or treatment for this abnormality
– HDL cholesterol <1.03 mmol/L in men and <1.29 mmol/L in women or treatment for this abnormality
– Fasting plasma glucose >5.6mmol/l

The metabolic syndrome underlies the pro-atherogenic risk factor profile displayed in people with diabetes. In addition to impaired glucose regulation, people with Type 2 diabetes are more like to be hypertensive and centrally obese, have an adverse lipid profile, microalbuminuria and raised inflammatory markers (Kalofoutis C, Piperi C et al. 2007). 50% of people with diabetes are hypertensive at the time of diagnosis (Stults and Jones 2006), while the lipid profile often includes low circulating HDL cholesterol, with a moderate elevation in triglyceride levels (Solano and Goldberg 2006). Diabetes itself causes a 2-4 fold increase in the risk of CVD, and in the presence of concomitant hypertension, total mortality and stroke, that risk is doubled, while the CHD risk is tripled, and the development of microvascular complications is hastened (Reboldi, Gentile et al. 2009).

The association of diabetes with the metabolic syndrome is strong and may account for the increased prevalence of cardiovascular disease in this group (Isomaa, Almgren et al. 2001; Stern MP, Williams K et al. 2004).

1.2.6 Managing cardiovascular risk in Type 2 diabetes

In the UK, guidelines from NICE and SIGN provide guidance to clinicians for the management of cardiovascular disease in people with Type 2 Diabetes (NICE CG87 2008; SIGN 116 2010). As people with Type 2 diabetes are known to be at increased risk for CVD, the overarching principal is one of aggressive management of any pertinent vascular risk factors, to the same extent as secondary prevention of CVD in the non-diabetic populations. Risk factors are generally managed using the same agents and targets for blood pressure and lipids are broadly the same as the general population, unless complications co-exist (NICE CG87 2008), for example, blood pressure targets are 140/80mmHg unless renal, eye or cerebrovascular disease are present at which point in becomes 135/85mmHg.

There are differing opinions in the literature concerning the value of cardiovascular risk prediction in individuals with diabetes. Because individuals with diabetes demonstrate a similar risk profile as those without diabetes that have had an event,

several guidelines recommend managing all people with diabetes as high risk. However, other guidelines take a different approach, whereby there is considered to be a spectrum of risk with the population of diabetes that still requires quantification. They support the argument that it is important to perform risk prediction in people with Type 2 diabetes because as in the general population, there is evidence that intensive therapy can reduce cardiovascular and all-cause mortality (Gæde, Lund-Andersen et al. 2008). Unfortunately, not all health systems can afford a blanket policy of treatment and so varying elements of risk prediction must be performed in different populations to identify those at highest risk. As discussed in chapter 1, unlike in the general population, the use of risk prediction scores in Type 2 Diabetes is not clear cut, and is limited by their accuracy in this population. However, we know that people with Type 2 diabetes have an increased risk of cardiovascular disease. Therefore, the development of scores specifically for use in people with Type 2 diabetes has been tackled by several groups, including the UK Prospective Diabetes Study (UKPDS).

Some clinical guidelines recommend that people with Type 2 Diabetes be considered as high risk from the point of diagnosis. The Scottish Intercollegiate Guidelines Network (SIGN) published a guideline on assessing cardiovascular risk (SIGN 97 2007). This guideline, advised that adults with Type 2 diabetes who are over the age of 40 years do not require risk assessment, as they are automatically at a high CVD risk (>20%) due to their clinical history. The risk assessment that they discuss is based on risk scores alone, and does not include the use of adjuncts such as intima media thickness. The 2009 NICE guidelines for managing CVD risk in people with Type 2 diabetes suggest that people with Type 2 diabetes should be considered as high risk for CVD, unless he or she:

- is not overweight
- is normotensive (< 140/80 mmHg in the absence of antihypertensive therapy)
- does not have microalbuminuria
- is a non-smoker
- does not have a high-risk lipid profile
- has no history of cardiovascular disease **and**
- has no family history of cardiovascular disease. (NICE Clinical Guideline 87 2009)

In this situation, they recommend that it would be of use to perform annual cardiovascular risk estimation using the UKPDS risk score in people who meet these criteria (NICE CG87 2008) (however, it should be noted that the advice in this guideline was updated in 2014 to recommend that the QRisk2 score be used for risk assessment in people with diabetes (NICE CG 181 2014)). The American Diabetes Association (ADA) states only that there should be assessment of risk factors (American Diabetes Association - Standards of Medical Care in Diabetes 2011). Guidelines from the International Diabetes Federation recommend the use of diabetes specific risk scores (International Diabetes Federation 2005), while others recommend scores such as Framingham or DECODE (Rydén, Standl et al. 2007).

In 2009, Chamnan et al published a systematic review focusing on cardiovascular risk assessment scores in people with diabetes, in which they examined scores that were developed in both individuals with diabetes and those developed in a general population (Chamnan P, Simmons RK et al. 2009). They highlight that there is evidence of improvement in the risk of fatal and non-fatal cardiovascular events in people with diabetes following multifactorial interventions, such as the Steno-2 study (Gæde, Vedel et al. 2003; Gæde, Lund-Andersen et al. 2008). However, for chiefly economic reasons, many countries have to practice a rationing approach to managing cardiovascular risk, with risk assessed prior to prescription of preventive therapies, allowing the highest risk patients to be prioritised for treatment (Chamnan P, Simmons RK et al. 2009). This has led to increased use of cardiovascular risk scores

in people with diabetes. The authors also highlight two other reasons for the use of a risk prediction tool (Chamnan P, Simmons RK et al. 2009). Firstly, they may be used to provide an individual's risk (absolute risk) and secondly, they may be used to encourage individuals to improve their lifestyle, in which case the score is required to reflect the modifiable risk, rather than a risk predicted from fixed variables.

1.2.7 Cardiovascular risk prediction scores in Type 2 diabetes

Several groups have attempted to develop risk algorithms that are specific to the diabetic population using diabetic cohorts in the development, in order to produce a risk score that might be more accurate in this group. Although there have been several scores developed that have good discriminatory values, there is still no strong evidence as to which score is best suited for use in Type 2 diabetes (van Dieren S, Beulens JWJ et al. 2012).

Two major reviews have summarised the main risk scores used in and developed for diabetic populations (Chamnan P, Simmons RK et al. 2009; van Dieren S, Beulens JWJ et al. 2012) (the latter providing what is essentially an update of the former). The authors examined several different risk scores, including scores developed both in general populations and diabetic populations, in addition to reviewing scores that have been validated in diabetic populations. They noted that the predictive ability of scores varied in different populations and conclude that scores developed in general populations were likely to underestimate risk in people with T2DM. Similarly, those scores developed in diabetic populations required to be better validated in other populations before definitive conclusions can be made as to their use.

Their overarching conclusion of both reviews is that it may be more useful to have population specific scores rather than one universal score for all populations. This would seem to be a sensible approach to risk prediction. Some of the scores that were developed in diabetic populations that are included in their review are discussed below.

Risk scores developed in diabetic populations

There are several examples of risk scores that have been developed in diabetic populations rather than in general populations. The most commonly included risk factors are age, sex, duration of diagnosed diabetes, HbA1c and smoking (van Dieren S, Beulens JWJ et al. 2012).

An early score by Yudkin et al used a sample of 2138 American subjects with Type 2 diabetes to develop a model for predicting CHD risk over 10 years, including six predictor variables in the model (Yudkin and Chaturvedi 1999). Several years later, the UKPDS risk engine was developed in Oxford, and was based on data from 53 000 patient years from individuals with Type 2 diabetes enrolled in the UKPDS study (Stevens RJ, Kothari V et al. 2001). UKPDS found that previous risk scores were less accurate in people with diabetes and the use of simply a dichotomous variable for diabetes or glycaemia in previous risk scores was identified as a target for change. The authors instead used HbA1c as a diabetes marker and additionally replaced age with age-at-diagnosis and time since diagnosis of diabetes. The inclusion of these variables aimed to address the role that each plays in the development of the complications of diabetes (Stevens RJ, Kothari V et al. 2001). The first version was developed in a population of 4540 men and women with Type 2 Diabetes but no history of cardiovascular disease (Stevens RJ, Kothari V et al. 2001). An additional version that is specific for stroke was developed in 4549 people with T2DM and no previous stroke (Kothari V, Stevens RJ et al. 2002).

The ARIC investigators also published a model developed in people with diabetes (Folsom AR, Chambless LE et al. 2003). They based the model on 1273 subjects with Type 2 diabetes in the US and it predicted risk of CHD over 10 years. They achieved an area under the curve of 0.76 for men and 0.78 for women. Another score that predicted CHD risk (although over only 5 years) was the DARTS score (Diabetes Audit and Research in Tayside, Scotland) (Donnan, Donnelly et al. 2006). This score included a similar number of predictors to the initial ARIC score and the resulting AUC for the score was 0.71.

The Hong Kong Diabetes registry was used to create 3 scores – one for stroke (Yang, So et al. 2007), one for CHD (Yang, So et al. 2008) and one for heart failure (Yang, Ma et al. 2008). These studies were published on data from just over 7000 people with Type 2 diabetes living in China. They predicted risk over 5 years and achieved AUC ranging from 0.70 for CHD to 0.85 for heart failure. These results were similar to those of the Swedish National Diabetes Register (Cederholm, Eeg-Olofsson et al. 2008). 11,646 people with Type 2 diabetes were used to develop the score using a Cox model and it predicted the risk of CVD over a 5 year period. The reported AUC was 0.70.

More recently a study published in New Zealand developed a risk model in a cohort of people with Type 2 diabetes living in New Zealand (Elley, Robinson et al. 2010). CVD and CHD risk was predicted over 5 years and used 10 predictors. The AUC for CVD was 0.68 and for CHD 0.71. A further score from Australia by Davis et al as part of the Freemantle diabetes study (Davis, Knuiman et al. 2010) reported an AUC of 0.80 in the prediction of CVD. Additionally, the ADVANCE collaborative group (Kengne, Patel et al. 2011) reports the use of 10 predictors to create a cox model that predicted 4 year risk of CVD. A similar AUC was seen in both the initial testing and validation in an independent cohort (0.71 and 0.69 respectively).

Scores externally validated in a diabetic population

Van Dieren et al highlight in their comprehensive review article that of the 12 risk scores they identified that have been developed in diabetic populations, and the 33 general population risk scores that account for diabetes, 31% had been externally validated in a general population (van Dieren S, Beulens JWJ et al. 2012), including the ADVANCE, Fremantle, DCS, DARTS, UKPDS, HMRS, Framingham, CUORE, Decode and PROCAM scores. There was great variation in the discriminative ability of these scores in diabetics, including the even the UKPDS and Framingham scores, which had AUC ranging from 0.65-0.76 for the UKPDS score and 0.56-0.80 for the Framingham score, with poor calibration reported for both studies (van

Dieren S, Beulens JWJ et al. 2012), suggesting that risk scores do not perform well in patients with diabetes.

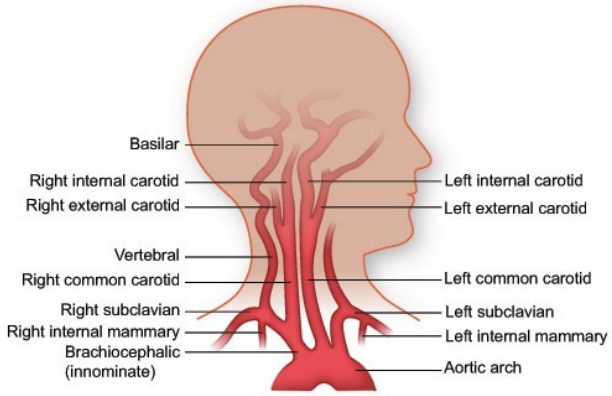
The poor performance of risk scores in people with diabetes, as well as the increased incidence of cardiovascular disease in this population, has opened the door to exploration of methods to improve risk prediction in people with diabetes. Novel biomarkers such as carotid intima media thickness (which has been extensively studied in the general population as a marker of vascular risk) and carotid plaque, are starting to be explored in addition to traditional cardiovascular risk factors (Yamasaki Y, Kodama M et al. 2000; Bernard S, Sérusclat A et al. 2005).

1.3 Carotid ultrasound and cIMT

Visualisation of the carotid artery using ultrasound is regularly undertaken as part of investigations for transient ischaemic attacks (TIA) although the primary objective in that situation is usually assessing blood flow in the vessel and any stenosis resulting from atherosclerosis of the wall, with a view to performing carotid endarterectomy. However, in 1984 Pignoli et al suggested that measuring the thickness of the carotid wall may be a more accurate measure of atherosclerosis given that atherosclerosis is primarily a pathology of the artery wall and there is often luminal expansion to compensate for thickening of the vessel wall (Pignoli P, Tremoli E et al. 1986).

1.3.1 Anatomy of the neck

Figure 1-5 Arteries of the neck (reproduced from http://www.texasheartinstitute.org/HIC/Topics/cond/CarotidArteryDisease.cfm)

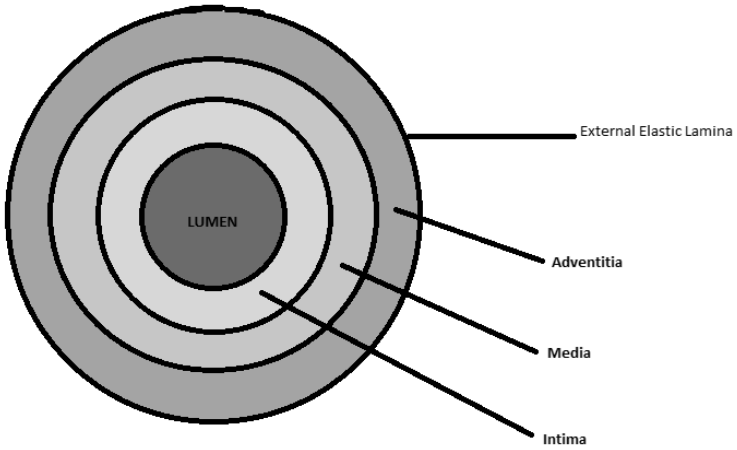


The carotid arteries are the major arteries of the neck (figure 1-5). The brachiocephalic trunk branches from the aorta, and bifurcates into the right common carotid artery and the right subclavian artery. The left common carotid artery and the left subclavian artery are branches of the aortic arch itself. On both the right and left sides, as the common carotid artery extends upwards into the neck, it bifurcates into the internal and external carotid arteries, with the internal artery entering the skull to provide the cerebral blood supply, and the external artery supplying structures external to the skull.

The three key anatomical areas that are considered most important when measuring carotid intima media thickness are the common carotid artery, the carotid bifurcation and the internal carotid artery. The common carotid artery is the most accessible portion of the carotid artery in terms of surface anatomy. It runs along the course of the sternocleidomastoid muscle on the anterior aspect of the neck and as such, it is ideally located for non-invasive assessment by ultrasound. The bifurcation is located at the level of the thyroid cartilage, and the internal carotid extends up behind the angle of the jaw.

1.3.2 Structure & pathology of the carotid artery wall

Figure 1-6 Diagrammatic representation of the anatomical structure of the carotid artery wall



The carotid wall shares common anatomy with all arterial structures. The wall comprises three layers – the tunica intima, tunica media and tunica adventitia (figure 1-6) – with each layer performing a unique role. The intima is the layer adjacent to the lumen of the vessel and consists of a layer of endothelial cells and an elastic layer known as the internal elastic lamina. The endothelium is the biologically active section of the intima and it is supported by the elastic lamina. The media is primarily composed of smooth muscle cells surrounded by a layer of extracellular matrix comprising elastin, collagen and proteoglycans. The media has an important structural role but is also responsive to intimal injury, when smooth muscle cells proliferate and it promotes inflammatory cell migration. The external elastic lamina separates the media from the adventitia. The adventitia is primarily composed of collagen. Autonomic nerve fibers extend into the media.

The intima media thickness is defined as the distance between the luminal edge of the intima and the adventitial edge of the media (Wikstrand J 2007) however, the pathology underlying the thickening of the intima media complex is not fully understood. Thickening of the intima media complex is most often associated with atherosclerosis whereas thickening of the media is often due to hyperplasia related to shear stress on the vascular wall as a result of hypertension (Johnsen SH and Mathiesen EB 2009). Therefore, thickening of the intima-medial complex could be due to either or both of these processes, highlighting that it is not well defined whether or not cIMT thickening is a precursor of atherosclerosis or simply a response to stress on the wall. This is further complicated because when measuring cIMT, it is not always possible to delineate the intima from the media and it is therefore difficult (or perhaps impossible) to attribute any thickening to one or the other processes.

1.3.3 Ultrasound measurement of cIMT

Traditionally, the detection and quantification of atherosclerotic disease in arteries was done by invasive methods, including contrast angiography. However, since the mid-1980s, work has been advancing to develop reliable, non-invasive methods for the quantification of carotid intima media thickness, as well as atherosclerotic plaques.

Ultrasound modes used in measurement of cIMT

There are several different modes of ultrasound that have a variety of different uses. A-mode ultrasound is used primarily for therapeutic ultrasound treatment and is not usually involved in imaging. In B mode ultrasound, a linear array of transducers simultaneously scans the desired area of investigation, allowing a 2 dimensional picture to be formed on screen. It is this mode that is most commonly used in diagnostic ultrasound. Current technology allows for real time image analysis, producing a moving 2D image on screen. M mode ultrasound, which is a form of ultrasound that detects motion, is primarily used in echocardiography and fetal ultrasound to demonstrate heart motion.

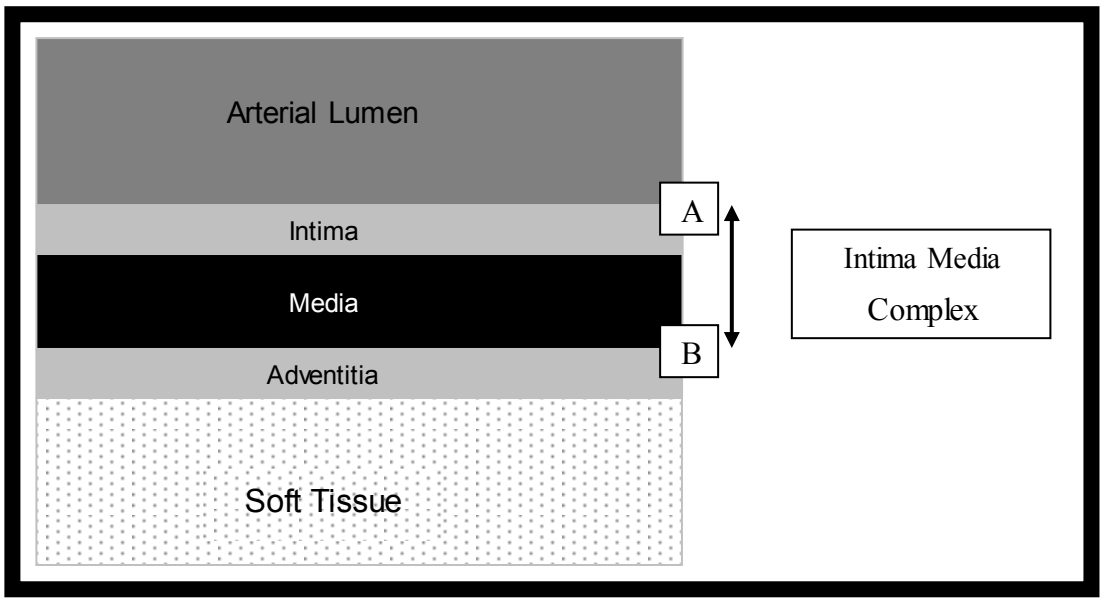
Doppler mode ultrasound is perhaps most commonly associated with carotid imaging. Measurement and visualisation of blood flow in vessels of the neck is made possible by utilising the principles of the Doppler Effect. This mode of ultrasound is commonly used when investing stenosis of the carotid artery in patients affected by transient ischaemic attacks (TIA). It is also possible to discern the outline and luminal projection of arterial wall plaques. However, it does not provide a detailed assessment of plaque thickness and cannot provide any more information regarding the vessel wall. B mode ultrasound however, can provide a more detailed image of the artery wall, hence its use in the measurement of cIMT. It enables direct visualisation of the artery wall (Pignoli P 1984), enabling measurement of intima-media thickness.

Use of B mode ultrasound in measuring cIMT

The initial advances in this field were made by Pignoli et al. The group looked at measuring cIMT *in vitro* and *in vivo*, using B mode ultrasound. Their initial work, published in 1984, demonstrated a significant association between pathological measurement of cIMT and B mode ultrasound measurement of the complex. This original study measured cIMT in a small number of normal and moderately diseased arteries *in vitro* (Pignoli P 1984). They continued to take this work forward by examining the relationship between measurements made both *in vitro* and *in vivo*, and determining what anatomical structures account for what images on the ultrasound.

The characteristic B mode image of the intima media complex of the arterial wall is of a “double line pattern”. This pattern consists of two parallel echogenic lines that are separated by an area of hypogenicity (figure 1).

Figure 1-7 Graphic representation of B mode ultrasound of cIMT



In figure 1-7, the first line of the double echo represents the intima of the artery wall and the leading edge of this structure (marked A) represents the intimal-luminal interface. The area of hypogenicity (black section) represents the media of the wall and finally the second line of the echo (second light grey section) represents the adventitia. The edge between the media and adventitia is called the media-adventitial interface (marked B).

Pignoli et al took specimens from men aged 20-74, and included a mix of normal and pathological specimens. They measured the intima media thickness, in a marked region of the artery wall, using both gross pathology and histology. Once this was done, they prepared the arterial segments and placed them in water tanks. They then measured the same segment of the arterial wall using a B mode ultrasound. This enabled them to compare histological and pathological cIMT with that measured by B mode ultrasound.

The next step was to measure *in situ* specimens in cadaveric specimens. They measured the cIMT by B mode ultrasound in common carotid arteries that were cannulated with an inflatable balloon catheter and found that the cIMT was not

significantly different in the *in situ* and excised specimens. This was then extended to assessing cIMT in 10 living subjects. This demonstrated that the characteristic "double line pattern" was visible in living specimens and that the mean cIMT was similar to that found in the *in vitro* study.

And so, Pignoli et al had shown B mode ultrasound to be a valid and useful tool in measuring cIMT *in vivo*. It was this work that laid the foundations for the use of B mode ultrasound as a reliable, and importantly, non-invasive method for such measurements. These findings were further confirmed by Persson et al in 1994 (Persson J, Formgren J et al. 1994). The group compared measurement of cIMT using B mode ultrasound with the same distance measure using light microscopy. They found that cIMT measured by B mode ultrasound correlated highly with that defined by light microscopy (r 0.82, $p<0.001$) – however, one must bear in mind that whilst there is a good correlation between these measures, this does not necessarily mean that there will be good agreement as even poorly agreeing measures can produce good correlation (Bland JM and Altman DG 1986). They noted that cIMT measured by light microscopy was consistently smaller, however, this was attributed to tissue shrinkage (Persson J, Formgren J et al. 1994).

The main advantages of using ultrasound for this purpose lie in its flexibility, low cost and low risk. There is little to no long term effects of ultrasound exposure and new technological advances mean that ultrasound can be easily done at the bedside if required. Compared to other imaging modes, ultrasound is also inexpensive, making it more accessible. The major disadvantage to its use is unreliable penetration of deep structures in obese patients. Whilst not a major concern when imaging the neck, this must be considered, especially given the relationship between obesity and potential vascular risk.

The carotid artery branches from the common carotid artery into the internal and external carotid artery. The point of branching is known as the carotid bifurcation. The common carotid artery is most easily visualized using ultrasound due to its

position in the neck. The bifurcation and internal carotids are more difficult to visualize due to their anatomical obscurity and consequently they cannot be measured in all individuals. Kanters et al found that there can be a higher proportion of missing values when cIMT is measured in these areas, as well as a higher degree of measurement variation (Kanters SD, Algra A et al. 1997). A moderate correlation between cIMT at different sites was noted by Howard et al, however, cIMT at once site could not predict cIMT at another (Howard G, Burke GL et al. 1994). This has led to the cIMT in the common carotid artery being favoured as a site of measurement due to ease of access and reliability.

Summary measures of intima media thickness

Despite the publication of various guidelines concerned with the measurement of cIMT, there is no unified summary measure of cIMT. Because of the variety of protocols which studies use to measure cIMT, different methods are used to summarize cIMT (Lorenz MW, Markus HS et al. 2007). The simplest of these is the mean – an average of all the measurements taken for cIMT. For example, in the Edinburgh Type 2 Diabetes Study, 6 measurements were made in total (3 on the left, 3 on the right). The mean cIMT would be the mean of those 6 readings. In other studies, it would be the mean of the mean cIMT at each segment of the carotid tree. In some cases, the maximum cIMT is used. This too is often used to different effect in different studies. In some, it is the higher of the mean of the left and mean of the right. In others, it represents the mean of the maximum right and maximum left readings. This heterogeneity leads to many difficulties when it comes to comparing studies of cIMT.

1.3.4 Measurement variation

Measurement variation is a problem which is widespread in all research methodology. In epidemiological studies, error and variability can lead to bias and lack of precision when measurements are analysed.

Physiological measurements are prone to variability from many sources, including the machine or equipment used and the subjective view of the individual taking the measurement. In terms of carotid intima media thickness, variation can be introduced by the ultrasound machine used, the ultrasonographer taking the readings, the skill of the reader performing measurements using software after image acquisition and even the patient themselves. The first of these – the ultrasound machine – is not of as much importance as it was previously. Machines are built to an industry standard and error and variation are often due to selection of inappropriate settings (eg gain) or poor maintenance of machines. Physical factors and anatomy of the patient contribute to variability in several ways. Firstly, a more physical than anatomical property, is movement of the carotid artery. In comparison to other arteries, there is relatively little movement of the carotid artery; nonetheless, this remains a source of error (Kanters SD, Algra A et al. 1997). However, a more important factor to consider is the position of the carotid segments in the neck. Whilst the carotid is a very superficial artery, not all segments are as easily accessible by ultrasound as the common carotid. Measurements of the internal and external carotids, and the carotid bifurcation, are more prone to missing data due to inaccessibility. Several studies have documented this and the ACAP Study found that the CCA segments could be visualized in 99% of cases, the bifurcation 88% and the ICA 67%, which highlights the differences in accessibility of each of these segments (Espeland MA, Craven TE et al. 1996). Part of this may be due to an inability of the patients to move into or maintain the position required for measurement of cIMT.

The largest source of measurement error and variability however, is the sonographer and reader. Human error is a major factor in many physiological measurements and no less so in cIMT measurement. The actual imaging of the cIMT is usually undertaken by a sonographer. They may or may not perform cIMT measurement there and then. If not, the image is stored, either as a longitudinal image or as a video, and measurement is performed later by a reader (either the same sonographer or an independent reader), or automated edge detection software. Many large studies

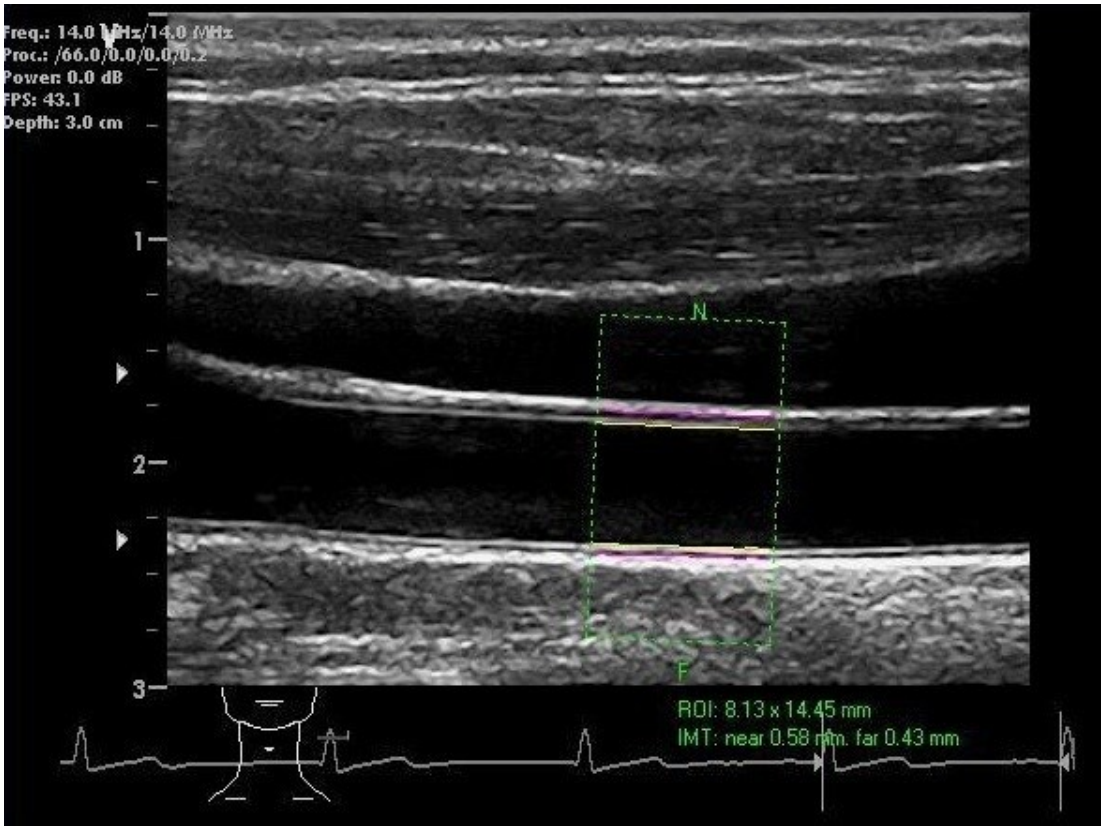
who have measured cIMT have looked more specifically at variability in cIMT within their studies. An example would be the study by Salonen et al in 1991. In this study, inter-observer variation was the main source of variability, with only 4% attributable to intra-observer variation (Salonen R, Haapanen A et al. 1991). The findings of the Rotterdam study were similar. They measured cIMT using the controls described above – a single radiographer and multiple off line readers - and demonstrated that 87% of variability in cIMT following repeat scanning was due to between-subject variation and 13% was due to inter-observer variation in both readers and sonographers (Bots ML, Mulder PG et al. 1994). A study by Lundby-Christensen et al examined reproducibility of cIMT in those with and without Type 2 diabetes (Lundby-Christensen L, Almdal TP et al. 2010). They found that reproducibility was good in both people with and without diabetes.

As would be expected, it is easier to control for many of these sources of variation in a research setting than in clinical practice. The use of simple measures such as a single radiographer making measurements, using the same machine for each participant and using several readers to perform offline measurements can all reduce variation; however assessment of cIMT measurement accuracy should be an important feature of any research study.

1.3.5 Automated edge detection software

Tackling measurement variation has led researchers to investigate a variety of techniques aimed at reducing measurement variation. In clinical trials and epidemiological studies, it is usually relatively simple to have the same sonographer or reader perform measurements at each follow up, dependent on the length of the trial or study. However, this is not as applicable in a clinical setting. Work rotas, staff turnover and availability, and importantly, health care budgets all affect the availability of specific staff members for performing measurement of cIMT in specific patients. And so, focus has fallen upon finding a way to remove this source of measurement variation to allow cIMT measurement to be widely applied in clinical settings.

Figure 1-8Automated edge detection of cIMT (reproduced from Robertson et al, Appendix F)



There has been considerable interest in the development of automated and semi-automated measurement protocols for cIMT measurement (figure 1-8). By removing the human aspect of cIMT measurement, and replacing it with a computer algorithm, the inherent inter-reader variability could be removed. However, finding an algorithm that is capable of analysing all scans of cIMT done on all types of ultrasound machine is a difficult task. As such, many ultrasound machine manufacturers have developed their own in-house software to be used on their own machines. These allow real time measurement of cIMT by the algorithm and remove human error. However, many measurements are still made on machines on which such software cannot be used.

There have been recommendations from both the ASE and Mannheim consensuses that edge detection software should be used where possible to make measurements of cIMT (Touboul PJ, Hennerici MG et al. 2007; Stein JH, Korcarz CE et al. 2008; Touboul PJ, Hennerici MG et al. 2012). Initially, there were only programmes that were based on user dependent edge detection techniques. However with time, there has been evolution from those which require a significant level of user input, to systems which use algorithms to allow user independent identification of the lumen-intima interface and the media-adventitia interface of the cIMT. Several different methods are discussed in a 2010 review of computer aided cIMT measurement by Molinari et al (Molinari, Zeng et al. 2010).

On ultrasound imaging, the adventitia and the lumen have differing pixel intensities, with the lumen having bright pixel intensity in comparison to the lumen. The underlying basis of edge detection software lies in the detection of this pixel gradient as the unique intensity profiles make both the lumen and adventitia easily identifiable. The intima media thickness is the area that lies between these 2 landmarks. As described in the review by Molinari, several groups have approached this challenge from a number of different angles. Liguori et al, who applied an edge detection technique, describe a measurement uncertainty of 0.02mm. However, the major problems that existed with their approach were noise interference and that the system was not fully automated (Liguori, Paolillo et al. 2001). More recent publications have also employed edge detection techniques using a gradient based algorithm have shown improved measurement error (Stein, Korcarz et al. 2005) (Faita, Gemignani et al. 2008).

Many of these systems still rely on the user acquiring the image initially and then selecting the region of interest in which the system can make measurements, which still introduces an element of potential error. However, fully automated systems have been considered by several groups, including Molinari et al, who have worked to develop a fully automated edge detection system (Molinari, Zeng et al. 2010), in which the user is not required to provide any further input beyond scan acquisition.

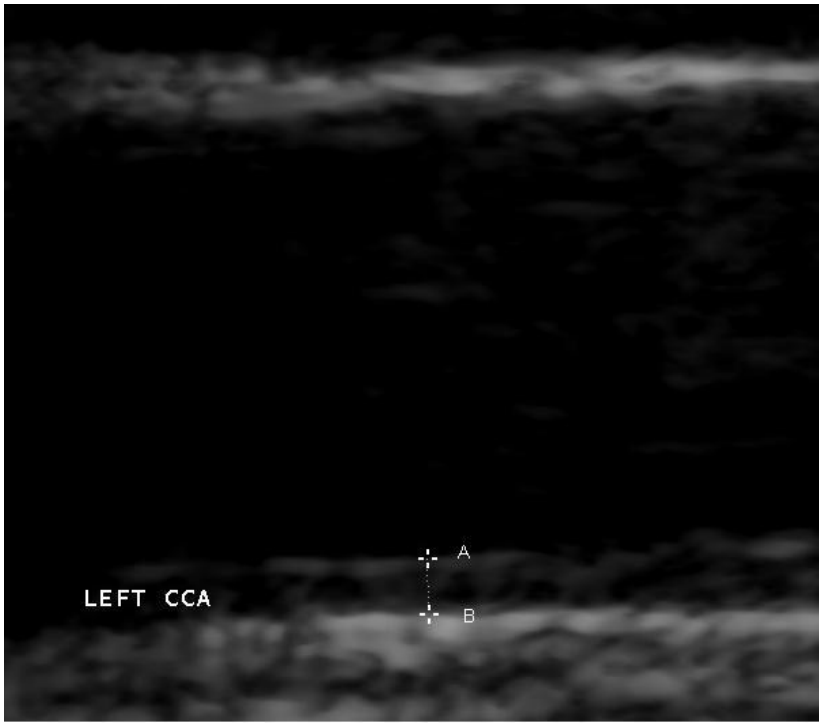
Although several groups have developed potential edge detection systems, based on a variety of algorithms, there is still a requirement to compare these to the current methods of cIMT measurement, which is manual measurement. Peters et al found semi-automated and manual measurements to be highly reproducible (Peters SA, den Ruijter HM et al. 2011). The relationship between cIMT and cardiovascular risk factors was also preserved by the use of semi-automated measurements. A similar finding was seen in the MESA study, between edge detection readings and manual readings (Polak JF, Pencina MJ et al. 2011).

The crucial factor that will support the use of automated methods in clinical practice is the reduction in inter-reader bias.

Multiple versus single measurement of cIMT

Traditionally, ultrasound measurement of cIMT involves taking small numbers of measurement of cIMT online, using calipers (figure 1-9).

Figure 1-9 Measurement of cIMT using calipers (A intima lumen interface, B media adventitia interface)



For example, in the Edinburgh Type 2 Diabetes Study, 3 measurements were taken on each side of the neck (common carotid). Other studies may take up to 12 measurements on each side (common, bifurcation and internal carotid). Taking only small number of measurements poses several problems. As has been previously discussed, there are important differences between intima-media thickening and plaque/atherosclerosis. While it is recommended that cIMT measurements are made in areas free of plaque, intimal thickening is not uniform throughout the length of the vessel wall. Whilst plaque is defined as a focal thickening >1.5mm, taking one, three or even twelve measurements may not provide a good representation of a constantly varying intimal medial thickness.

It is important to consider the importance of this however. Whilst it may not be a problem if the measurements taken capture the largest cIMT of the vessel, true risk may be missed if measurements are made in a thinner area. Some obvious problems arise when considering how to measure cIMT down the length of the vessel. Plaque is the first of these obstacles that must be overcome. Some degree of user input may still be necessary to define areas free of plaque in which measurements can be made.

Automated measurement of cIMT provides the ability to take multiple measurements in a relatively short period of time. Measurements can be made at predefined intervals and an average produced. However, the amount of input required from the user depends on the way in which the software works. The necessity of the absence of plaque in the measurement of cIMT means that user input may be required to select an area for analysis that is free of plaque.

There has been limited work looking specifically at the association between edge detected cIMT measurements and cardiovascular risk factors, compared with manual cIMT measurements. In 2011, Polak et al published a study which addressed this using an edge detection system to measure cIMT thickness on over 5000 people (Polak JF, Pencina MJ et al. 2011). The cIMT measurements made by edge detection software were on average 0.191mm larger than the manual measurements

and showed less inter-reader variability, although interestingly, they were no more reproducible (as has been shown in other studies). The associations of edge detected cIMT were almost as strong as those from manual cIMT with the exception of diabetes and HDL cholesterol, which were stronger for edge detected cIMT.

1.3.6 Use of cIMT in risk prediction - consensus statements

The use of cIMT in clinical practice has not currently reached a high degree of saturation. Clinical trials more commonly utilize cIMT as a surrogate end point for cardiovascular disease. Clinical trials, indeed any kind of research study, demand a high degree of accuracy in measurement of any factor and so in an attempt to standardize the way in which measurements of cIMT are made, a number of guidelines and consensus statements have been developed by different groups, including the American Society of Echocardiography, among others (Touboul PJ, Hennerici MG et al. 2004; Roman MJ, Naqvi TZ et al. 2006; Touboul PJ, Hennerici MG et al. 2007).

In 2004 a group of European researchers drafted the Mannheim Consensus (Touboul PJ, Hennerici MG et al. 2004). As mentioned, they aimed to standardize cIMT measurement with the aim of allowing more accurate analysis and meta-analysis of cIMT data. They also addressed the issue of classifying early and late atherosclerotic change, defining plaque and cIMT separately. They highlight that there are many differences between plaque and cIMT, including different associations with established vascular risk factors, as well as differing associations with incident vascular disease (Ebrahim, Papacosta et al. 1999; Taylor 2002; Den Ruijter, Peters et al. 2013). In terms of measurement of cIMT, the consensus recommends that measurements be made in the common carotid artery (as this is the most easily visualized of the carotid segments) in a 10mm segment free from plaque with the double echo visible. Measurements should also be made on the far wall. In an update of this statement published in 2007, they address which measure of cIMT to make (ie mean cIMT, maximum cIMT etc). Whilst they make no definite conclusion, they state that mean cIMT may be less susceptible to outliers than max

cIMT (which may represent a more advanced disease state) and also that cIMT and plaque should always be considered separately (Touboul PJ, Hennerici MG et al. 2007). A 2011 update this guideline published in 2012 highlights the importance of harmonizing the methods of collecting cIMT across epidemiological and interventional studies to facilitate easier comparison of results. They also provide further criteria for distinguishing early plaque from thickened cIMT. It also recommends against serial cIMT measurement in individual patients (Touboul PJ, Hennerici MG et al. 2012)

Further guidelines have also been released by the American Society of Echocardiography (Roman MJ, Naqvi TZ et al. 2006; Stein JH, Korcarz CE et al. 2008). A key suggestion from these guidelines is that cIMT measurement is most likely to be of benefit in those people deemed to be at intermediate risk by cardiovascular risk scores as this group represents both those at true high risk, true intermediate and true low risk. Therefore additional information from cIMT measurements may allow clinicians to tease out the true risk status of an individual. The ASE guidelines of 2008 also highlight that it is important to measure carotid plaque as well as cIMT.

The Mannheim Consensus does not make reference to what should be considered either a "normal" cIMT or a "high risk" cIMT. IN contrast, the ASE guidelines for the use of carotid ultrasound suggest that a cIMT value in the 75th percentile should be considered high risk

1.3.7 Association of cIMT with vascular risk factors

cIMT is known to be associated with the traditional cardiovascular risk factors. Increasing age, male sex, smoking, blood pressure, BMI, WHR, sedentary lifestyle, family history, ethnicity and presence of diabetes are all predictive of cIMT (Mannami T, Konishi M et al. 1997). Several studies have demonstrated an association of cIMT with increasing age, including the National Institute for Longevity Sciences-Longitudinal Study of Aging (NILS-LSA) (Ando, Takekuma et

al. 2000) and the Edinburgh Artery Study (EAS) (Allan, Mowbray et al. 1997). These studies also demonstrated that cIMT was higher in men than in women. The AXA study found associations between cIMT and BMI, systolic and diastolic blood pressure, plasma lipids, glucose and smoking, although some sex differences existed for several risk factors (Gariepy J, Salomon J et al. 1998). The Insulin Resistance Atherosclerosis Study (IRAS) demonstrated an important association between cIMT and established diabetes (Wagenknecht, D'Agostino Jr et al. 1998), which will be discussed later in this thesis. Lifestyle factors such as dietary cholesterol, BMI and smoking were associated with progression of cIMT in the Monitored Atherosclerosis Regression Study (MARS) (Markus, Mack et al. 1997). Hypercholesterolaemia has also been associated with increased cIMT (Wendelhag, Wiklund et al. 1993; Joensuu T, Salonen R et al. 1994; Gariepy, Simon et al. 1995).

A study in 2000 by Baldassarre investigated the association between cIMT measured in routine clinical practice and cardiovascular risk factors in 963 participants from a general population sample. The authors report that cIMT correlated with systolic blood pressure, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides and blood glucose (Baldassarre, Amato et al. 2000). cIMT was linearly associated with the number of risk factors present ($p<0.003$). They also showed that cIMT was higher in men than women and that those with CHD and PAD had higher cIMT than controls.

Ebrahim et al examined the association between cIMT in the common carotid and bifurcation with CV risk factors (Ebrahim, Papacosta et al. 1999). They found in 800 men and women drawn from a national cohort (British Regional Heart Study) that cIMT of the common carotid had a different pattern of risk factor association compared with cIMT of the bifurcation. cIMT of the bifurcation had a stronger association with age than common carotid cIMT. In addition, cIMT in the bifurcation showed a significant association with plasma lipids, whereas common carotid IMT did not.

In 2010, a cross sectional analysis of cIMT and vascular risk factors in the IMPROVE study demonstrated that cIMT was positively associated with latitude, age, gender, pack years and hypertension, and negatively associated with education level (Baldassarre, Nyyssönen et al. 2010). The authors comment that the association of cIMT with latitude reflects the known north-south gradient of CHD mortality (Sans, Kesteloot et al. 1997; Baldassarre, Nyyssönen et al. 2010). The PARC study demonstrated an association between cIMT and age, male sex, smoking, total cholesterol, HDL cholesterol and systolic blood pressure on linear regression (Touboul, Vicaut et al. 2007).

cIMT and ethnicity has also been studied and several studies have demonstrated that there are differences in cIMT according to ethnic origin. It has been demonstrated that cIMT in black people is greater than that in white people (Howard, Sharrett et al. 1993; Urbina, Srinivasan et al. 2002). Both these groups had a higher cIMT than that of Hispanic people (D'Agostino, Burke et al. 1996).

1.3.8 Association of cIMT with incident coronary events

In order to further explore the relationship between cIMT and vascular risk, several large epidemiological studies have expanded on the cross sectional work by the studies that have been discussed so far.

In the first instance, studies have shown that there is a correlation between cIMT and the extent of coronary artery atherosclerosis (Geroulakos G, O'Gorman DJ et al. 1994; Coskun U, Yildiz A et al. 2009). However, there are large studies that have examined the relationship between cIMT and incident vascular disease (Salonen JT and Salonen R 1993; Chambless LE, Heiss G et al. 1997; O'Leary DH, Polak JF et al. 1999; Chambless LE, Folsom AR et al. 2000; Iglesias del Sol A, Bots ML et al. 2002; Hollander M, Hak AE et al. 2003; Rosvall M, Janzon L et al. 2005; Rosvall M, Janzon L et al. 2005; Lorenz MW, von Kegler S et al. 2006; Folsom, Kronmal et al. 2008; Polak, Pencina et al. 2011). These studies are summarized in table 1-5.

One of the earliest studies examining this relationship was a Finnish study of 1257 men free of vascular disease and aged 43-60 years (Salonen JT and Salonen R 1993). After 3 years of follow up, a 0.1mm increase in carotid IMT represented an 11% increase in risk of myocardial infarction. Following this study, the Atherosclerosis Risk in Communities Study (ARIC) found a similar relationship. They followed up 12841 men and women free of cardiovascular disease at recruitment and aged 45-64 years. Mean cIMT was associated with an increased risk of cardiovascular disease. This relationship persisted after adjustment for age, sex and race (HR 5.07 (95% CI 3.08-8.36) and HR 1.87 (95% CI 1.28-2.69) for men and women respectively). They performed additional analysis controlling for established cardiovascular risk factors and found that whilst a higher cIMT (>0.8mm) maintained a relationship with incident events, this was not the case for cIMT <0.8mm (Chambless LE, Heiss G et al. 1997).

Another epidemiological study that addressed this relationship was the Cardiovascular Health Study (CHS). In their study of 4476 subjects free from CHD (mean age 72.5 years) they followed up incident vascular events, including stroke and myocardial infarction over approximately 6 years. Like the ARIC study, they also adjusted the results for conventional cardiovascular risk factors and found that those with an cIMT in the higher quintiles had increased risk for MI or stroke when compared with the lower quintiles (adjusted RR 3.15 (95% CI 2.19-4.52) (O'Leary DH, Polak JF et al. 1999). They also noted that they relationship appeared to be linear.

In the Rotterdam Study, increased cIMT was predictive of myocardial infarction, even after adjustment for age and sex. The Rotterdam study is a prospective study that measured cIMT in 5851 men and women aged 55 and above and followed up incident events for an average of 4.6 years. cIMT was measured in the common carotid, bifurcation and internal carotid, and in addition a combined cIMT value was derived from these three measures. Of these, only cIMT in the common carotid artery (RR 1.44), bifurcation (RR1.34) and the combined measure (RR 1.47) were

significantly associated with incident MI (Iglesias del Sol A, Bots ML et al. 2002). Interestingly, these results were in contrast to an earlier publication from the Rotterdam study that found that adding cIMT to established vascular risk factors did not improve prediction of CHD (Iglesias del Sol A, Moons KG et al. 2001).

Following on from the work of the Rotterdam study was the Carotid Atherosclerosis Progression Study (CAPS). Researchers studied 6962 people who were initially drawn from a primary health care scheme. cIMT was measured at all carotid segments. They followed up 5056 participants for myocardial infarction, stroke and death for an average of 4.2 years. Of the segments measured, all were found to have increased HR for all outcomes before adjustment. Adjusted HRs remained significant only for cIMT at the CCA and bifurcation for MI and a combined outcome of MI, stroke or death (Lorenz MW, von Kegler S et al. 2006).

The Malmo Diet and Cancer Study (MDCS) enrolled 5163 subjects into their 10 year cohort (Rosvall M, Janzon L et al. 2005). They measured cIMT in addition to carotid plaque and stenosis, and found that all three were associated with incident fatal and non-fatal MI, or CHD death. The adjusted HR for cIMT was 1.23 (95% CI 1.07-1.41), which persisted on additional adjustment for carotid plaque.

In 2007, in order to review the evidence for the use of cIMT in risk prediction, Lorenz et al undertook a systematic review and meta-analysis of large epidemiological studies examining cIMT in relation to incident events. They identified that the studies included used variety of ultrasound protocols and summary measures of cIMT which introduced a degree of heterogeneity between the studies. When they estimated relative risk they found that cIMT was predictive of MI (age and sex adjusted RR 1.26 (95% CI 1.21-1.30)) (Lorenz MW, Markus HS et al. 2007). This led them to conclude that cIMT may be of use in cardiovascular risk prediction.

Following the publication of this review, there have been several other large epidemiological studies that have published data concerning cIMT and incident vascular events. The Framingham study published data from almost 3000

participants in the Framingham Offspring study in 2011. In addition to assessing the relationship between cIMT and incident events, they also looked at the effect of cIMT on reclassification of risk (Polak, Pencina et al. 2011) after measuring cIMT in both the common and internal carotid arteries. The authors report that both common carotid (HR 1.13 (1.02 to 1.24)) and internal carotid cIMT (HR 1.21 (1.13-1.29)) predicted coronary events but that only the internal carotid cIMT improved risk prediction above traditional risk factors (NRI 7.6%, P<0.001).

In 2012, MESA published the results of a further study examining the role of novel risk markers in cardiovascular risk assessment in those people deemed to have an intermediate risk of vascular disease. In this study, carotid intima media thickness did not associate with incident cardiovascular events (HR 1.17, 95% CI, 0.95-1.45) (Yeboah J, McClelland RL et al. 2012). The overall result of this study was that coronary artery calcium was the best predictor of vascular risk in this group and provided the greatest improvement in risk classification.

A large meta-analysis by Den Ruijeter et al comprising data from 14 population-based cohorts (45, 828 individual participants) examined the association between cIMT and incident MI and stroke. They identified 4007 incident events over a follow up period of 11 years and fitted a model that was adjusted with common Framingham risk factors and then added cIMT to estimate absolute 10 year risk. The net reclassification improvement was small (0.8%; 95% CI, 0.1%-1.6%) and specifically in those at intermediate risk, it was 3.6% (95% CI, 2.7%-4.6%) (Den Ruijter HM, Peters SA et al. 2012). The conclusion of this meta-analysis was that while there was an increase in prediction, it was unlikely to be of any clinical use.

Another recent meta-analysis performed in the Netherlands also examined the association between cIMT and future vascular events. They too found that cIMT did not add to the predictive value of traditional risk factors (van den Oord, Sijbrands et al. 2013). While the meta-analysis revealed that a 1 SD increase in cIMT was predictive for myocardial infarction (HR 1.26, 95% CI 1.20–1.31), as well as for

stroke (HR 1.31, 95% CI 1.26–1.36), and the HR for the combined end point of MI and stroke was 1.26 CI 1.17-1.36, there was no significant increase in the areas under the curve increased from 0.726 to 0.729 ($p = 0.800$).

The IMPROVE study examined the use of both a range of cIMT measures and a measure known as interadventitia common carotid artery diameter (ICCAD). Using data from cohort study covering 5 European countries (3,703 subjects with a median age 64.4 years) with median follow up of 36.2 months, and 215 first cardiovascular events, they found that an average of 8 maximal cIMT measurements either alone or combined with ICCAD improved classification of events and nonevents better than common carotid IMT and they recommend a strategy involving cIMT and ICCAD in addition to Framingham risk factors in predicting risk (Baldassarre D, Hamsten A et al. 2012).

Table 1-5 Prediction of cardiovascular events by cIMT in epidemiological studies

Study	Subjects (n)	Outcome	Adjusted* HR for cIMT (95% CI)	RR (95% CI)
Kuopio Ischaemic Heart Disease Study (KIHD)	1257	MI	-	-
Atherosclerosis Risk in Communities (ARIC)	12841	MI	Women 5.07 (3.08-8.36) † Men 1.87 (1.28-2.69) †	-
Atherosclerosis Risk in Communities (ARIC)	14214	Stroke	Women 8.54 (3.52-20.74) † Men 3.62 (1.45-9.15) †	-
Cardiovascular Health Study (CHS)	4476	MI/ Stroke	-	3.15 (2.19-4.52) ‡
Rotterdam Study	5851	MI	-	Combined cIMT 1.38 (1.21-1.58) ‡
Rotterdam Study	5479	Stroke	-	2.23 (1.48-3.36) †
Malmo Diet and Cancer Study (MDCS)	5163	MI	1.23 (1.07–1.41) ‡	-
Malmo Diet and Cancer Study (MDCS)	5163	Stroke	1.21 (1.02-1.44) ‡	-
Carotid Atherosclerosis Progression Study (CAPS)	6962	MI/Stroke/death	1.16 (1.05-1.27) ‡‡	-
Lorenz Meta-Analysis	37197	MI Stroke	-	1.26 (1.21-1.3) ‡ 1.32 (1.27-1.38) ‡
Multi-Ethnic Study of Athersclerosis (MESA)	6698	All CVD	1.3 (1.1-1.4) ‡	-
Framingham Offspring Study	2965	All CVD	Mean CCA 1.13 (0.000-0.007) ‡ Max ICA 1.21 (1.13-1.29) ‡	-

CCA – common carotid artery, ICA – internal carotid artery, MI – Myocardial Infarction, CHD-coronary heart disease, CVD-cardiovascular disease HR hazard ratio, RR relative risk
*=age sex and risk factor adjusted, except ARIC – age, race and centre adjusted only,
†-highest vs lowest cIMT ‡ - per 1SD increase in cIMT ‡‡ - per 0.15mm increase in cIMT

1.3.9 Association of cIMT with incident stroke

As well as an association with coronary artery disease, studies have also examined the relationship between cIMT and stroke, given the more direct anatomical relationship between the carotid artery and the cerebral vasculature. In 2003, the Rotterdam study assessed both carotid plaque and cIMT. When they compared the association between both and incident stroke, they found that a cIMT >0.84mm was a stronger predictor than plaque, with a relative risk of 2.42 after adjusting for established CV risk factors (highest tertile versus lowest tertile) (Hollander M, Hak AE et al. 2003). Other studies that have produced results supporting this finding include the MDCS study which demonstrated a hazard ratio of 1.21 for cIMT in prediction of incident stroke. This was found to persist even when the results were adjusted for carotid plaque (Rosvall M, Janzon L et al. 2005). In support of this, Prati et al found that in their study of 1348 participants, common carotid artery cIMT >1mm had a RR of 10.4% versus cIMT <1mm for ischaemic stroke, TIA and vascular death (Prati, Tosetto et al. 2008). However, when they added cIMT or plaque to Framingham risk factors, IMT only improved prediction in those with a Framingham risk >20%. Chien et al found a similar HR per 1mm SD change in cIMT for stroke of 1.47 (1.28-1.69) in 2190 Chinese participants (Chien, Su et al. 2008).

Not all studies have supported the findings so resolutely. Both the ARIC and CHS studies have demonstrated a relationship between stroke and IMT that was not linear. In ARIC, whilst increasing cIMT and stroke incidence were associated, the relationship was not as strong when conventional risk factors were taken into consideration (Chambless LE, Folsom AR et al. 2000). These findings were mirrored by the CHS which demonstrated a non-linear relationship between cIMT quintiles (O'Leary DH, Polak JF et al. 1999).

In contrast, the more recent MESA study has published conflicting results on cIMT and stroke. In their 2008 publication, they report a hazard ratio per 1mm SD change in cIMT of 1.4 (1.2-1.8) for stroke in 6698 participants after median follow up of 3.9 years (Folsom, Kronmal et al. 2008). However, a subsequent paper published in

2011 from the same study (including 5520 participants) could not find an association between cIMT and stroke with a non-significant hazard ratio of only 0.89 (95%CI 0.8-1.3) after adjusting for traditional risk factors, although they did see an significant association for change in cIMT (Polak, Pencina et al. 2011)

1.3.10 cIMT and clinical risk prediction

Before cIMT can be considered for use in clinical practice, its ability to predict vascular events must be compared with the current accepted methods of risk prediction. The most common methods of risk prediction (as have already been discussed) are risk scores which incorporate the major cardiovascular risk factors. Therefore, cIMT must be compared with these factors. One way of assessing this is to add cIMT into models containing risk factors. This method has been recommended by the recent USPSTF statement (United States Preventative Services Task Force 2009). In addition, net reclassification index and clinical net reclassification can be assessed. This is the number of people who are reclassified based on risk. Clinical NRI applies specifically to those deemed to be at intermediate risk.

Several studies have adopted this approach, including ARIC and CAPS. In the ARIC data, adding both cIMT and plaque to traditional risk factors improved prediction of vascular events. Overall, 23% of cases had their risk reclassified. This resulted in a net reclassification index of 9.9% and clinical NRI of 21.7% (Nambi V, Chambless L et al. 2010). In addition, they found that measuring cIMT in the CCA was sufficient for using in clinical risk prediction (in comparison to all segment cIMT).

Not all studies have found such a positive finding and have cast doubt on the usefulness of cIMT in risk prediction. The CAPS study found that cIMT in addition to established risk factors, did not improve risk classification, with a net reclassification index of -1.41% (Lorenz, Schaefer et al. 2010). A 2010 narrative review of the data published on cIMT and plaque in risk prediction found that although cIMT did predict CHD independently, it contributed only modestly to risk prediction and they concluded that carotid plaque may be a more useful measure of vascular risk (Simon A, Megnien JL et al. 2010). A systematic review of examined

cIMT in addition to other novel risk markers and they had an opposite conclusion – that they found strong evidence that cIMT could improve risk prediction (Peters SAE, den Ruijter HM et al. 2012). The NRI of the studies included ranged from -1.4 to 11.6%.

Several guidelines have been highlighted by the Mannheim Consensus, which address the use of cIMT in assessing CVD risk in clinical practice including the Report of the National Cholesterol Education program Adult Treatment Panel III, which recommends its use to detect subclinical atherosclerosis, which aids decision making in the management of blood lipids (van den Oord, Sijbrands et al. 2013); and ESH/ESC guidelines used in Spain that recommend cIMT for the use in detecting target organ damage in hypertension (Antithrombotic Trialists' (ATT) Collaboration, Baigent C et al. 2009). However, the ACC/AHA published recommendations in 2013 that were not in support of cIMT in the assessment of cardiovascular risk of first CVD event, citing the basis for this decision as primarily a 2012 individual participant data meta-analysis published by Den Ruijter et al of the USE cIMT initiative (Den Ruijter HM, Peters SA et al. 2012), in combination with concerns about measurement accuracy (Goff, Lloyd-Jones et al. 2013).

1.3.11 cIMT progression and vascular risk

Progression or indeed regression of cIMT has been used as a surrogate cardiovascular outcome in the trials of several drugs (ENHANCE and METEOR trials). The assumption underlying its use is that progression of cIMT is associated with increased vascular risk and regression with decreasing risk, however, the evidence underlying this relationship is not clear. Several large meta-analyses have attempted to address this and the results have been mixed. A large meta-analysis recently published included 41 studies and addressed whether cIMT regression predicted reduction of cardiovascular events. The authors reported that although there was reduction in the number of events, there was no relationship between the regression of cIMT and the events, suggesting that reduction in cIMT does not reflect a reduction in cardiovascular events (Costanzo P, Perrone-Filardi P et al. 2010). A further meta-analysis including 28 studies identified that mean change in cIMT was

associated with a lower likelihood of non-fatal MI (Goldberger, Valle et al. 2010). However, this was noted only in selected RCTs that were included in their analysis. Because of these inconsistent findings, they conclude that while there is some evidence that change in cIMT is associated with change in event likelihood, they urge caution in the use of cIMT as a surrogate endpoint. In 2012, the PROG IMT collaboration published a meta-analysis of data from 36 948 individuals from 16 cohorts (Lorenz, Polak et al. 2012). The authors report that after mean follow up of 7 years, change in cIMT from two ultrasound scans was not associated with increased cardiovascular risk, and draw no firm conclusions on its use as a surrogate end point. They also reported that the "average" cIMT (the average of the two ultrasound readings) did independently associated with cardiovascular risk (adjusted HR 1.16 (1.10-1.22), suggesting that cIMT may still be useful in risk prediction rather than as a surrogate endpoint.

1.4 Carotid plaque

Atherosclerotic plaque development is the end product of atherosclerotic change in the vessel wall. Plaques form on the vessel wall as a consequence of exposure of the wall to vascular risk factors such as hypertension, dyslipidaemia or inflammation (see section 1.4). Interest in the presence of atherosclerotic plaques in the carotid artery is growing and there is interest in both plaque burden and plaque morphology. Several authors have suggested that plaque represents a more advance stage of disease, and as such may be more predictive of vascular events than cIMT (Ebrahim, Papacosta et al. 1999).

There are many different aspects of plaque that can be measured using carotid ultrasound. Plaque thickness is perhaps the simplest facet, and is primarily assessed by measuring either the thickness of the plaque itself or the degree of carotid stenosis – that is, the encroachment of the plaque on the lumen of the vessel. The Mannheim consensus defines plaque as a focal protrusion into the lumen of >1.5mm or >50% of the surrounding IMT (Touboul PJ, Hennerici MG et al. 2012). There is also interest in plaque area, plaque volume and plaque morphology, and the relationship of these aspects of plaque with cardiovascular disease. In terms of plaque morphology,

plaque echogenicity and structural appearance have attracted most interest, particularly when considered in the context of cerebrovascular disease. However, there has also been limited work that has assessed the impact of carotid plaque morphology on the future risk of coronary artery events.

1.4.1 Quantitative measures of carotid plaque

Similarly to cIMT, carotid plaque can be assessed using B mode ultrasound of the carotid artery. In addition, Doppler ultrasound can be used to assess stenosis of the artery lumen and make quantitative assessments of plaque. Several aspects of carotid plaque can be measured – maximum plaque thickness, total plaque area and total plaque volume are three commonly measurable variables. In addition, plaque morphology (ie the composition) can also be assessed. Plaque is sometimes described by the plaque "burden", which is a loose term used to describe the number, size and/or volume of plaques present at specific anatomical sites, commonly the carotid artery. Age is sometimes substituted for plaque burden however, it is the plaque burden which is the true risk factor – and individuals of the same age will have differing plaque burden. The different methods for measuring plaque are discussed below. Consensus statements have attempted to address plaque measurement but there are no firm recommendations. The Mannheim consensus suggests the measurement of the following plaque characteristics: Plaque location, thickness, area and number, scanned in longitudinal and cross-sections must be recorded, but highlight that there is not enough current evidence to support the measurement of plaque morphology as of yet (Touboul PJ, Hennerici MG et al. 2012).

Maximum plaque thickness

Maximum plaque thickness is the maximum thickness of any given plaque, from the media adventitial interface to the lumen-intima interface. It is similar to intima media thickness but is measured specifically in a focal plaque. The reading captures only the thickness of the plaque at the maximum point.

Total plaque area and plaque volume

Total plaque area involves measurement of the surface area of plaque in an artery. Similarly, total plaque volume is a measure of the volume of plaque present. Both can be estimated using carotid ultrasound, with total plaque area and plaque volume represent the 2D and 3D quantification of plaque burden, respectively; whereas plaque thickness represents only a 1 dimensional measure of plaque and is more alike to intima media thickness, even though it is a specific measure of a plaque (Pollex RL, Spence JD et al. 2005). Plaque area is typically measured on a cross sectional longitudinal image of the carotid artery and involves measurement of all visible plaques. The perimeter of each visible plaque is traced and the areas summed to create total plaque area (Spence, Eliasziw et al. 2002). Spence et al also performed a study in 1686 subjects where they measured total plaque area at baseline and followed them up for a mean of 2.5 ± 1.3 years for incident cardiovascular events (Spence, Eliasziw et al. 2002). After adjusting for baseline characteristics, they found that the risk of cardiovascular disease increased with each quartile of plaque area. They also noted that those people who showed a progression in plaque area with time had an increased risk of vascular events compared with those who had no change or regression (15.7% vs 7.6% and 9.4% respectively). An additional study by Spence et al found that over 1 year, plaque area increased by 2 times as much as plaque thickness, which suggests mean that plaque area may be a more sensitive indicator of atherosclerosis (Spence 2002).

Total plaque volume is a similar measure to plaque area but requires the use of 3D ultrasound to quantify the plaque volume along a longitudinal section of the carotid artery. 3D ultrasound uses a series of cross sectional slices which are added together to determine the volume. Thus, accuracy of volume is dependent on the number of cross sectional slices that are taken. Spence et al examined the reliability of 3d ultrasound measurement of plaque volume and found intra and inter-observer variability of 94% and 93.2% respectively (Landry, Spence et al. 2004). Measurement variation reduced as plaque size increased. A systematic review by Makris et al, identified 7 studies examining reproducibility of carotid plaque volume measurements using 3D ultrasound (Makris, Lavida et al. 2011). They found that 3D

ultrasound measurements were reliable although they note heterogeneity between studies and recommend that further evidence is needed to assess whether 3D ultrasound is superior to 2D ultrasound assessment of carotid plaque.

1.4.2 Plaque morphology

B mode ultrasound can also be used to assess the morphology of carotid plaque which can suggest the structure and composition of the plaque and there is evidence to suggest that plaque morphology might be useful in assessing how vulnerable a plaque is (Grønholdt MLM, Nordestgaard BG et al. 2001; Liapis, Kakisis et al. 2001; AbuRahma, Wulu et al. 2002; Honda O, Sugiyama S et al. 2004). Plaques which are more likely to rupture tend to be rich in lipid and haemorrhage (Epstein, Fuster et al. 1992; Davies 1996).

Assessing plaque morphology on ultrasound brings with it many similar limitations to those seen in intima media measurement. Classification of a plaque as echolucent or echogenic by a sonographer can be a subjective process, although the use of predetermined criteria can reduce this to some extent. However, the development of computer quantification of echolucency has helped to a great extent. Gray scale median (GSM) is measured after image normalization and there have been demonstrated links between low GSM (echolucent plaque) and plaque instability (Hall HA and Bassiouny HS 2012). The Mannheim Consensus update of 2012 notes that the value of recording plaque texture remains uncertain and that this is an area that requires further research (Touboul PJ, Hennerici MG et al. 2012) although some studies have started to explore this area. For example, Prati et al developed a risk score incorporating various aspects of plaque composition including stenosis degree, plaque surface irregularity, echolucency and texture. They found that the total plaque risk score was a powerful predictor of cerebrovascular events (Prati, Tosetto et al. 2011).

Echogenicity

Plaque echogenicity can be characterised on the basis of it echogenicity on ultrasound. Low echogenicity plaques appear on ultrasound as being black or nearly black (echolucent) and are usually referenced in comparison with blood. In contrast,

high echogenicity plaques appear as white (echogenic) and can be referenced with bone. Echolucent plaques tend to be lipid rich and are prone to rupture (Grønholdt MLM, Nordestgaard BG et al. 2001; Honda O, Sugiyama S et al. 2004), whereas echogenic plaques tend to be calcified and fibrinogen rich.

Heterogeneity

Plaque heterogeneity is a way of classifying the texture or composition of the plaque. Similarly to echogenicity, plaque heterogeneity can be classified into 2 broad categories. Heterogeneous plaque is defined as plaque with >20% of the plaque differing in echogenicity to the rest of the plaque. Homogeneous plaques have a structure that is consistent throughout. An example of a heterogeneous plaque might be a plaque with a lipid core that also possesses intra-plaque haemorrhage as well as a calcified portion. A homogenous plaque would be a plaque composed of only a lipid core. Heterogeneous plaques are also considered to be at increased risk of adverse clinical consequences (Petersen C, Pecanha PB et al. 2006).

Relationship of ultrasound appearance of plaques with histology

Several studies have demonstrated the link between B mode ultrasound appearance of plaques and histological appearance of plaque. Many of these studies are based upon work done in the exploration of the association between plaque type and carotid endarterectomy. In 1995, a large multi-centre study by the European Carotid Plaque Study Group published data on 270 carotid endarterectomy specimens and found that ultrasound determined plaque morphology was associated with histological findings. Plaque echogenicity on ultrasound was inversely associated with plaque composition (European Carotid Plaque Study Group and Sillesen 1995).

One must then look to examine the relationship between histological plaque appearance and future vascular risk. A paper by Hellings et al published in 2010 described the association between plaque histology and future cardiovascular risk in a prognostic study (Hellings WE, Peeters W et al. 2010). Subjects who had undergone carotid endarterectomy had their plaque specimens analysed histologically and those who had plaque haemorrhage or intraplaque vessel formation had a higher risk of the primary outcome (vascular event including CVD

death, non-fatal stroke and non-fatal MI, or vascular intervention) with a HR of 1.7 (95% CI 1.2 – 2.5) and 1.4 (95% CI 1.1 – 1.9) respectively. This relationship was independent of clinical risk factors and medication use. Interestingly, other histological aspects of plaque such as macrophage infiltration, large lipid core, calcifications, collagen and smooth muscle cell infiltration were not associated with an increased risk of a vascular outcome.

In 2007, a study was published of pathological specimens taken from symptomatic carotid endarterectomy patients that were examined using ultrasound prior to excision (Snow, Ben-Sassi et al. 2007). Of 33 predominantly echolucent plaques on USS, 27 were haemorrhage or lipid rich. Of 17 plaques that were characterised as echogenic, 11 were found to be predominantly fibrotic. They concluded that ultrasound plaque assessment may be useful in identifying potentially unstable (echolucent) plaques and help in the selection of individuals for carotid endarterectomy.

In 2011, a Japanese study that examined the ability of diagnostic tools to predict plaque type (in order to stream line selection of patients for carotid endarterectomy) demonstrated that plaque morphology on carotid ultrasound was closely associated with histological findings following carotid endarterectomy, although ultrasonography was not sufficient for all patients and they suggest that a combination of US and MRI could be useful (Arai, Yamaguchi et al. 2011).

1.4.3 Reproducibility of plaque assessment

An early study by Joakimsen et al examined the reproducibility of carotid plaque assessment using a ultrasound (Joakimsen O, Bønaa KH et al. 1997). They found that plaque occurrence displayed good inter and intra-observer agreement (K value 0.72 95%CI 0.6-0.84 vs 0.76 95%CI 0.63-0.89). There was only a moderate degree of agreement with regards plaque thickness, with mean absolute differences ranging from 0.25-0.55mm. Plaque morphology classification however showed a high degree of agreement. Therefore, ultrasound assessment seems to be a good way of assessing plaque occurrence and morphology but not such a good way of measuring plaque thickness.

1.4.4 Association of carotid plaque with vascular risk factors

Like cIMT, there have been several studies that have demonstrated an association between carotid plaque and cardiovascular risk factors. Increasing age is associated with increased plaque presence (Lemne, Jogestrand et al. 1995; Bonithon-Kopp, Touboul et al. 1996; Mannami T, Konishi M et al. 1997; Aminbakhsh, Frohlich et al. 1999; Homma S, Hirose N et al. 2001; Sun, Lin et al. 2002). Systolic blood pressure, smoking, total/HDL cholesterol ratio and BMI have also been associated with plaque presence (Bonithon-Kopp, Touboul et al. 1996; Mannami T, Konishi M et al. 1997; Aminbakhsh, Frohlich et al. 1999; Ebrahim, Papacosta et al. 1999; Sun, Lin et al. 2002). The British Regional Heart Study (BRHS) demonstrated a linear relationship between increasing plaque presence and increasing BRHS risk score (which contains traditional CV risk factors) (Zureik, Touboul et al. 1999).

In Canadian study of 168 Oji-Cree adults with an average age of 38.2 years, total plaque area was strongly associated with age, sex, smoking and total cholesterol, but not hypertension (Al-Shali K, House AA et al. 2005). Total plaque volume however was associated only with age, sex and diabetes.

A study by Ebrahim et al in 1999 showed that 57% of men and 58% of women had carotid plaque. Prevalence increased with age and cIMT was on average 0.1mm higher in people with plaque compared with those without and there was an inverse association between plaque prevalence and HDL cholesterol (Ebrahim, Papacosta et al. 1999). The presence of plaques was associated with cardiovascular risk factors (smoking, deprivation and fibrinogen), as well as prevalent vascular disease. A later study by Virani et al in 2011 found that in the ARIC study, maximum plaque thickness (defined as the maximum plaque thickness recorded in 12 segments of the carotid artery) was strongly associated with blood lipids including total cholesterol, LDL and HDL cholesterol after full risk factor adjustment (Virani SS, Catellier DJ et al. 2011). They also identified that plaques found on MRI to have a lipid rich core (those that would be described as echolucent on US) had a strong relationship with lipid ratios such as total/HDL ratio after full adjustment for CV risk factors.

Spence et al identified that the distribution of carotid plaque area increased with age and was higher in men than in women (Spence 2006).

Data from the PIVUS study that plaque size (as quantified by plaque volume) demonstrated different associations with vascular risk factors than plaque echogenicity (Andersson, Sundström et al. 2009).

Data from the Tromsø Study established that HDL cholesterol was associated with echolucent carotid plaques (adjusted OR 0.69 (95%CI 0.52-0.93) in 6727 participants in a population health survey (Mathiesen EB, Bønaa KH et al. 2001).

1.4.5 Carotid plaque and prediction of coronary events

Carotid plaque has been investigated in relation to prediction of coronary events. Because there is no definitive measure of carotid plaque, the studies vary in exactly which measure is used in prediction. Several studies have linked carotid plaque with an increased risk of coronary events (Honda O, Sugiyama S et al. 2004; Seo Y, Watanabe S et al. 2006; Reiter, Effenberger et al. 2008). Honda et al established that echolucent (lipid rich plaques) as identified by low integrated backscatter on US predicted coronary artery plaque complexity and also the development of coronary artery complication sin a sample of 71 individuals with ACS and 215 with stable CAD.

A German study by Reiter et al examined echolucency and the risk of major cardiovascular events in high risk patients. They determined the change in plaque composition over 7.5 months and found an association with future major cardiovascular events in those people with increasing plaque echolucency. However, there was no relationship between absolute levels of GSM at either baseline or follow up (Reiter, Effenberger et al. 2008).

In a related study, Swedish investigators examined the relationship between non-stenotic *femoral* plaques and future vascular risk. They used B mode ultrasound to assess plaque in the femoral arteries and record plaque occurrence, plaque size and plaque characteristics including echogenicity and echolucency. They found that plaque occurrence and size were predictive of future vascular events, as was plaque

echolucency to some extent, although there was no significant difference between echogenic and echolucent plaque (Schmidt, Fagerberg et al. 2005).

Spence et al also found an association between plaque area and risk of stroke, MI or death. In a study of 918 patients, the higher quartile of plaque area was associated with a 3 fold increase in risk for stroke, MI or death (Spence 2002).

Hirano et al published a study of 413 patients with CAD and carotid plaque and assessed the predictive ability of plaque echolucency and plaque size for incident coronary events (Hirano M, Nakamura T et al. 2010). They found that in multi-risk factor adjusted models, the model with both plaque echolucency and plaque thickness was a better predictor of incident events (AUC 0.80) than either the model with just plaque thickness (AUC 0.74) and the model with only plaque echolucency (AUC 0.76).

The MESA study explored carotid plaque metrics measured using ultrasound (plaque presence, thickness and stenosis) in relation to incident CVD. They demonstrated that all measures of plaque were predictive of coronary artery disease and cardiovascular disease but not stroke (Polak, Szklo et al. 2013). The authors also found that the NRI for CVD was less than that for CHD suggesting plaque was more useful in predicting CHD. More recently, the authors reported the results of a study of carotid plaque morphology determined by magnetic resonance imaging in relation to incident vascular disease (Zavodni, Wasserman et al. 2014). The authors reported that when MRI plaque remodeling index and lipid core were added to a model containing traditional risk factors, the AUC was increased from 0.696 to 0.734, suggesting the plaque characteristics were useful in improving prediction of cardiovascular risk.

1.4.6 Carotid plaque and prediction of stroke

Carotid plaque is known to be a pathological cause of stroke and TIA, with narrowing of the carotid arteries by enlarging plaques causing critical stenosis and reduction in blood flow to the brain (ischaemic stroke). In addition, thrombus or plaque material may embolise and are carried straight to the cerebral arteries, where

partial or complete occlusion can lead to stroke. The mechanism is dependent on the characteristics of the plaque present in the carotid artery. Stable, fibrotic plaques tend to cause stenosis whereas lipid rich, haemorrhagic plaques are more likely to rupture and embolise their contents. Thus plaque morphology has been explored in relation to stroke, with several studies reporting that when compared with echogenic plaques, echolucent plaque has been shown to be associated with a higher risk of cerebrovascular ischaemic events (Pedro LM, Pedro MM et al. 2000; Grønholdt MLM, Nordestgaard BG et al. 2001; Mathiesen EB, Bønaa KH et al. 2001). .

In a 1998 study, Polak et al reported that hypoechoic (echolucent) plaque was associated with incident stroke (RR 1.72) after adjusting for cardiovascular risk factors (Polak JF, Shemanski L et al. 1998). The authors also reported that stenosis was associated with incident stroke. Additionally, Hollander et al published data from the Rotterdam study in 2002 which demonstrated a relationship between carotid plaque, stroke and cerebral infarction. In 4217 asymptomatic adults older than 55 years of age, carotid plaque at any area increased the risk of stroke and cerebral infarction (Hollander M, Bots ML et al. 2002). In support of these results, Shaalan et al have reported that plaques with more calcification were more stable and were associated with a lower risk of ischaemic events than plaques with less calcification (Shaalan, Cheng et al. 2004).

There is also evident that heterogeneous plaque is associated with an increased risk of stroke. Petersen et al established a relationship between heterogeneous plaque and death in a study of 541 hospitalised cardiology patients in Pisa, Italy (Petersen C, Pecanha PB et al. 2006). In multifactorial models, plaque heterogeneity had a relative risk of 1.6 95% CI 1.2-2.14) for death.

Some studies have examined multiple facets of carotid plaque. Mathieson et al showed that in the Tromsø study, echolucent plaques were associated with an increased risk of future cerebrovascular events, independent of the degree of arterial stenosis and cardiovascular risk factors (Mathiesen EB, Bønaa KH et al. 2001). They also determined that total plaque area appeared to be predictive for first ever stroke, with fully adjusted HR (for highest quartile vs no plaque) of 1.73 (95%CI

1.19-2.52) for men and 1.62 (95%CI 1.04-2.53) for women (Mathiesen EB, Johnsen SH et al. 2011). Similarly, Prati et al investigated the ability of carotid plaque morphology to predict future stroke over and above Framingham Risk factors. They developed a plaque score (total plaque risk score (TPRS)) that included degree of stenosis, plaque surface irregularity, echolucency and texture. They found that a high TPRS was a strong predictor of cerebrovascular events. When they added TPRS to conventional Framingham risk factors, the area under the ROC curve was significantly increased compared with just the Framingham risk factors (0.90 vs 0.88, p=0.04) (Prati, Tosetto et al. 2011).

1.5 Carotid plaque or cIMT in prediction of vascular risk?

Carotid intima media thickness and carotid plaque both represent alteration to the artery wall but their differing relationships with cardiovascular risk factors and CVD mean it is unclear which might be of most use in the prediction of vascular events. Indeed, Ebrahim et al hypothesised in 1999 that it may be the presence of plaque at the carotid bifurcation, rather than the cIMT, that represents a higher risk of disease and suggested that efforts be made to address this (Ebrahim, Papacosta et al. 1999).

It has been argued that carotid intima media thickness does not represent early atherosclerosis, with much of the thickening being due to medial hyperplasia in response to hypertension, and that rather, it may in fact represent a precursor to atherosclerosis (Spence JD 2012). Plaque on the other hand is a manifestation of atherosclerosis, so it has been suggested that carotid plaque might be a better choice as a predictor of future vascular disease than intima media thickness (Inaba, Chen et al. 2012).

In 2011, Mathieson et al explored the association between carotid plaque, cIMT and first ischaemic stroke in the Tromsø Study (Mathiesen EB, Johnsen SH et al. 2011). They followed up 3240 men and 3344 women over a 10 year period after measuring cIMT and carotid plaque at baseline. They found that carotid plaque area had a higher hazard ratio for stroke than cIMT per 1 SD increase (for men and women respectively, HR 1.23 95% CI 1.09–1.38 and 1.19 (95% CI, 1.01–1.41 vs HR 1.08

95% CI 0.95–1.22 and HR 1.24 95% CI 1.05–1.48). In addition, there was a higher risk for those in the highest quartile of plaque area versus no plaque (HR 1.73 95% CI 1.19–2.52 for men and 1.62 95% CI 1.04–2.53 for women) whilst there was no difference across the quartiles of cIMT. They also demonstrated that plaque area was a better predictor for MI than cIMT in the general population (Johnsen, Mathiesen et al. 2007).

Other studies that have examined the predictive ability of plaque versus cIMT were summarized in a meta-analysis by Inaba et al that compared cIMT with carotid plaque in the prediction of coronary artery events. They identified 11 population based studies and found that carotid plaque was better at predicting future MI than cIMT. They also undertook a meta-analysis of 27 diagnostic studies that examined coronary artery disease and found an increased, but non-significant, diagnostic odds ratio of carotid plaque for detecting CAD (Inaba, Chen et al. 2012).

A study by Nambi et al used data from the ARIC study to examine the effect of adding cIMT and carotid plaque to TRFs in cardiovascular risk prediction (Nambi V, Chambless L et al. 2010). They found that net reclassification index was similar for TRFs + cIMT and TRFs + plaque. However, NRI was higher when both cIMT and plaque were added to TRFs, suggesting that adding both cIMT and carotid plaque to TRFs would improve risk stratification.

The emerging evidence that plaque may be more predictive of vascular events led to an editorial comment by J. David Spence (Spence JD 2012) on the meta-analysis performed by Inaba et al (Inaba, Chen et al. 2012) in which he highlights an important point – why is plaque not measured at the same time as cIMT, as no additional equipment is required? Therefore, the evidence is not clear as to which might be of more use and indeed, whether or not combing cIMT and plaque would in fact provide additional value.

1.6 cIMT and Type 2 diabetes

Given that cardiovascular risk scores are known to underperform in people with Type 2 diabetes, and the continued excess morbidity from vascular disease, there is a need to find additional sources of information regarding future vascular risk in this group. The United States Preventive Services Task Force (UKPSTF) released a recommendation statement regarding the use of non-traditional risk factors in the prediction of cardiovascular risk. Carotid intima media thickness is included in their list of non-traditional risk factors, along with high-sensitivity CRP (hs-CRP), ankle-brachial index (ABI), leukocyte count, fasting blood glucose level, periodontal disease, coronary artery calcification (CAC) score on electron-beam computed tomography (EBCT), homocysteine level, and lipoprotein(a) level. They highlight that there is still insufficient evidence to make a definitive statement on the use of non-traditional risk factors, including cIMT, and highlight where there are still gaps in the available research. With regards diabetes, they stated that there is a need to clarify clinicians' views on whether diabetes is a CHD risk equivalent state or is instead a risk factor for CHD. As a result, they suggest that non-traditional risk factors associations with cardiovascular risk should be assessed in diabetic populations (United States Preventative Services Task Force 2009).

1.6.1 cIMT in Type 2 diabetes

Several studies have demonstrated increased cIMT in people with Type 2 diabetes in comparison with non-diabetic individuals. A 1994 study by Pujia et al measured cIMT in 54 subjects with NIDDM and 54 controls and found that cIMT was significantly higher in subjects with NIDDM compared with controls (0.765 vs 0.692mm respectively), along with higher plasma triglycerides and lower HDL cholesterol (Pujia, Gnasso et al. 1994). cIMT was positively correlated with age, sex and negatively correlated with HDL cholesterol. Another early article published by Niskanen et al in 1996 demonstrated that in 84 individuals with NIDDM compared with 119 non-NIDDM individuals, those with diabetes had an increased cIMT at both the common carotid and bifurcation (Niskanen L, Rauramaa R et al. 1996).

In 2006, Brohall et al conducted a systematic review and meta-analysis of studies examining IMT in Type 2 diabetes (Brohall G, Odén A et al. 2006). They identified 23 studies comprising 24111 subjects. The overall finding was that subjects with diabetes had a cIMT which was on average 0.13mm higher (95% CI: 0.109–0.1310) than subjects without diabetes. In addition, those with impaired glucose tolerance (IGT) also had a significantly higher cIMT, on average 0.04mm. In a follow up study by Brohall et al, they examined cIMT in people with IGT compared with controls and in addition performed a meta-analysis. They found that in the case control sub study, there was no increase in the occurrence of subclinical atherosclerosis. The meta-analysis revealed a contrasting result, demonstrating a small increase in the CCA IMT in people with IGT (Brohall G, Schmidt C et al. 2009). Einarson et al performed a meta-analysis examining the relationship between cIMT and blood glucose. They used 15 592 patients from 11 studies and found a correlation between cIMT and post-prandial glucose. The effect size between diabetics and normal was 0.294 (0.245-0.343) and between IGT and normal was 0.137 (0.072-0.202) (Einarson TR, Hunchuck J et al. 2010). These results suggest that there is a small but significant relationship between glucose and cIMT. A 1999 study by Temelkova-Kurktschiev et al also found that cIMT was significantly higher in 71 people with newly diagnosed Type 2 diabetes when compared with matched controls (mean cIMT 0.95 vs 0.85mm respectively) (Temelkova-Kurktschiev, Koehler et al. 1999).

Lee et al have also demonstrated that people with diabetes had a higher cIMT than those without diabetes, and indeed, those with diabetes and CAD had a higher cIMT than those with diabetes and no CAD, and both had higher cIMT than non-diabetic individuals (Lee E, Emoto M et al. 2009). On multifactorial logistic regression, they found that a high cIMT >1.3mm was associated with concurrent CAD (OR 2.205 (1.52-3.20) $p<0.001$).

A Spanish study published in 2011 compared cIMT in people with diabetes, people with hypertension and controls. They found that cIMT was greatest in people with diabetes compared with those with hypertension and controls (0.781mm v 0.738mm

and 0.686mm respectively), although progression of cIMT was greatest in people with hypertension (Gómez-Marcos, Recio-Rodríguez et al. 2011).

1.6.2 Association of cIMT with CV risk factors in people with Type 2 diabetes

Before cIMT can be investigated in association with incident disease, it is important to establish the relationship between cIMT and cardiovascular risk factors. The use of cIMT as a surrogate marker in clinical trials suggests that cIMT is known to be associated with cardiovascular risk factors and cardiovascular disease in people with Type 2 diabetes. This section describes published literature on the relationship between cIMT and risk factors in people with Type 2 diabetes. While there are several papers that report this, very few publications address this topic as a primary aim.

In their study of normotensive Type 2 diabetic participants, Kong et al found a positive association of cIMT with increasing age, male sex and smoking (Kong, Elatrozy et al. 2000), while Wagenknecht et al have reported an association between cIMT and duration of diabetes in their cross sectional study of 489 people with Type 2 diabetes (Wagenknecht, D'Agostino et al. 1997)

Niskanen et al demonstrated in 1996 that mean cIMT in 84 older adults with diabetes was associated with a variety of factors including post-glucose 1 hour plasma insulin, serum LDL triglyceride and apolipoprotein B (Niskanen L, Rauramaa R et al. 1996). However, in 2000, Guvener et al found reported that the major determinants of cIMT in people with Type 2 diabetes were age and BMI (Güvener, Tütüncü et al. 2000).

Smoking has also been associated with cIMT in diabetes as in the general population. Karim et al found that mean carotid IMT was higher in smoking than in non-smoking subjects with diabetes, although the difference was not statistically significant after adjusting for age, gender and other confounders (Karim, Buchanan et al. 2005).

A 2006 cross sectional study by Yokoyama et al assessed the relationship between components of the metabolic syndrome and cIMT in people with Type 2 diabetes and no history of CVD (Yokoyama, Kuramitsu et al. 2007). They found that cIMT

increased according to the number of metabolic syndrome components present (p=0.002) and the presence of higher blood pressure and higher abdominal obesity influenced cIMT.

The association between glucose fluctuations in people with Type 2 diabetes and cIMT was explored by Chen et al in 2010 (Chen, Zhang et al. 2010). Using data obtained from continuous glucose monitoring, a case control study of 36 subjects with diabetes and 10 controls several glucose fluctuation parameters increased with increasing cIMT and the authors hypothesize that glucose fluctuations may accelerate the development of atherosclerosis in older people with Type 2 diabetes. This echoes the findings of a 1999 study that examined the association of post prandial glucose levels with cIMT in 403 people aged 40-70 years without diabetes. They found an association between post prandial hyperglycaemia and increased cIMT, suggesting that hyperglycaemia exerts a damaging effect on the endothelium (Hanefeld, Koehler et al. 1999). A similar study by Esposito et al examining post meal glucose peaks found that incremental glucose peaks were correlated with cIMT (Esposito K, Ciotola M et al. 2008).

A 2009 study by Butt et al examined cIMT in 200 people with Type 2 diabetes and described the association with cardiovascular risk factors. cIMT was only correlated with duration of diabetes, BMI and HDL cholesterol, with an inverse correlation being seen in the case of HDL cholesterol (Butt MU and Zakaria M 2009). A study in 380 Korean subjects with newly diagnosed Type 2 diabetes assessed the association between cardiovascular risk as determined by the UKPDS risk engine and cIMT, among other non-invasive markers (Seon, Min et al. 2011). After adjusting for age, both the 10 year CHD risk and 10 year stroke risks correlated with cIMT. In addition, cIMT correlated with age, HbA1c and gender.

Inflammatory markers have also been shown to be associated with cIMT in people with diabetes. A study of 105 patients with Type 2 diabetes by Kang et al demonstrated that serum high sensitivity CRP was correlated with parameters of cIMT and also BMI, WHR as well as plasma lipids (Kang ES, Kim JH et al. 2004).

1.6.3 Association of cIMT with prevalent vascular disease in people with Type 2 diabetes

As well as an association with cardiovascular risk factors, it has been important to establish an association with cIMT and cardiovascular disease in other vascular beds.

In 2003, Hunt et al examined carotid intima-media thickness in 1127 non-diabetic, 66 pre-diabetic and 303 diabetic individuals (Hunt KJ, Williams K et al. 2003). They found that age and sex adjusted mean cIMT of both the ICA and CCA was higher in those with pre-diabetes than those without diabetes, although only the ICA IMT remained significantly higher after adjustment for established CV risk factors.

An early Japanese study examined the relationship between cIMT and coronary artery disease in 80 people with Type 2 diabetes (40 with CAD determined by coronary angiography and 40 with no known CAD) (Mitsuhashi, Onuma et al. 2002). Those with known CAD had significantly higher cIMT compared with those without CAD. In addition they noted that cIMT was higher in those who had had CABG compared with those who had another procedure. cIMT, along with hypertension, hyperlipidaemia and hyperuricaemia, was significantly associated with prevalent CAD on forward stepwise logistic regression, although this was not significant when all variables were entered on logistic regression.

Lee et al examined cIMT and plaque in relation to ischaemic stroke in patients with Type 2 diabetes (Lee EJ, Kim HJ et al. 2007). They found that those subjects with MR diagnosed stroke had significant differences in sex, current smoking, hypertension and HDL cholesterol compared with controls. Those with ischaemic stroke had significantly higher mean CCA IMT compared to controls. On logistic regression, there was an association between mean cIMT and ischaemic stroke (OR 5.29 (95% CI 1.05-26.7)), although this relationship did not persist after adjustment for cerebrovascular risk factors (OR 1.64 (95% CI 0.14-19.4)). A similar pattern was seen for max cIMT.

In 2009, Djaberi et al assessed cIMT in 150 asymptomatic diabetic patients, who also underwent CT coronary angiography. cIMT was increased in people with obstructive stenosis compared with no atherosclerosis and they found a cut off cIMT

value of 0.67mm gave a good sensitivity and specificity when predicting obstructive coronary stenosis. cIMT was an independent predictor of coronary atherosclerosis ($p<0.01$) (Djaberi R, Schuijf JD et al. 2009). In 2010, they examined data evaluating the relationship between cIMT and myocardial perfusion in 98 people with Type 2 diabetes. Increased cIMT was associated with abnormal myocardial perfusion on SPECT scanning. An increased cIMT was associated with both the extent of abnormal perfusion, measured by summed stress score (SSS), and the prevalence of abnormal perfusion (Djaberi, Schuijf et al. 2010).

In a small study of 91 Japanese subjects with Type 2 diabetes, multi-slice CT coronary angiography and carotid ultrasound were used to assess coronary artery stenosis and carotid IMT (Kasami, Kaneto et al. 2011). Those subjects that demonstrated a higher degree of coronary stenosis had a higher max cIMT than those with a lesser degree of stenosis, and for the middle grades of stenosis this relationship persisted after adjustment for age, sex, duration of diabetes, hypertension and dyslipidaemia.

1.6.4 cIMT and risk prediction in diabetes

The association between cIMT and future vascular risk in people with Type 2 diabetes has not been well documented. The literature was systematically searched to identify publications reporting the results of longitudinal studies investigating cIMT as a potential predictor of cardiovascular risk in people with Type 2 diabetes.

Search strategy

Titles and abstracts of studies listed in Medline and Pubmed from 1946 until 31st December 2013 were searched using the following search terms: [(carotid intima media thick* OR carotid atherosclerosis) AND (cardiovascular* OR coronary heart* OR stroke OR myocardial inf* OR MI) AND (type 2 diab* OR niddm)]. Reference lists of key papers and reviews were searched for additional sources.

Selection Criteria

Criteria for selection were: 1) studies of humans, 2) studies of type 2 diabetes, 3) ascertainment of incident cardiovascular events, 4) cIMT measured using ultrasound and 5) reports results in addition to risk factors.

Included studies

579 studies were identified. The titles and abstracts were assessed for inclusion using the selection criteria. Six studies were identified that met the criteria. Of those, 4 were prospective studies and 1 was a retrospective study. A summary of the included studies is provided in table 1-6.

Table 1-6 Summary of studies of cIMT and cardiovascular risk that were identified by literature search and met selection criteria

	Study Design	Follow up (years)	N	Age	CVD outcome	CIMT measure
Yamasaki	Prospective	3.1	287	61.6	CHD or cerebrovascular	CCA, ICA, bifurcation
Bernard	Prospective	5	229	55.5	CVD and CAD	CCA
Ataoglu	Retrospective	10	102	53	CVD	CCA
Malik	Prospective	6.4	881	65	CHD and CVD	CCA and ICA
Yoshida	Retrospective	5.4	783	-	CVD	CCA
Den Ruijter	Meta-analysis	8.7	4220	61	CVD (MI or stroke)	CCA

Japanese researchers measured cIMT in 287 subjects with Type 2 diabetes who were free of cardiovascular disease (Yamasaki Y, Kodama M et al. 2000). They measured it at baseline and after mean follow up of 3.1 years. They noted an annual

progression of cIMT of 0.04±0.004mm/year and identified risk factors that were associated with progression of cIMT. The risk factors identified were baseline cIMT, HbA1c and age. In addition they examined the factors that predicted incident non-fatal CHD and found that after adjusting for risk factors, baseline cIMT and a low HDL level were independent predictors.

In 2005, Bernard et al published a study of cIMT in 229 patients with Type 2 diabetes aged 35-75 years who were free of any cardiovascular complications but with at least one cardiovascular risk factor. They found that age, cIMT, carotid plaques, number of plaques, Framingham Risk score and a suboptimal exercise tolerance test were associated with incident vascular events. cIMT was an independent predictor of CV events and the predictive ability of cIMT was similar to that of the Framingham score. When added to a Cox model containing Framingham risk score, the combination of cIMT and Framingham risk factors improved prediction of events compared to Framingham risk factors alone (Bernard S, Sérusclat A et al. 2005). This suggests that using cIMT to predict future vascular risk in people with Type 2 diabetes may be of use.

In 2009, a Turkish group published the results of a retrospective cohort study that addressed the addition of cIMT to FRS for predicting vascular events in Type 2 diabetes (Ataoglu, Saler et al. 2009). They found that in 102 subjects who were followed up for 10 years, cIMT could be useful in addition to Framingham risk scoring. However, caution must be applied to these results as they are the results of a retrospective cohort and the effect of bias and confounding may be greater in this cohort than in a prospective study.

More recently, a study by the MESA researchers included 881 individuals with diabetes (Malik S, Budoff M et al. 2011). The authors examined the association of both cIMT and coronary artery calcium with CAD and CVD over 6.4 years of follow up. Conversely to previous studies, they identified that cIMT was not predictive of events in people with Type 2 diabetes (HR 1.7 (0.7-4.3) for 4^{th} vs 1^{st} quartile of cIMT).

In 2012 another retrospective Japanese study, including 738 people with T2DM but free of CVD, demonstrated that cIMT added to the predictive ability of the FRS in the study sample (Yoshida, Mita et al. 2012). They used Cox proportional hazards models to examine the predictive ability of cIMT and brachial-ankle pulse wave velocity for cardiovascular events and found that cIMT and not abPWV predicted the events. They then examined the additional effect of cIMT on FRS and found that adding cIMT to FRS improved prediction of cardiovascular events.

The smaller sample size of the previous study was tackled by a large meta-analysis published by the USE-IMT collaboration, which demonstrated that in a cohort of 4420 people with diabetes identified from the cohorts in the collaboration, cIMT did not add to cardiovascular risk prediction in individuals with diabetes (Den Ruijter, Peters et al. 2013). While, the authors reported an adjusted hazard ratio of 1.22 (1.14-1.32) of cIMT for incident cardiovascular events, the effect on reclassification was limited. They identified that a model containing Framingham risk factors alone had an AUC of 0.68 and that on addition of cIMT, AUC increased to 0.69, while the net reclassification of the cIMT model was small (1.7%). However, this study has several limitations. Because the individuals are drawn from general population cohorts, the definition of diabetes varied over each study which may lead to inaccuracy in the diagnosis of diabetes in the group. They also identify other limitations. Information regarding severity or duration of diabetes is missing and data acquisition protocols will have varied with each study, leading to cIMT measurement variation across the studies (Den Ruijter, Peters et al. 2013). The authors additionally note that they have not addressed carotid plaque in their analysis, which may be a potential avenue for future exploration in people with diabetes.

Summary

This search of the literature has confirmed that the evidence for the use of cIMT as a predictor of cardiovascular events in people with Type 2 diabetes is limited and mixed. There is heterogeneity among the studies in terms of the population studied and populations tend to be at the older end of the spectrum of diabetes. The results of

the meta-analysis, when taken into context with current research in cIMT in the general population, suggests that cIMT may not be as useful as first thought in the prediction of cardiovascular risk. However, the answer is still not clear cut and this will be only be addressed by replication of results in further diabetes populations.

1.7 Carotid plaque and Type 2 diabetes

Although there is a growing body of evidence for the use of carotid plaque in the prediction of cardiovascular risk in the general population, as described in section 1.4 of this chapter, carotid plaque in persons with diabetes has been not been as extensively studied.

1.7.1 Association of carotid plaque with CV risk factors and prevalent CVD in people with Type 2 diabetes

There are few studies specifically examining the relationship between carotid plaque and cardiovascular risk factors in individuals with Type 2 diabetes. In 2012, Cardoso et al reported the independent associations of cardiovascular risk factors with plaque score (a composite of plaque presence and degree of stenosis) in 441 people, aged up to 80 years, with Type 2 diabetes (Cardoso, Marques et al. 2012). They found that the main correlates of plaque score were older age, cigarette smoking and the use of antihypertensive medications.

Additionally, a recent prospective study by Irie et al found that gender, BMI and low-HDL-cholesterol are important determinants of the content of the vascular wall in diabetic subjects (Irie, Katakami et al. 2014).

Pollex et al demonstrated that people with Type 2 diabetes and impaired glucose tolerance had greater plaque development than normoglycaemic controls (Pollex RL, Spence JD et al. 2005). That study examined plaque in 49 Oji-Cree Canadians and 49 age and sex-matched controls and measured total plaque volume. They found that total plaque volume and cIMT were only moderately correlated, and interestingly noted that use of carotid plaque as an outcome would require a smaller sample size than that of cIMT (Pollex RL, Spence JD et al. 2005). However, it was noted that the

difference between the 2 groups in baseline plasma lipid levels could explain the difference in plaque volume seen.

A 2007 study by Ostling et al performed carotid ultrasonography on people with a known right carotid artery plaque (47 people with diabetes and 51 without diabetes). They assessed plaque echogenicity using standardised gray-scale median values (GSM). A lower GSM indicates a more echolucent plaque. The investigators noted that GSM values were significantly lower in the group with diabetes, compared with those without diabetes (37.0±14.8 v 45.5±15.4, P=0.007), indicating a higher prevalence of echolucent plaques in subject with diabetes. When they looked at the association between plaque echogenicity and vascular risk factors, they found that only triglycerides were associated with plaque echogenicity (Ostling G, Hedblad B et al. 2007).

Several studies have examined the relationship between carotid plaque and prevalent disease (both presence and extent). Akazawa et al performed B mode ultrasound of the carotid artery and multi-slice CT coronary angiography on 277 Japanese subjects with Type 2 diabetes but without known coronary artery disease. They created a plaque score (the sum of plaque thickness in the carotid artery) and found that when plaque score was inserted into a multiple regression model containing traditional cardiovascular risk factors, it was a significant predictor of extent of disease in the coronary artery (Akazawa, Tojikubo et al. 2012). Lee et al performed a cross sectional study to examine the CCA-cIMT and plaque score in relation to ischaemic stroke in people with Type 2 diabetes (Lee EJ, Kim HJ et al. 2007). Using brain MRI and carotid ultrasonography, they found that those with Type 2 diabetes and acute stroke had a higher CCA-cIMT and plaque score than those with diabetes who were free of stroke. Unadjusted odds ratios suggested that carotid plaque scores were risk factors for acute ischaemic stroke in people with Type 2 diabetes (OR 3.14 (95% CI 1.67-5.93). However, once cerebrovascular risk factors were accounted for, the association became non-significant (OR 2.14 (95% CI 0.80-5.73).

A Chinese study performed dual source computer tomography angiography in 125 people with Type 2 diabetes and suspected cerebrovascular disease (He C, Yang Z et

al. 2010). They examined the prevalence of carotid and cerebrovascular plaques. They found that 91.2% of subjects (n=114) had atherosclerotic plaques detected. 45% of those plaques were non-calcified, 39% were calcified and 16% were of mixed composition. In addition, they noted that there was an extensive distribution of plaque – 55.8% of people had a 1-5 diseased segments, and 30.7% had 6-10 diseased segments. The most common site of plaque was the cavernous ICA.

A small Czech study of 38 subjects who underwent stress myocardial single positron emission computed tomography (SPECT) reported an association between ultrasound detected atheromatous plaque and an abnormal SPECT scan (Charvat, Michalova et al. 2006). An abnormal SPECT scan suggests a myocardial perfusion defect, suggesting ischaemic heart disease. And so this result suggests that carotid plaque is associated with prevalent ischaemic heart disease in this study. The major limitation of this work is the study sample size but it does give an indication of a relationship between plaque and cardiovascular disease in people with Type 2 diabetes.

1.7.2 Carotid plaque and risk prediction in people with Type 2 diabetes

Publications reporting the prospective association between carotid plaque and future vascular risk in people with Type 2 are sparse. The literature was systematically searched to identify publications reporting the results of longitudinal studies investigating carotid plaque as a potential predictor of cardiovascular risk in people with Type 2 diabetes

Search Strategy

Titles and abstracts of studies listed in Medline and Pubmed from 1946 until 31st December 2013 were searched using the following search terms: [(carotid plaque OR carotid atherosclerosis) AND (cardiovascular* OR coronary heart* OR stroke OR myocardial inf* OR MI) AND (type 2 diab* OR niddm)]. Reference lists of key papers and reviews were searched for additional sources.

Selection Criteria

Criteria for selection were: 1) original research, 2) studies of humans, 3) studies of type 2 diabetes, 4) ascertainment of incident cardiovascular events, 5) carotid plaque measured using ultrasound and 6) reports results in addition to risk factors.

Included Studies

529 studies were identified. The titles and abstracts were assessed for inclusion using the selection criteria. Two studies were identified that met the criteria. A summary of the included studies is provided in table 1-7.

Table 1-7 Summary of studies of carotid plaque and cardiovascular risk that were identified by literature search and met selection criteria

	Study Design	Follow up (years)	N	Age	CVD outcome	Plaque measure
Katakami	Prospective	7.9	85		CVD	Echolucency
Irie	Prospective	4.6	287	65	CVD	Echolucency

Only 2 prospective publications were identified. The first publication by Katakami et al, reported the results of a pilot study in 85 adults with Type 2 diabetes who were asymptomatic of cardiovascular disease and who were followed up for around 8 years. Using the calibrated-IBS (a measure of the echolucency of a plaque) and plaque thickness to assess carotid plaque, the authors report that incident events were higher in individuals with low calibrated IBS values. They also report that both calibrated IBS value and plaque thickness were independently predictive of events even after adjusting for Framingham risk score (HR 0.80 (0.7-0.9) and 1.94 (1.12-3.21) respectively) (Katakami, Takahara et al. 2012). The negative relationship between IBS and risk is because a low IBS value represents more echolucent (ie high risk) plaque.

Building on the results of the pilot study by Katakami, Irie et al published data on a study of 287 adults with diabetes. Plaque echolucency was determined using gray scale median (a computer aided method of quantifying plaque echogenicity) (Irie, Katakami et al. 2013). The advantage of this method over the calibrated IBS method used in their pilot study (Katakami, Takahara et al. 2012) was that it did not require specific software or hardware and so might be a more generalizable method. The authors reported risk factor adjusted hazard ratios of 4.55 for echolucent plaque and 1.44 for plaque thickness in a model for cardiovascular events. Adding plaque thickness to traditional risk factors significantly increased the area under the curve (AUC 0.60-0.73, $p<0.05$) and addition of plaque echolucency to both risk factors and plaque thickness increased the AUC to 0.82 ($p<0.05$).

Summary

The systematic review performed here highlights just how lacking data for the use of carotid plaque in risk prediction in people with Type 2 diabetes is. While the results of the study presented above are promising, the small sample size and specific population under study limit the generalizability of the results. The evidence base in the general population is also small and it is still not clear whether cIMT or plaque may yield better prediction for CVD. What is clear is that much work is required to characterise this relationship in people with Type 2 diabetes before any firm conclusions can be made.

1.8 Chapter summary

Cardiovascular disease is a major cause of morbidity and mortality. It is particularly prevalent in individuals with Type 2 diabetes, and despite increased management of established cardiovascular risk factors, it is still the main cause of death in this group. Identification of people with Type 2 diabetes who are at higher risk may allow for more intensive management of risk factors and reduce the occurrence of events. However, risk scores do not adequately predict risk in people with Type 2 diabetes so researchers have turned to non-invasive markers of risk in attempts to improve prediction. One such marker is carotid intima media thickness, which is frequently used as a surrogate marker of vascular disease in clinical trials, and there is evidence

from general population studies that this may be useful in addition to current risk prediction methods, although this evidence is not definitive. The USPSTF recommends that cIMT be assessed in people with diabetes in order to fully assess its value as a prognostic marker. There is some evidence that cIMT is associated with both cardiovascular risk, prevalent disease and future vascular risk but it is not conclusive. Carotid artery plaque is also of interest as a potential marker of vascular risk. It is also measurable by ultrasound and may be more predictive than cIMT for vascular disease. However, evidence for its use in Type 2 diabetes is limited and more research is required.

Chapter 2: Aims and Objectives

The emergence of cIMT and carotid plaque as novel markers of cardiovascular risk in the general population has stimulated interest in their use in higher risk populations such as those with Type 2 diabetes. However, the USPSTF statement of 2009 highlights that there is a lack of evidence for the use of more novel markers such as cIMT in people with Type 2 diabetes (despite their use as surrogate cardiovascular outcomes in clinical trials in diabetic populations). In addition, the Mannheim consensus statement on cIMT and carotid plaque highlights that while there is a wide range of evidence for the use of cIMT, the evidence base for the use of carotid plaque in risk prediction remains sparse (Touboul PJ, Hennerici MG et al. 2012). Therefore, the overriding aim of this thesis is to explore the associations of both cIMT and carotid plaque with cardiovascular risk factors (both traditional and novel), prevalent cardiovascular disease and future cardiovascular risk in a large cohort of older people with Type 2 diabetes, using data from the Edinburgh Type 2 Diabetes Study.

2.1 Aims

This thesis will address several key aims:

1. To determine the frequency and distribution of cIMT and carotid plaque in older people with Type 2 Diabetes and investigate methods of cIMT measurement in the ET2DS
2. To determine the association of cIMT and carotid plaque with traditional cardiovascular risk factors and with more novel biomarkers of cardiovascular risk (those being explored in studies as of potential use in CV risk scores), including ABI, ACR, IL-6, CRP and NT proBNP
3. To determine association of cIMT and plaque with prevalent cardiovascular disease (CAD and cerebrovascular disease) in people with Type 2 diabetes

4. To assess the potential of cIMT and plaque as predictors of incident cardiovascular events and possible associations over and above UKPDS variables

2.2 Objectives

The aims described above will be achieved through a series of individual objectives:

1. Describe the study sample demographics and determine intra-reader variability in cIMT measurement in the ET2DS and investigate the effect of multiple measurements on cIMT values
2. Describe the frequency, distribution, prevalence and change in cIMT and plaque over time in the ET2DS
3. Determine the cross sectional association of cIMT and plaque with traditional and novel CV risk factors, in people with Type 2 diabetes,
4. Determine the association between cIMT and plaque, with prevalent CVD eg MI/Stroke.
5. Determine the incidence of vascular events in the ET2DS and describe associated cardiovascular risk factors
6. Determine the association between cIMT, carotid plaque and incident vascular events
7. Use statistical modelling to examine the predictive ability of cIMT and plaque for cardiovascular events over and above traditional cardiovascular risk factors

Chapter 3: Methods

3.1 Introduction

This chapter describes the methods of the research included in this thesis. The research was performed using individual participant data from the Edinburgh Type 2 Diabetes Study (ET2DS), a large, population-based cohort study that was established in Edinburgh in 2006. The main objectives of the ET2DS are to investigate the relationship between a wide range of risk factors and the long term complications of Type 2 diabetes, with a particular focus on cognition, liver disease and vascular disease. Prior to this thesis, subjects were assessed at baseline and year 1, and I subsequently participated in further data collection at year 4 follow up clinics. Data for this thesis have been drawn from all three data collection points of the ET2DS and focuses exclusively on the vascular aspects of the study. Methods relevant to the research included in the thesis are described below and draw on published material (Price JF, Reynolds RM et al. 2008; Marioni, Strachan et al. 2010) as well as describing my own contribution to follow-up data collection and the statistical analysis undertaken.

3.2 Edinburgh Type 2 Diabetes Study

The ET2DS comprises 1066 men and women with Type 2 diabetes aged 60-74 years and living in Edinburgh and the Lothians at baseline (2006-2007). The full study protocol is detailed in the 2008 publication by Price et al (Price JF, Reynolds RM et al. 2008).

3.2.1 Recruitment, clinic invitations and attendance

Potential eligible participants were identified from the Lothian Diabetes Register (LDR), a large computerised database containing details of over 20,000 people with Type 2 diabetes living in Edinburgh and the Lothians. Exclusion criteria were minimal and included subjects in whom it was not possible to confirm a clinical diagnosis of Type 2 Diabetes, non-English speakers (due to the nature of cognitive

tests undertaken), corrected visual acuity worse than 6/36, those unwilling to give consent and those physically unable to take part in the examination.

The aim of the study was to recruit 1000 subjects in order to achieve approximately 90% power at a two-sided 5% level of significance, to detect a Pearson correlation coefficient of $\geq$ 0.10, between continuous outcome measures (e.g. cognitive test scores) and predictor variables (Price JF, Reynolds RM et al. 2008). It was estimated that a sample size of 800 would retain 90% power to detect a correlation coefficient of $\geq$ 0.12 between risk factors and outcome measures after drop outs and loss to follow up. This sample size, with the same levels of power and significance, was determined to be sufficient to detect any risk factor that contributed 1% or more to the variance in outcome, both at baseline and at follow-up (Price JF, Reynolds RM et al. 2008).

A sample of men and women aged between 60 and 74 years on 1st August 2006 was obtained by randomly selecting participants by sex and 5 year age bands from the list of eligible participants extracted from the LDR. 5454 subjects were initially invited to take part in the study, 3286 of whom replied. 1252 expressed an interest in participation in the study and of those, 1077 attended baseline clinics. Four participants were unable to take part in the examinations for physical or emotional reasons and 7 did not meet the criteria for Type 2 Diabetes after detailed review of clinical evidence leaving a total of 1066 participants at baseline (figure 3-1). Baseline clinic appointments took place between 14th August 2006 and 29th August 2007 at the Wellcome Trust Clinical Research Facility (WTCRF) at the Western General Hospital (WGH), Edinburgh.

Participants were invited to attend a further research clinic one year after recruitment. Of the 1066 that attended at baseline, 939 (88%) agreed to attend the year one clinic. (12 subjects were not invited to the year 1 clinics; 2 had died, 5 refused to be contacted out with the originally planned 4-year follow up, 3 were deemed unsuitable for contact by the study team and 2 withdrew from further contact after the baseline clinic). Of those that were invited but did not attend the year 1 clinic, 19 could not be contacted, 23 were unable or unwilling to attend on health grounds, 38 were

unable or unwilling to attend for other reasons, 21 cancelled or did not attend the appointment and 13 had died. A further 1 person was unable to complete the examination (figure 3-2). Year 1 clinic appointments took place between 26th October 2007 and 27th August 2008 at the Wellcome Trust Clinical Research Facility (WTCRF) at the Western General Hospital (WGH), Edinburgh.

In 2010, appointments were sent out for year 4 follow up appointments. Because of the extended time between the follow up clinics, prior to contacting subjects for the year 4 follow up clinic, I prepared a newsletter about the study which was sent out in December 2009 to all subjects, reminding them that clinics would be starting again, and asking them to inform the study of any change in contact details or circumstances. This provided an opportunity to identify any subjects who were deceased, in addition to those who were not able or willing to continue in the study at this point. Any mail that was returned was tracked using the NHS Lothian health records (Med Trak) to identify any change of address or death. When participants were contacted with appointments, those who chose not to attend were recorded as declined. Of the 1066 people who attended baseline clinics, 974 were invited to attend the year 4 clinics. Of those that were not invited, 81 had died and 10 had withdrawn after the baseline clinic. Of the 974 that were available to be invited, 15 people could not be contacted, 30 people withdrew from the study and 100 declined to attend. Of those that withdrew themselves, 9 gave no reason, 6 were too unwell, 2 had dementia and one did not have diabetes. Of those that were withdrawn by the study team, 5 were too unwell to undergo assessment and 7 had dementia. Of the 100 that declined to attend, 39 were unwell, 43 gave no reason, 10 cited personal reasons, 5 were full time carers and 3 gave other reasons (figure 3-3). In total, 830 participants returned for the year 4 follow up. Clinical examinations for the year four follow up were undertaken by me and three other researchers between 17th May 2010 and 20th May 2011 at the WTCRF. Those who were unable to attend due to ill health or other reasons were sent a self-completion questionnaire. For those who did not return the questionnaire, a questionnaire was sent to their GP. Participants who did not attend appointments were telephoned and offered a further appointment.

Prior to each data collection clinic attendance (at each wave) and following confirmation of an appointment time via telephone, participants were sent an appointment letter and a self-completion questionnaire, along with an information leaflet, a urine specimen container, with instructions for collecting early morning urine (baseline and year 4 only), and a map of the hospital. Each participant was asked to fast overnight, or for at least 4 hours prior to the clinic visit (depending on appointment time) to facilitate blood sampling and ultrasound scanning. Those subjects who fasted overnight were instructed to omit diabetic medications and to bring them to the appointment to take when a light breakfast was provided.

Data were collated in a password protected Microsoft Access database. All data were manually entered and double data entry examination was completed to ensure data had been entered accurately.

3.2.2 Ethical approval

All participants in the ET2DS provided informed consent prior to each wave of the study. In addition the study had full ethical approval from the Lothian Medical Research Ethics Committee. Full ethical permission was granted for ISD data linkage performed at baseline and follow up.

Figure 3-1 Recruitment Flow Chart

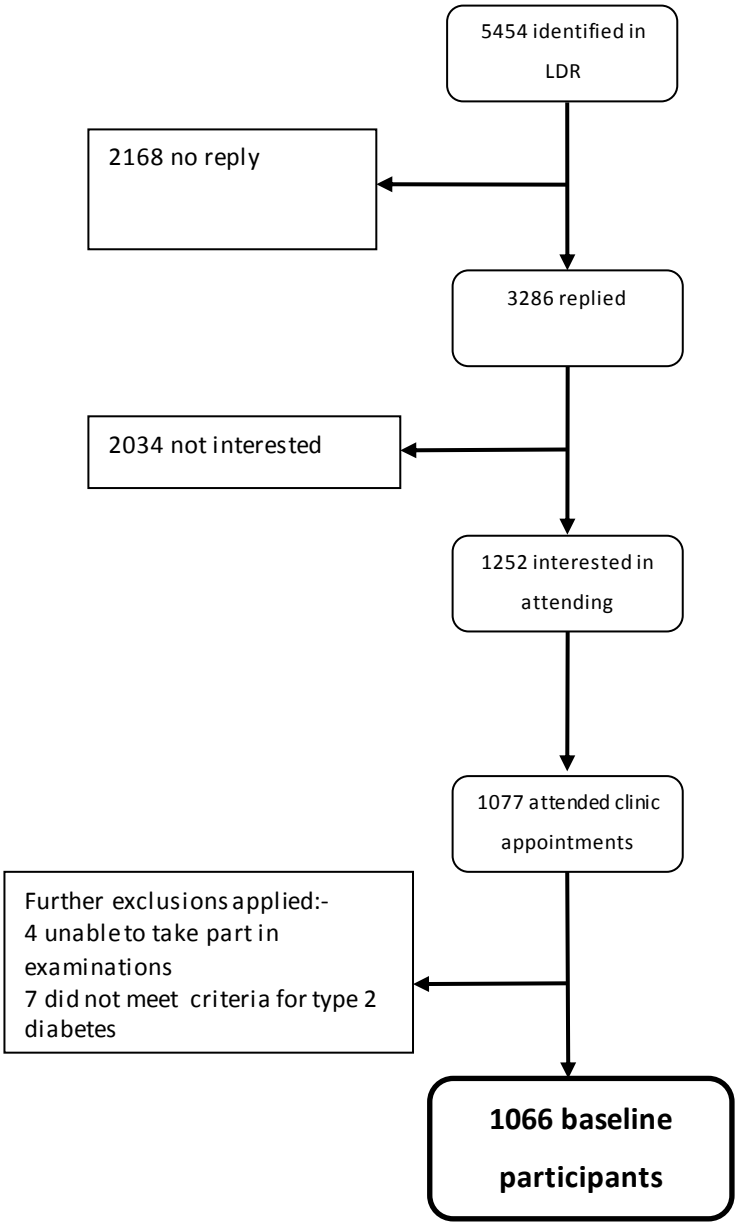


Figure 3-2 Flow chart of Year 1 attendance

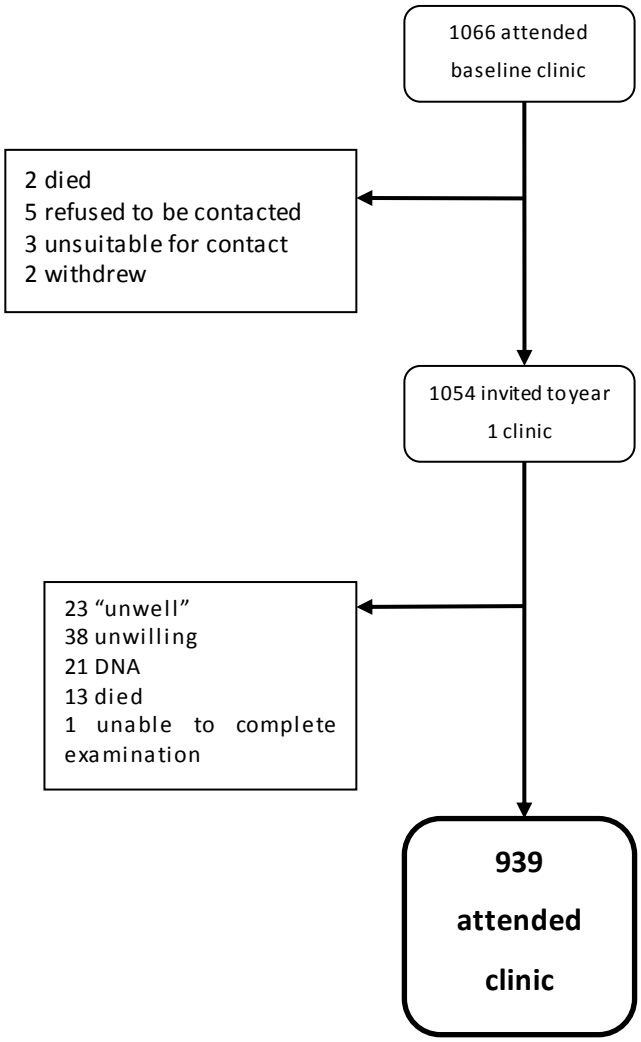
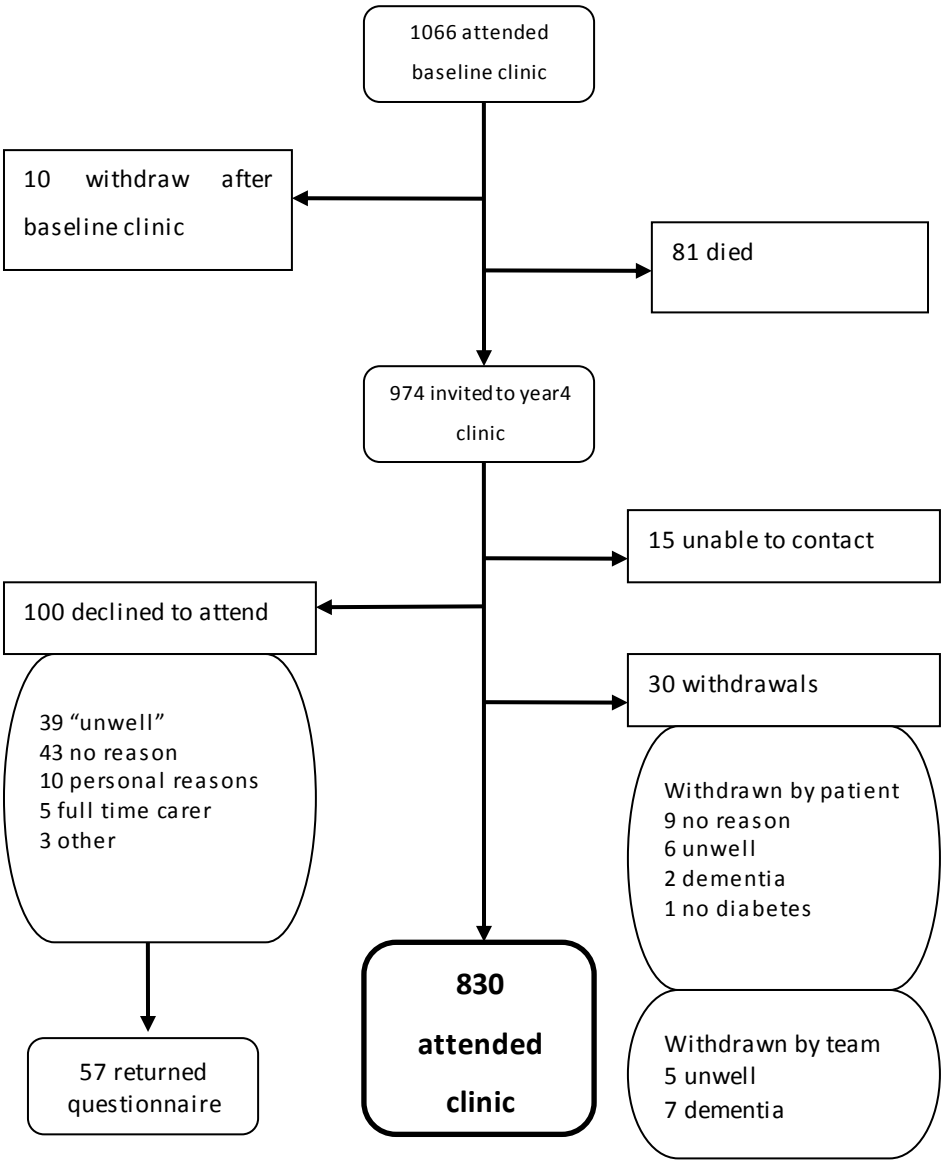


Figure 3-3 Flow chart of attendance at year 4 clinics



3.3 Clinical examinations

3.3.1 Height & weight

Height at baseline was recorded using a wall mounted height tape. Subjects were asked to remove shoes and to stand with their heels and back against the wall. Eyes were lined up with the centre of the ear to allow measurement of maximum height. Measurements were recorded to the nearest 0.1cm. Weight was measured without outdoor clothing, shoes or heavy items and recorded on the data collection form to the nearest 0.1kg.

3.3.2 Electrocardiogram

An ECG was recorded using a GE Marquette MAC 1200 ECG machine. Electrodes were positioned following preparation of the skin (cleaning, drying, exfoliating or shaving as required). Four electrodes were applied to the limbs and six to the chest. The ECG was obtained with the patient lying still and relaxed. ECGs were coded by a senior clinician using the Minnesota coding system (Prineas RJ, Crowe RS et al. 1982) and entered manually into the database.

3.3.3 Brachial blood pressure

Brachial blood pressure was measured in the right arm, directly after ankle brachial pressure index was measured. The cuff was applied to the right arm and using a stethoscope, systolic pressure was read when clear, repetitive tapping sounds appeared for 2 consecutive beats. The diastolic pressure was taken when the tapping sounds were no longer audible.

3.3.4 Ankle brachial pressure index

Following 5 minutes of rest, the sphygmomanometer cuff was placed around the arm and the hand held Doppler was placed over the brachial artery at the point of maximum pulsation. The cuff was inflated to 30mmHg above the estimated systolic pressure. The pressure was reduced at a rate of 2-3mmHg per second and systolic pressure was recorded when clear sounds first appeared. Measurements were recorded to the nearest 2mmHg. This was repeated in the both arms and both feet (dorsalis pedis and posterior tibial pulses) to obtain 6 readings. ABI was calculated

using the lowest ankle pressure as the numerator and the highest brachial pressure as the denominator.

3.4 Self-completed questionnaires

Subjects were sent out a questionnaire prior to attending the baseline, year 1 and year 4 clinics (appendices A, B and C). At baseline, in addition to basic demographic questions (date of birth, education level, marital status, ethnicity) this questionnaire included: questions regarding previous medical diagnoses and procedures; date of diagnosis of diabetes; current and recent medications; alcohol consumption; smoking history (have they ever smoked and if so, how many cigarettes/cigars/pipe per day and for how long); the WHO chest pain questionnaire (Rose G, McCartney P et al. 1977) and the Edinburgh Claudication Questionnaire (Leng GC and Fowkes FG 1992). At year 1, questions regarding current medication use were repeated, in addition to other conditions not related to this thesis. At year 4, the self-completion questionnaire included the same questions as baseline and in addition, questions regarding new medical diagnoses in the past 4 years as well as further questions regarding current medications, and use of specific medications in the past 6 months. In the baseline and year 4 questionnaires, the responses regarding events, diagnoses and operations were coded using ICD 10 and OPCS codes, prior to data entry. Medications were coded using BNF coding. The chest and leg pain questionnaires were coded at the time of the clinic. Any questions that the subject could not answer were completed with the assistance of study staff and all responses checked before the subject left the clinic, where possible.

3.5 Blood & urine sampling and processing

3.5.1 Venepuncture & urine sample collection

Venous blood samples were obtained on the day of clinic attendance. After a period of fasting, a tourniquet was applied above the antecubital fossa and an appropriate sampling site identified. Skin was cleaned using alcohol wipes and venepuncture

undertaken using a Monovette blood collection system. Samples for processing and storage by study staff (for later analysis) were immediately labelled with the study number and the date the blood was taken and placed directly onto ice for processing at the end of the clinic. Samples sent to the Western General Hospital Combined Haematology and Biochemistry Laboratory were labelled with the minimum required data set and sent to the laboratory at the end of each clinic. Reasons for any tubes not being filled were noted, as was the time of venepuncture. Attempts were made to obtain blood samples from each participant. Where blood sampling was not possible on the day of clinic attendance, subjects were invited back to a further appointment for blood sampling. When that was not possible, home visits were carried out where possible in order to obtain samples. Samples processed by study staff were centrifuged before being separated and stored at -80C. CRP, IL-6 and NTproBNP were measured in these samples.

An early morning urine specimen was collected for measurement of urinary albumin and creatinine for calculation of the albumin: creatinine ratio (ACR). Participants were sent instructions and a sample tube to collect an early morning urine sample. Samples were stored on ice on receipt at the clinics and frozen at -80C for later analysis.

3.5.2 Analysis of samples

Serum isotope dilution mass spectrometry–traceable creatinine, plasma HbA1c, total cholesterol and HDL cholesterol were measured in venous blood and creatinine measured in urine (for calculation of albumin:creatinine ratio) and analysed according to standard protocols in the Department of Biochemistry, Western General Hospital, Edinburgh, UK. Serum creatinine measurement was used to estimate glomerular filtration rate (eGFR) using the CKD-EPI equation ((Levey, Stevens et al. 2009)). Assays for plasma CRP and IL-6 were performed in the University Department of Medicine, Glasgow Royal Infirmary, as described by Marioni et al (Marioni, Strachan et al. 2010). CRP was assayed using a high-sensitivity immunonephelometric assay (Tzoulaki, Murray et al. 2007), while IL-6 antigen levels were determined using high-sensitivity ELISA kits (R&D Systems, Oxon,

U.K.). Plasma NT-proBNP concentrations were determined using the Elecsys 2010 electrochemiluminescence method (Roche Diagnostics, Burgess Hill, UK) calibrated using the manufacturer's reagents. Manufacturer's controls were used with limits of acceptability defined by the manufacturer. Low control CV was 6.7% and high control CV was 4.9%.

3.6 Ultrasound scanning

Ultrasound of the carotid arteries was performed at both year 1 and year 4 by the same specially-trained ultrasonographer. Images were captured using a Siemen's Elegra Ultrasound. A 2-5-MHz transducer was used.

3.6.1 cIMT measurement at Year 1 and Year 4

The cIMT was measured according to the ET2DS ultrasound SOP (appendix D). The subject was positioned at 45 degrees, with the head turned to the side contralateral to measurement. The probe was placed on the neck until the carotid artery could be visualised. The probe was then turned so that the artery wall was parallel with the transducer. Measurements were made of the intima media thickness at a point 1cm below the bifurcation of the common carotid, in an area free of plaque. Three measurements were made on each side. An image was frozen and saved for each measurement. Images were securely stored on a University of Edinburgh hard drive.

3.6.2 Carotid plaque assessment at Year 1 and Year 4

Plaque was defined in the SOP as a focal structure encroaching into the arterial lumen of at least 0.5 mm or 50% of the surrounding IMT value, or with a thickness >1.5 mm as measured from the media-adventitia interface to the intima-lumen interface, and was judged subjectively by the ultrasonographer. Carotid plaque was assessed as present or absent in both right and left common carotids, internal carotids, external carotids and carotid bifurcations.

Plaque morphology was assessed subjectively by the sonographer. Echolucency was defined as presence of one or more plaques appearing black or almost black as flowing blood (compared with echogenic plaque which appears white or almost white, similar to the far wall media-adventitia interface). Heterogeneous plaque was recorded if one or more plaques were present in which echogenicity of more than 20% of the plaque area differed substantially from the echogenicity of the rest of the plaque.

Plaque thickness was also assessed. The plaque with the maximum thickness was identified on each side and measured using cross hairs placed by the ultrasonographer. Plaque thickness was recorded for both left and right.

3.6.3 Viewing and storage of carotid ultrasound images

At year 1, all ultrasound images were downloaded from the ultrasound in a proprietary format. Prior to use in this thesis, they were required to be converted to DICOM format in order for use with University software. File conversion was undertaken by Dr Calum Gray of the Clinical Research Imaging Centre, University of Edinburgh.

At year 4, images were downloaded directly from the ultrasound machine in DICOM format onto MO discs and transferred by me to a networked hard drive using an MO disc reader. Images were recorded and saved by me in separate files for each subject, labelled by subject number. I stored the images on a networked drive, which was backed up, and a copy was also stored on a portable hard drive (Freecom Hard Drive Classic 3.5" 500GB USB-2.0).

Following this, I was able to view both year 1 and year 4 images using MicroDicom viewer. Analyze 10.0 software was used to prepare images for use with the IMT measurement software (detailed later in this thesis).

3.6.4 IMT measurement validation at Year 4

In order to assess intra-observer variability in the measurement of cIMT, I selected individuals to undergo repeat cIMT measurement at year 4. Subjects who reattended the clinic for other reasons were invited to be rescanned at the time of return. 52

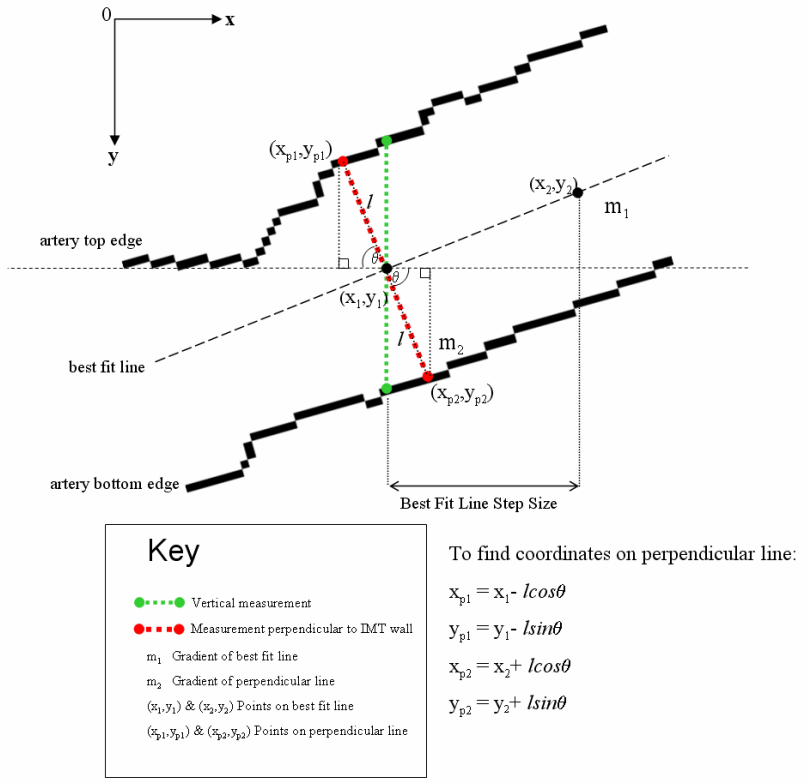
subjects were recruited to this validation study and each subject returned to the clinic at a time suitable for them. They underwent repeat cIMT measurement using the same ultrasound protocol as was used at the previous 2 measurement sessions. The sonographer was blinded to the previous year 4 measurements. Data were recorded in a Microsoft Access database.

3.7 IMT measurement software

In collaboration with Dr Calum Gray and Dr Tom MacGillivray of the Image Analysis team at the Clinical Research Imaging Centre (CRIC), part of the Wellcome Trust Clinical Research Facility in Edinburgh, I contributed to the development of a computer programme that could be used perform serial measurement of IMT along a length of carotid artery wall using the frozen images stored at the time of ultrasound assessment. The aim of developing this software was to allow for a comparison between taking only 2 or 3 measurements at points on the vessel wall and taking multiple measurements along the wall. Broadly speaking, once an outline of the IMT of vessel wall is identified by the user, the software finds a line of best fit along the midpoint of the wall section identified, and takes measurements of the cIMT at an angle perpendicular to this line (see software SOP, appendix E).

3.7.2 Program algorithm

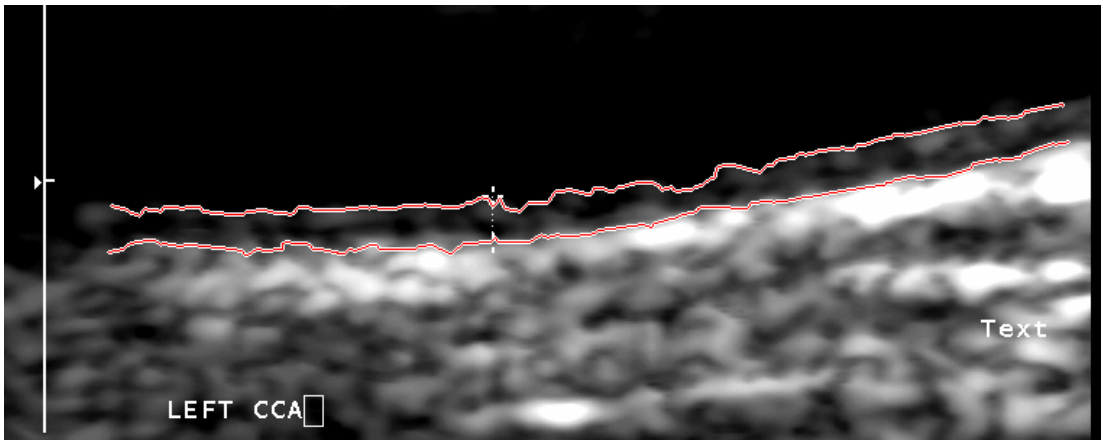
Figure 3-4 Schematic for calculating cIMT (reproduced from software SOP, Appendix E)



The assumption of the program is that measurements of IMT taken along the vessel wall must be taken perpendicular to the edges as far as possible to take into account any bend in the wall, which allows the measurement of IMT to be a meaningful value (figure 3-4). Users have control over the best fit line pixel distance and the number of pixels between each cIMT reading. This can be defined after the image is prepared, allowing the number of readings taken per image to be altered as required. A line of best fit is made between the two user drawn lines and then perpendicular lines were drawn between the edges and the best fit lines. These are deemed to be the intima media thickness.

3.7.3 Preparation of images

Figure 3-5 Outline of interfaces following manual tracing (reproduced from software SOP, Appendix E)

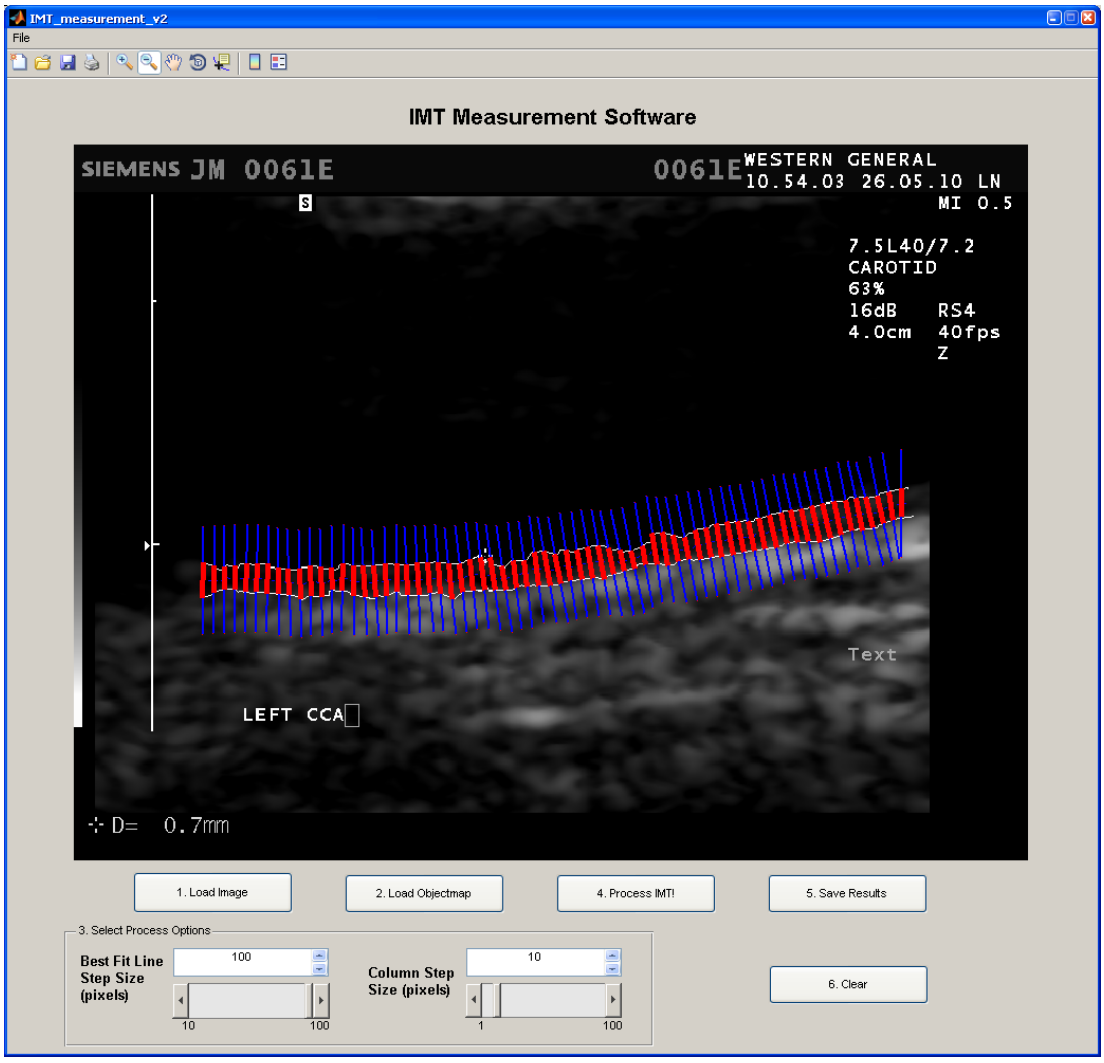


Analyze 10.0 was used to create an object map of each image (figure 3-5). This involved tracing the outline of the lumen-intima media border using a spline. Then, using a new spline, the line of the media-adventitial interface was traced. The sections of artery wall that were identified for measurement were free of plaque, as judged by each user, in keeping with the study SOP for measurement of IMT. As long a section of artery wall as possible was measured. The splines were then toggled if appropriate to create a more accurate fit and then these edits were applied to the object map. The object map was then saved, along with a copy of the image.

Once I was experienced in processing the images, I trained a further two readers to assist in preparing the images. Two hundred and thirty five participants' images were prepared. The time that it took to prepare each set of images limited the number of participants' images that could be included in the sample. This limited the statistical analysis that could be performed as the way in which the images were selected was not completely random (as preparing the images took a lot longer than was initially expected).

3.7.4 Processing of images

Figure 3-6 Screenshot of software measurement output (reproduced from software SOP, Appendix E)



Following preparation of the images, processing of the images by the software was performed in batches overnight. The name of the image file and the object map file were inserted into an Excel spread sheet which was then used by the software to process the files in batches. The number of measurements could be easily altered if needed and the measuring process rerun if required. However, for the purposes of this thesis a line of best fit distance of 50 pixels and measurement distance of 10 pixels was chosen for analysis. Once measured, the programme automatically

exported the measurements to an excel spread sheet where they were stored. In addition to the raw individual measurements, the mean, maximum and minimum values were summarised, as well as number of measurements and standard deviation of the mean.

3.7.5 Limitations of IMT software

The major limitation of this process was image quality and the possibility of human error introduced when the IMT borders were traced. The machine on which the images were obtained could not produce images of a high enough quality to allow automatic edge detection to be used. Human error was introduced at the point of line selection. However, these limitations were felt to be acceptable in the context of this research as the comparable measure is the sonographer measurements which are open to the same limitations, and the main aim of the research was to find the overall effect of increasing the number of measurements used to calculate the mean cIMT.

3.8 Determination of prevalent and incident events

Prevalent and incident cardiovascular events used in this thesis were identified using several sources of information to ensure that all possible events were captured. At both baseline and year 4 follow-up, events and diagnoses that were reported by participants in the self-completed questionnaire or on ECG were identified. Attempts were made to confirm these diagnoses using data linkage from ISD. Additionally, any events that were not reported by subjects but were present on ISD linkage were identified. Further evidence was obtained from general practitioners and hospital notes where required.

I was responsible for the identification and confirmation of incident cardiovascular events following the year 4 follow up clinic, while baseline events had been determined by members of the baseline study team on completion of the baseline clinics.

3.8.1 Non-fatal events

All possible cardiovascular events were confirmed by an events committee comprising 2 clinically qualified members of the ET2DS team, as recommended by the FDA in 2010 (Centre for Drug Evaluation and Research 2008). Data was collected by one team member and the criteria applied. Cases were then discussed by the 2 panel members and decisions in cases where more detail was need were discussed following further data collection from hospital notes. In the case of any discrepancies in decisions making, a third clinically qualified consultant was available to adjudicate.

Questionnaire and Examination

Baseline

At baseline, subjects completed a questionnaire (appendix A) and were asked if they had ever had a doctor diagnosis of angina, MI, stroke, coronary intervention and peripheral intervention. They were also asked to report current medication use and whether they were taking medication for angina, hypertension, raised cholesterol or if they were taking aspirin. They also completed the WHO chest pain Questionnaire (Rose G, McCartney P et al. 1977). The ECG taken as baseline was also available to provide information, having been coded using the Minnesota coding system by clinically trained members of the study team (coded as ischaemic if Minnesota codes were 1.1 to 1.3; 4.1to 4.2; 5.1 to 5.3; 7.1, and coded as Q-waves if Minnesota codes were 1.1.1 to 1.2.5; 1.2.7; or 9.2 plus 5.1 or 5.2 (Prineas RJ, Crowe RS et al. 1982).

Year 4

The self-reported questionnaire at year 4 (appendix C) asked subjects to report whether they had had a doctor diagnosis of angina, MI, stroke, coronary intervention and peripheral intervention in the 4 years since their baseline clinic attendance. They were also asked about current medication use, completed the WHO chest pain questionnaire again, and a repeat ECG was taken and coded using the Minnesota coding system by a clinically trained member of the study team (as at baseline).

Information Services Division Data Linkage

At baseline, data concerning deaths and hospital discharges were obtained from the Information Services Division (part of NHS National Services Scotland).

Baseline Data Linkage

Identifying data were sent to ISD and data linkage performed to capture all hospital discharge codes (both ICD 9 and 10) and OPCS 4 codes for each subject, from the earliest point available up until baseline. The SMR linked dataset was used to perform the linkage (SMR01, 04, 06 and GROS deaths records). All ICD 10 codes for cardiovascular disease (I20-I25, I61, I63-I66, I252, I679, I694, G45 and G659) or where not available, ICD 9, were extracted manually from the ISD data linkage.

Year 4 Data Linkage

Prior to performing linkage, an application was made to ISD. Full ethical permission was granted. Information was obtained regarding all hospital discharges and procedures for each subject between 1st January 2007 and 20th May 2011. Information was also obtained regarding all deaths in that period. Subjects were again linked to the SMR linked dataset. All ICD 10 codes for cardiovascular disease (I20-I25, I61, I63-I66, I252, I679, I694, G45 and G659) were extracted manually from the ISD data linkage.

Hospital Notes and GP Information at Year 4

In situations where there was discrepancy between data linkage and self-report of an event, or where individuals did not meet the full criteria (detailed later) for a given event, I searched individual hospital notes for information to help confirm or refute a diagnosis. If hospital notes were uninformative or unavailable, GPs were asked to confirm any events.

3.8.2 Fatal Events

Next of Kin Reporting of Deaths

During the process of appointment making, reports received from spouses/family members of a participant's death were noted in the database, along with a provisional cause of death if given. In addition, correspondence in response to the study newsletter also provided information of participant death.

ISD Linkage to Death Information

Linkage to the combined dataset at ISD allowed identification of all registered hospital and out-of-hospital deaths, and provided a cause of death.

Confirmation of Cause and Date of Death

All deaths notified by next of kin or by ISD data linkage were investigated. Those deaths notified by ISD data linkage had the date of death and underlying cause of death noted. Those deaths notified by next of kin were cross referenced with the ISD linkage and those that were not present on both lists were investigated further using medical records so that cause and date of death could be confirmed.

3.9 Criteria for definition of cardiovascular events

3.9.1 ET2DS criteria for prevalent CVD at baseline (2006/2007)

MI

Myocardial infarction was recorded if 2 out of 3 of the following criteria were met:

(i) Self-report (subject's recall of a doctor's diagnosis) of heart attack
(ii) Myocardial infarction indicated by WHO chest pain questionnaire
(iii) ECG evidence of ischaemia

OR if both of the following were present:

(i) Self-report (subject's recall of a doctor's diagnosis) of heart attack
(ii) Prior hospital discharge code for MI (ICD10 codes I21-I23, I252)

Stroke

Stroke was recorded if 2 out of 3 of the following criteria were present:

(i) subject recall of a doctor's diagnosis of stroke
(ii) prior hospital discharge code consistent with stroke (ICD10 codes I61, I63-I66,I679, I694)
(iii) confirmation by clinical notes reviews that event not due to transient ischemic attack (TIA).

TIA

Transient ischaemic attack was recorded if two out of three of the following 174 criteria were present:

(i) subject recall of a doctor's diagnosis of stroke

(ii) prior hospital discharge code consistent with TIA (eg. ICD10 codes G45, G659)

(iii) confirmation by clinical notes reviews that event due to TIA.

TIA was also recorded if subjects volunteered a self-reported history of TIA, "mini stroke" or "slight stroke" on the questionnaire.

Angina

Angina was recorded if two out of three of the following criteria were met:

(i) self-report of doctor-diagnosed angina or taking regular anti-anginal medication
(ii) angina indicated on WHO Chest Pain Questionnaire
(iii) Ischaemic ECG codes

OR if both the following criteria were met:

(i) self-report of doctor-diagnosed angina or taking regular anti-anginal medication
(ii) prior hospital discharge code for ischemic heart disease (ICD10 codes I20-I25).

3.9.2 ET2DS criteria for incident CVD at year 4 (2010/11)

I identified potential vascular events, operations and procedures using the self-reported questionnaire, GP questionnaire, ECG and hospital discharge coding. I then developed and applied the following criteria and searched hospital notes for additional information if the criteria were not immediately met. If there was doubt as

to whether criteria had been met even after searching hospital notes, possible cases were discussed by the events panel on an individual basis and a consensus decision was made.

Fatal MI

Either death certificate confirmed OR criteria for non-fatal MI within 4 weeks of unexplained/sudden death.

Non-fatal MI/ACS

Either ICD-10 code for new MI from ISD record linkage to discharges from Scottish hospitals (SMR), with date confirming that event occurred after baseline clinic visit PLUS one or more of the following (supporting evidence):

i) Subject report of a doctor diagnosis of MI on the 4-year self-completion questionnaire, with date consistent with ISD report OR NEW subject report of MI as cause of chest pain on the WHO CPQ
ii) Minnesota ECG codes for MI which were not present at baseline
iii) GP report of an MI on postal GP questionnaire (for clinic non-attenders only), with date consistent with ISD report

OR If criteria for MI met following scrutiny of the clinical notes (hospital and/or GP notes) of any subject with one or more individual indicators of a possible MI, except ECG (see (a) above) but not meeting the full criteria under (a).

Angina

Either A diagnosis of angina during follow-up required that subjects did not meet criteria for angina at baseline, plus either:

i) ICD-10 code for angina as PRIMARY diagnosis code from ISD record linkage to discharges from Scottish hospitals (SMR), with date confirming that diagnosis occurred after baseline clinic

OR At least two of the following:

i) Subject report of a doctor diagnosis of angina (or of taking medication for angina) with date consistent with diagnosis after baseline
ii) New ischaemic ECG at year 4
iii) Angina on WHO chest pain questionnaire

OR Clinical diagnosis of angina recorded by a doctor on scrutiny of the notes of any subject with one or more individual indicators of possible new angina (new ICD code for angina but not primary code, new self-report of doctor diagnosis or medication, new angina on WHO chest pain questionnaire) but not meeting full criteria under 1 or 2 above.

(N.B. If actual date of diagnosis of angina cannot be ascertained, then date should be date of follow-up clinical appointment)

New possible other IHD

A diagnosis of 'possible other IHD' during follow-up required that subjects did not meet any of the criteria for events listed under 'all IHD' at baseline (including 'possible other IHD'), plus:

i) ICD code for IHD with date consistent with diagnosis since baseline in subjects without MI, angina or coronary intervention at follow-up.

Fatal stroke

Death certificate confirmed or criteria for non-fatal stroke within 6 weeks of unexplained/sudden death

Non-fatal stroke

Either ICD-10 codes for stroke as PRIMARY diagnosis

OR Self-report of stroke confirmed as stroke on scrutiny of clinical notes

OR Non-primary ICD-10 codes for stroke, confirmed as stroke on scrutiny of clinical notes

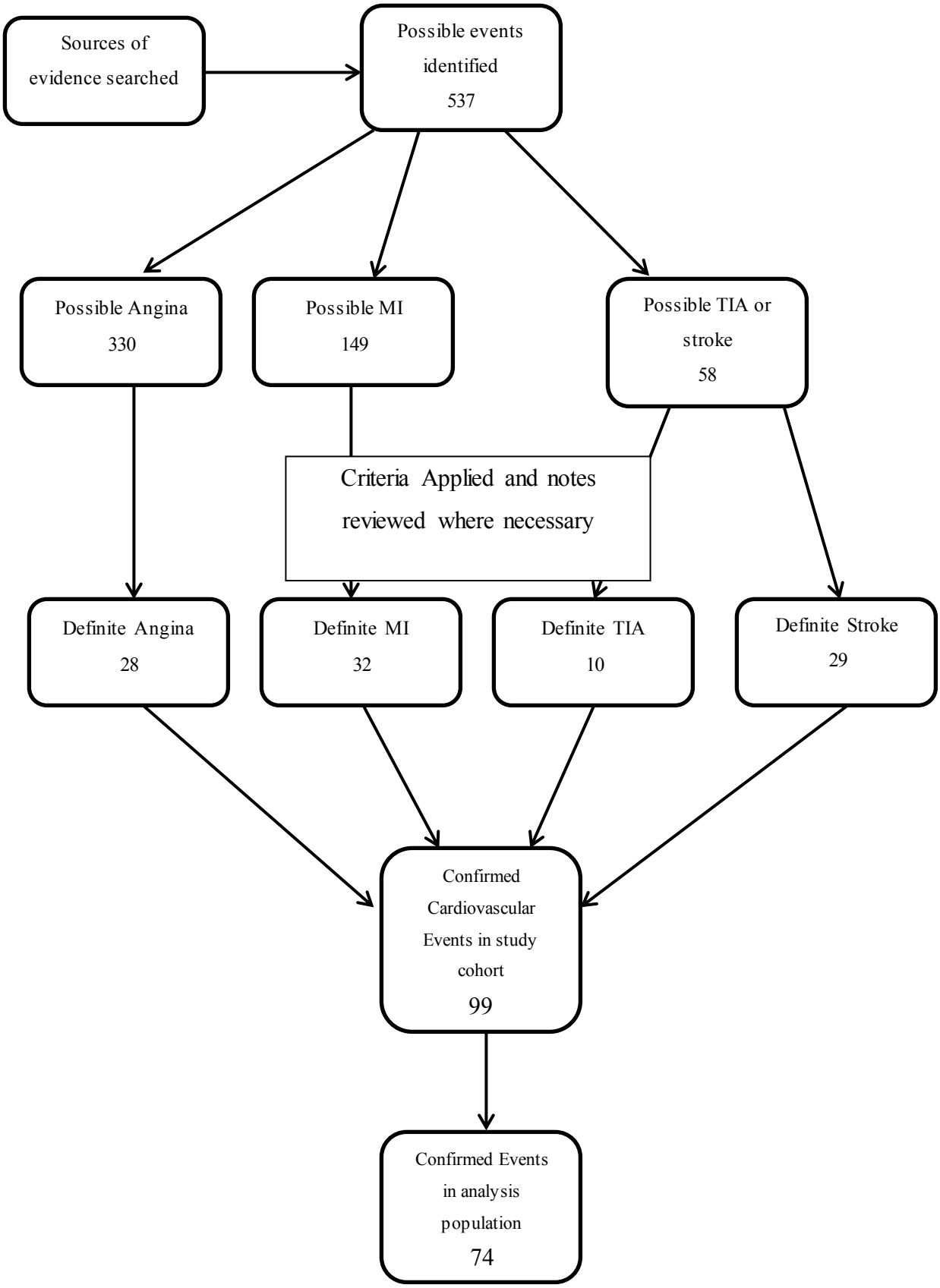
TIA

Either ICD-10 code for TIA as PRIMARY diagnosis on ISD data

OR Self-report of stroke confirmed as TIA on scrutiny of clinical notes

OR Non-primary ICD-10 code for stroke or TIA confirmed as TIA on scrutiny of clinical notes.

Figure 3-7 Flow chart of incident event identification



3.10 Data analysis

Data were collected and entered into a Microsoft Access database and exported to IBM SPSS v19 for data analysis.

3.10.1 Double data entry

In order to assess the accuracy of the data collected at year 4 and the resultant variables, the paper records of a random sample of 80 individuals (10%) were selected from the cohort and used for double data entry. I, along with three other study team members re-entered 20 sets of notes each into the database. Comparison of the resulting dataset with the original data set revealed an error rate of 0.007. On discussion with senior study team members, this was felt to be an acceptable level of error and it was agreed that the resultant variables were reliable for use.

3.10.2 Data cleaning and outliers

Following completion of data entry at year 4, all data in the database was analysed for outlying or unusual values. These were examined on an individual basis and compared with original copies of the results. Input errors were changed to the correct value on the original database, and detailed logs of any changes were kept. Those values which could not be explained by input error were removed from the database if not biologically plausible, and retained if simply high but true values. As part of this procedure I prepared the year 1 and year 4 cIMT and plaque and the year 4 renal function, lipids, ECG, ABPI and brachial blood pressure.

The year 1 cIMT measurements were examined for any outlying or unusual results (<0.5mm or >1.5mm), and those that were identified as having been incorrectly measured by the sonographer (following analysis of stored images) were removed from the database. Examples of this include where a plaque has clearly been measured instead of an area of IMT free of plaque, or where the measurement callipers were incorrectly placed. Measurements that were simply data input errors were corrected following comparison with images, and any other unusual values that

could not be explained in this way were dealt with on a case by case basis. Any values that could not be explained were removed from the database.

Year 4 IMT measurements were dealt with in the same way, as were plaque measurements.

3.10.3 Variables used in this thesis

Variables included in this thesis are summarized in table 3-1.

Demographic Variables – Age at year 1 is a continuous variable calculated as [date of year 1 clinic appointment – date of birth]. Sex is a binary variable (male or female). SIMD is a 5 level categorical variable (1 being most deprived quintile and 5 being least deprived).

Diabetes Variables – Duration of diabetes is calculated as [date year 1 appointment – date of diagnosis of diabetes] and is used as a log10 transformed variable in analyses. HbA1c is a continuous variable (%Hb) and diabetes treatment was recorded as a 3 level categorical variable – diet alone, oral medication alone or insulin ± oral medication.

Blood Pressure Variables – Systolic and diastolic blood pressure are continuous variables reported in mmHG. The use of antihypertensive medications is a binary variable (yes or no).

Lipid Variables – Total and HDL cholesterol are continuous variables (mmol/l) and use of lipid lowering medication is a binary variable (yes or no).

Smoking Variables – Smoking status is described as a 3 level categorical variable (current, ex or never smoker).

Obesity Indices – BMI is a continuous variables calculated using height and weight measured at baseline clinics, using the formula BMI = weight (kg) / [height (m)]^2.

Novel Markers – IL-6, ACR and CRP are continuous variables on a log scale (due to non-normal distribution). ACR and eGFR are also continuous variables. ABPI is calculated as [lowest ankle pressure (mmHg) / highest brachial pressure (mmHg)]. All novel markers were log10 transformed for use in analyses.

Carotid Ultrasound Variables –All cIMT variables are continuous variables. There is no definitive recommendation on which summary measure of cIMT to use. Several measures of cIMT will be used in this thesis. Mean cIMT is the mean of all 6 cIMT readings; maximum cIMT is the highest of the 6 IMT readings; maximum mean cIMT is the higher of mean left and mean right IMT; mean maximum cIMT is the mean of the maximum right and maximum left IMT. Plaque presence (at least 1 plaque in any segment of the carotid arteries) is reported as a binary variable (yes or no). Mean plaque thickness is the mean of the right and left plaque thicknesses. Maximum plaque thickness is the higher of the right and left plaque thicknesses. Plaque score is the sum of the areas in which at least one plaque is present throughout the left and right carotid tree (min 0 areas, max 8 areas), based on a method used by Lee et al (Lee EJ, Kim HJ et al. 2007). Plaque score was also used as a binary variable (score $\leq$4 or score >4). Plaque echogenicity is a 4 level categorical variable (echogenic/no plaque only, echolucent plaque only, heterogeneous plaque only, both types of plaque). It is also considered as a binary variable (low risk plaque (echogenic/no plaque) vs high risk plaque (echolucent, heterogeneous or both types).

Cardiovascular Event Outcomes – Event variables for prevalent and incident CVD are binary (yes or no). Outcomes are (for both prevalent and incident events):

- Any CVD – either angina, fatal or nonfatal MI, TIA, fatal or non-fatal stroke, and any cardiovascular death.
- Any CAD – either fatal or nonfatal MI or angina
- Any cerebrovascular – either fatal or non-fatal stroke or TIA
- Any fatal (incident only) – any fatal cardiovascular event (including other ischaemic heart disease)

In most cases, prevalent events were taken from baseline data. In addition, because cIMT was measured at year 1, any incident events recorded between baseline and year 1 were subsequently attributed to "prevalence".

Table 3-1 Collection time point for variables used in this thesis

Demographic	Baseline	Year 1	Year 4
Date of birth	BMI	ABI	IMT and Plaque
SIMD	Smoking variables	Systolic BP	Incident CVD events
Sex	NTproBNP	Diastolic BP	
Age	IL-6	Lipids	
	eGFR	HbA1c	
	ACR	Medication use	
	CRP	IMT & plaque	
	Prevalent CVD events	Duration DM	

SIMD=Scottish Index of Multiple Deprivation, BMI=body mass index, NTproBNP=amino terminal brain natriuretic peptide, IL-6=interleukin 6, eGFR=estimated glomerular filtration rate, ACR=albumin creatinine ratio, CRP=c reactive protein, CVD=cardiovascular disease, ABI=ankle brachial index, BP=blood pressure, HBA1c=glycated haemoglobin, DM=diabetes mellitus, IMT=intima media thickness

3.10.4 Statistical analysis

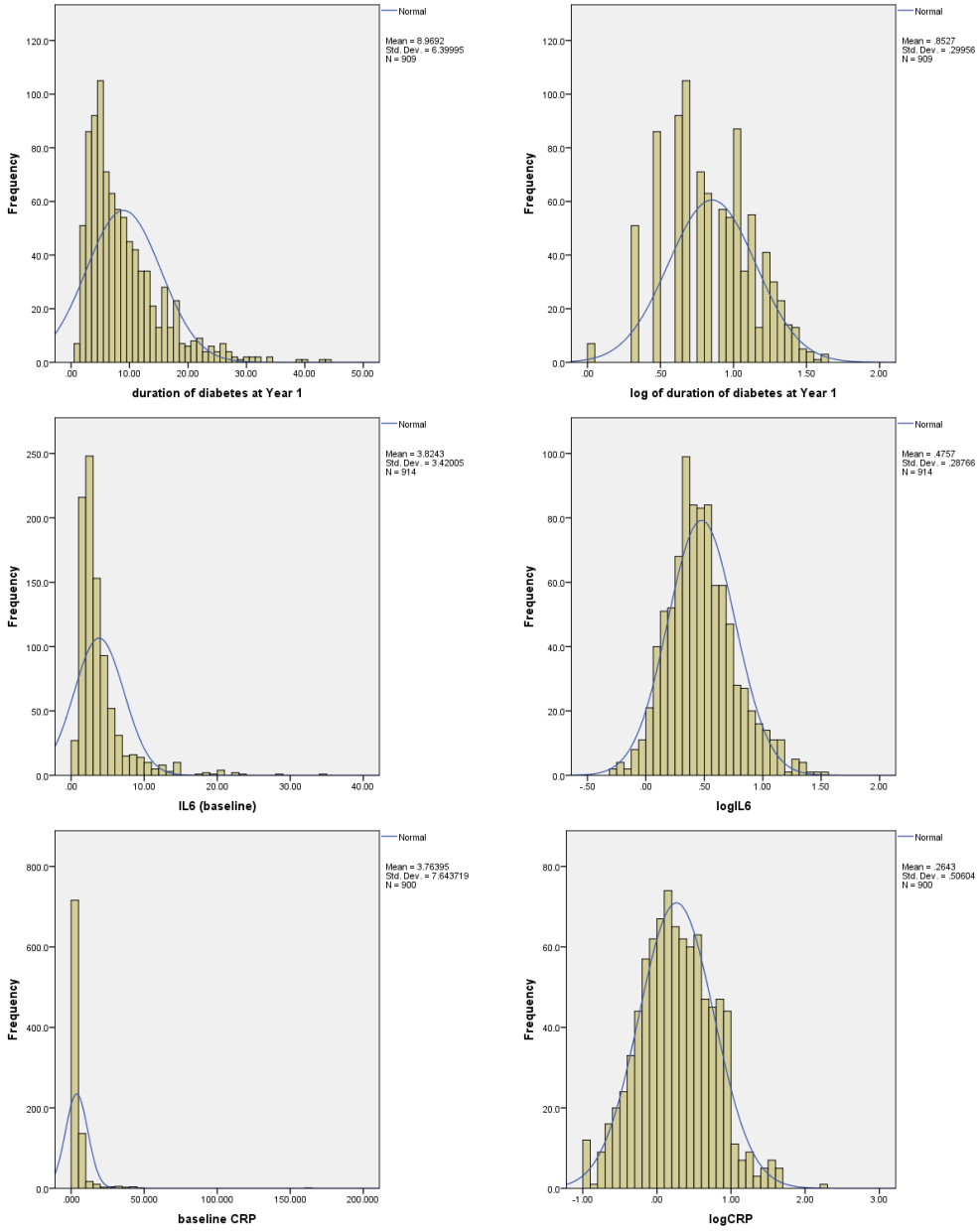
In the analyses presented in this thesis, a p value of less than 0.05 was generally taken to denote statistical significance, as is standard in most epidemiological research. Consideration was given to correcting for the effect of performing multiple analyses, using the Bonferroni method. At a level of <5% significance, there is a less than 5% chance the null hypothesis is rejected, even though it is true (type I error). As the number of analyses increases, the likelihood of a type I error increases and so in the Bonferroni method, the level of significance is divided by the number of analyses performed to reduce the level below which a result is considered significant, thereby reducing the likelihood of type I errors. However, the consequence of this is an increase in type II errors, whereby the null hypothesis is accepted, despite being untrue. (Perneger TV 1998) highlights that the main problem with this approach is that the point at which one limits the number of tests divided by (eg in on paper, or in one study or indeed, a researchers entire lifetime) becomes arbitrary and in the context of research undertaken with a predetermined hypothesis (as in this thesis), it is more important to consider the significance of test results in the context of other tests, taking care to keep the number of tests undertaken to the minimum required for the research. This was the approach adopted in this thesis.

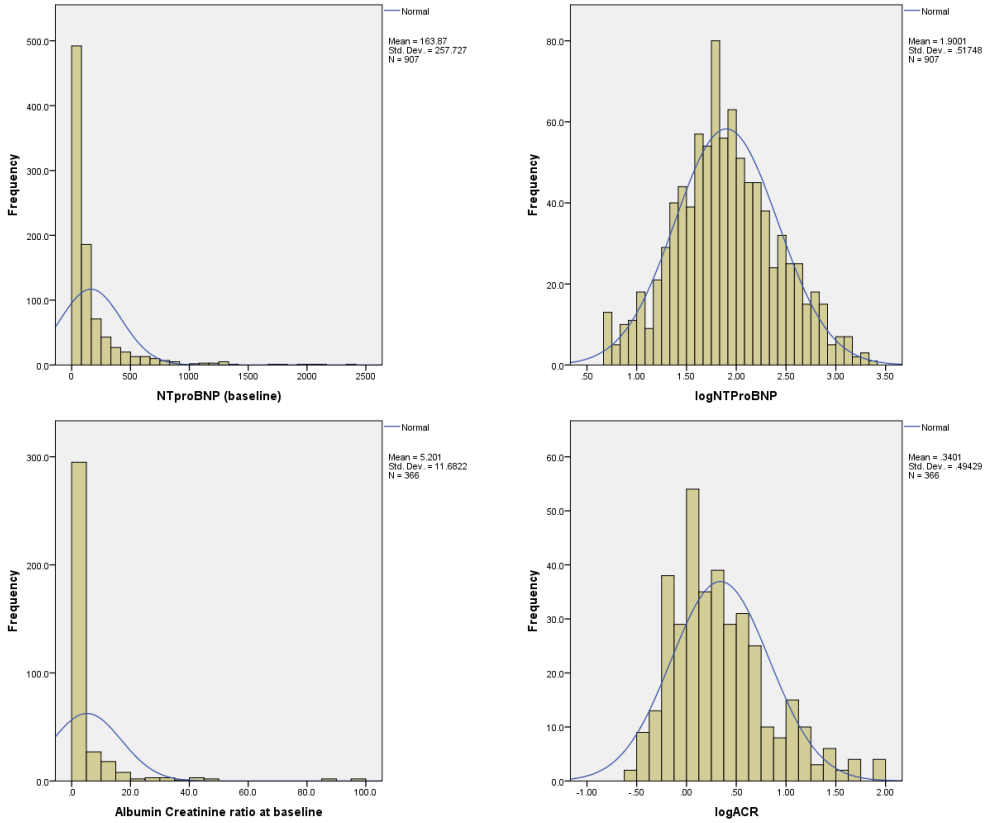
Descriptive analysis and cross sectional analysis of carotid variables with risk factors

Variables for analysis were drawn from the year 1 data rather than baseline where possible, as IMT was first measured at the year 1 clinic. Baseline demographics of the study population are described to allow comparison with those who attended year 1 follow up. Variables that were not measured at year 1 were substituted with the corresponding baseline variable (BMI, smoking status and pack years, NTproBNP, IL-6, eGFR, ACR and CRP).

Histograms of continuous variables were visually inspected for normality. Most variables were normally distributed. Those variables that were not normally distributed were duration of diabetes, ACR, NTproBNP, IL-6 and CRP. Log10 transformation was used to transform all the variables except pack years, which was square root transformed. Histograms of variables after transformation were inspected to confirm that a normal distribution had been achieved (figure 3-5). For normally distributed variables, mean and standard deviation are quoted, and for skewed variables, median and interquartile ranges are described.

Figure 3-8 Histograms of untransformed and transformed skewed variables





Unadjusted associations between continuous variables were assessed using Pearson correlation coefficients. The r value and corresponding p value are reported. Partial correlation was used to examine age and sex adjusted correlation coefficients. R values and p values are reported. For categorical variables, unadjusted and age and sex adjusted associations with continuous variables was assessed using ANOVA (β values and p values reported). Chi squared testing was used to examine relationships between two categorical variables (chi square and p value reported).

Linear regression models were used to examine the multifactorial determinants of the continuous IMT and plaque variables. Variables were entered into the model and β coefficients and p values reported. Model variance explained is also reported (r^2).

Association of carotid variables with prevalent CVD

Logistic regression models were used to assess the association between prevalent CVD and measures of cIMT and plaque. Four models were fitted:

Model 1	Unadjusted
Model 2	Age and sex
Model 3	model 3 Age, sex, SIMD, duration of DM, HbA1C, diabetes treatment, systolic blood pressure, diastolic blood pressure, Antihypertensive use, total cholesterol, HDL cholesterol, lipid lowering medication use, smoking, BMI
Model 4	Age, sex, SIMD, duration of DM, HbA1C, diabetes treatment, systolic blood pressure, diastolic blood pressure, Antihypertensive use, total cholesterol, HDL cholesterol, lipid lowering medication use, smoking, BMI, ankle brachial index, albumin creatinine ratio, interleukin 6, c reactive protein, NTproBNP

cIMT measurement validation

52 subjects were recruited to have a second IMT measurement performed. Subjects returned at varying intervals after the first scan. As only one sonographer was used, only intra-observer variability could be evaluated. IMT measurements were recorded in millimetres to 1 decimal point at both sessions. The mean values of cIMT were calculated for the first session and the second session. A mean of both the right and left measurements was calculated as well as a mean of all 6 measurements.

Kolmogorov-Smirnov tests were performed on the mean values and were found to be non-significant at the 99% level, so the data was assumed to be normally distributed, thus parametric tests could be used. Pearson correlation coefficients were calculated and the paired t test used to compare the means. Intra-class correlation was calculated to assess intra-observer variability. Limits of agreement between both sessions were evaluated using Bland Altman plots. Analysis was performed on all 52 subjects (regardless of time between scans) and on a subgroup who had their second scan within 4 months of the first.

cIMT measurement software analysis

Measurements of mean cIMT made using the cIMT software were compared with those made in the same 233 individuals by the sonographer. Pearson correlation coefficients and paired student t tests were used to compare inter-correlations and the mean of each measurement in the sub-population. Due to the small sample size, no further statistical analyses were performed.

Association of carotid variables with incident cardiovascular disease

The association between year 1 cIMT and plaque and future vascular events was determined using Cox Proportional Hazards modelling. Time to event was calculated by subtracting the date of year 1 appointment from the date of the first event, or from the date of censoring in the case of non-events (defined at 31st August 2011). Cardiovascular events were examined in 4 ways – any cardiovascular event (fatal and non-fatal MI, angina, fatal and non-fatal stroke or TIA), any CAD (Fatal or non-fatal MI or angina), any Cerebrovascular disease (Fatal or non-fatal Stroke or TIA) and any fatal event (fatal MI, fatal Stroke, fatal other IHD). Firstly, variables were entered into the model unadjusted. Then, they were adjusted for age, sex and previous CVD (to take account of the risk inferred by previous disease). Following this, full adjustment for variables (where available) used in the UKPDS risk score was undertaken. The UKPDS risk score variables were chosen because scores such as the Framingham score take limited account of diabetes status and were developed in general populations. As such, the UKPDS, which was developed in and is for use by individuals with diabetes, was felt to be a more appropriate representation of current risk prediction in people with Type 2 diabetes. Ethnicity was not included as the majority of the cohort was of the same ethnicity. Atrial fibrillation was also excluded as this information was not available at the time of analysis. Stepwise regression and construction of individual multifactorial models were not undertaken given the low proportion of overall variability in cIMT that was explained by the totality of the measured various risk factors.

Cox Regression Model A Unadjusted

Cox Regression Model B Age, Sex and Previous CVD adjusted

Cox Regression Model C Age, Sex, Previous CVD, duration of diabetes, HbA1c, systolic BP, total cholesterol, HDL, smoking status

The Cox proportional hazards assumption was assessed for included variables using log minus log plots. All variables met the proportional hazards assumption for each outcome event.

Following the construction of the Cox models, assessment of any improvement in the model on addition of the test variables was assessed by determining the area under the ROC curve for each model (AUC). The predicted individual risk (XBeta) for each model under investigation was saved as a new variable during Cox Regression and used to create a ROC curve. The area under each curve was calculated and compared.

In order to assess the potential effect on risk classification of any variables which survived Cox analysis, the net reclassification index was employed. Net reclassification index is a method which evaluates the proportion of subjects moving accurately or inaccurately from one risk category to another after change to a prediction model. In this thesis, participants were assigned to a level of risk (low, intermediate or high) based on arbitrary tertiles calculated from the X beta values of each model, as described by de Ruijter et al ((de Ruijter W, Westendorp RGJ et al. 2009). While this does not correspond directly to meaningful clinical risk levels, the small number of events led to a number of zero cells in the cross tabulations. The tertiles for each model were cross tabulated (separately for the event and non-event groups) and net reclassification index calculated using the following formula:

NRI= [Pup(events)-Pdown(events)]-[Pup(nonevents)-Pdown(nonevents)]
Where:
Pup(events)=[number of events moving up/number of events]
Pdown(events)= [number of events moving down/number of events]
Pup(nonevents)= [number of nonevents moving up/number of nonevents]
Pdown(nonevents)= [number of nonevents moving up/number of nonevents]

Chapter 4: Results 1 - Characteristics of study population and descriptive statistics for risk factor variables and carotid ultrasound parameters

This chapter describes the socio-demographic characteristics and representativeness of the ET2DS study population and the sub-population used for analyses presented in this thesis. It also presents descriptive statistics for included vascular risk factor variables which are both traditional cardiovascular risk factors (including those in the UKPDS risk score) and more 'novel' biomarkers (including ABI, ACR, eGFR, plasma IL6, CRP and NTproBNP), which are starting to be explored in terms of their potential as cardiovascular risk predictors in other study populations. It further describes the results of carotid ultrasound measurements at Year 1 and Year 4 follow up as well as change in ultrasound measurements between these time points. Finally, an assessment of measurement methods used in the study is presented.

4.1 ET2DS study population

4.1.1 Baseline socio-demographic characteristics

Baseline socio-demographic characteristics of the ET2DS study population are presented in table 4-1 (column 1). At recruitment, mean age of participants of the ET2DS (n=1066) was 67.9 years, and 547 (51.3%) participants were male. One hundred and twenty-seven (11.9%) were in the first quintile of the Scottish Index of Multiple Deprivation (most deprived), 208 (19.5%) were in the second quintile, 188 (17.6%) were in the third quintile, 194 (18.2%) were in the fourth quintile and 349 (32.7%) were in the top quintile (least deprived).

4.1.2 Representativeness

Representativeness of the recruited ET2DS population was assessed by researchers involved in the baseline phase of the ET2DS (Marioni, Strachan et al. 2010). The

results of this analysis are presented in table 4-1. Clinical and socio-demographic characteristics recorded on the Lothian Diabetes Register were compared between those individuals who had been invited, at random, to participate and had attended (participants n=1066), with those who had declined or did not respond ('non-participants', n=4386).

Mean age of participants and non-participants was similar (both 67.9 years), but participants were more likely to be male (51.3% vs. 41.9%). In addition, there was a higher proportion of individuals from less deprived backgrounds in the participant group. Mean systolic blood pressure was slightly lower in participants but mean cholesterol, mean HbA1c, duration of diabetes and proportion receiving insulin treatment were similar between participants and non-participants.

Table 4-1 Comparison of clinical characteristics recorded on the Lothian Diabetes Register in ET2DS participants and non-participants (adapted from (Marioni, Strachan et al. 2010))

	Participants (n=1028-1066)	Non-Participants (n=4020-4385)
Mean Age (years)	67.9 (4.2)	67.9 (4.4)
Male sex	51.3 (547)	41.9 (1839)
Deprivation rank		
1st quintile (most deprived)	11.9 (127)	16.8 (736)
2nd quintile	19.5 (208)	25.9 (1134)
3rd quintile	17.6 (188)	18.7 (820)
4th quintile	18.2 (194)	17.8 (782)
5th quintile (least deprived)	32.7 (349)	20.8 (897)
Duration of diabetes <5 years	48.4 (516)	48.7 (2135)
Insulin Treatment	17.4 (185)	16.1 (704)
Cholesterol (mmol/l)	4.3 (0.9)	4.2 (0.96)
Systolic blood pressure (mmHg)	133.3 (16.44)	137.2 (18.15)
HBA1c (%Hb)	7.4 (1.12)	7.4 (1.36)

Values are mean (SD) or % (n) HbA1c=glycated haemoglobin

For the purposes of the research presented in this thesis, baseline demographic and cardiometabolic characteristics were also compared between subjects attending baseline, one year follow up and four year follow up respectively (table 4-2), and between those that had a full set of valid IMT readings at the one year visit and those that did not (table 4-3).

Of the 1066 participants in the ET2DS, 939 (88.1%) attended the year 1 follow up clinic and 831 (77.9%) attended the year 4 follow up clinic. In general, mean baseline characteristics were similar across the cohort at all three time points. At year 1 and year 4, there was a slightly lower proportion of subjects who had been current smokers at baseline (both 12.5%) compared with the entire cohort (14.0%) and baseline systolic blood pressure was slightly lower at 132.5 ±15.7 mmHg in those at year 4 compared with the whole cohort (133.3 ± 16.4 mmHg). However, even these differences were small.

Table 4-2 Baseline characteristics of study participants attending baseline, 1 year and 4 year follow-up of the ET2DS

		All subjects (n=1066)	Year 1 participants (n=939)	Year 4 Participants (n=831)
Demographics	Age at Baseline (years)	67.8 (4.17)	67.8 (4.15)	67.7 (4.14)
	Sex (male)	546 (51.3)	487 (52.0)	430 (51.7)
Diabetes	Duration of Diabetes (years)	6.0 (8)	6.0 (8.0)	6.0 (8.0)
	HbA1C (%Hb)	7.39 (1.12)	7.37 (1.11)	7.39 (1.13)
	Diabetes Treatment			
	Diet Alone	201 (18.9)	182 (19.4)	166 (20.0)
	Oral Hypoglycaemics	689 (64.6)	607 (64.7)	530 (63.8)
	Insulin ± Oral Hypoglycaemics	176 (16.5)	149 (15.9)	135 (16.2)
Blood Pressure	Systolic BP (mmHg)	133.3 (16.3)	133.2 (16.2)	132.5 (15.7)
	Diastolic BP(mmHg)	69.1 (9.0)	69.0 (9.0)	68.9 (8.8)
Blood Lipids	Total Cholesterol	4.31 (0.90)	4.31 (0.89)	4.33 (0.91)
	HDL Cholesterol	1.30 (0.36)	1.29 (0.35)	1.29 (0.35)
Smoking	Smoking Status			
	Current smoker	148 (14.0)	117 (12.5)	104 (12.5)
	Ex smoker	501 (47.4)	443 (47.5)	392 (47.2)
	Never smoker	409 (38.7)	373 (40.0)	332 (40.0)
Obesity Index	BMI (kg/m^2)	31.3 (5.6)	31.3 (5.6)	31.2 (5.5)
Renal	ACR	1.8 (3.0)	1.8 (3.0)	1.8 (3.2)

BP=blood pressure, HDL=high density lipoprotein, BMI=body mass index
Values are mean (SD), median (Interquartile range), or n (%)

4.2 One year follow up study population

4.2.1 Missing data

cIMT

Of the 939 subjects that attended year 1 follow up, 916 subjects had valid cIMT readings at year 1 and 904 had valid plaque readings. In terms of cIMT measurements, the remaining 23 participants had no valid cIMT readings due to

problems with the measurement of cIMT at the clinic eg physical barrier to measurement or incorrect measurements taken. This also applied to those with missing plaque measurements. A comparison of baseline characteristics between those subjects with and without cIMT measurements at year 1 is made in table 4-3. There were no significant differences between the two groups, with the exception of smoking, where only 13.0% of people with cIMT measurements were current smokers compared with 22.4% of people without cIMT measurements.

Table 4-3 Comparison of baseline characteristics of subjects with and without valid cIMT measurements at year 1 follow up of the ET2DS

		Subjects with valid year 1 cIMT measurements (n=916)	Subjects without valid year 1 cIMT measurements (n=150)	P value
Demographics	Age at Baseline (years)	67.9 (4.2)	67.9 (4.4)	NS
	Sex (male)	51.7 (474)	48.7 (73)	NS
Diabetes	Duration of Diabetes (years)	6.0 (8.0)	6.0 (7.8)	NS
	HbA1C (% Hb)	7.38 (1.12)	7.53 (1.12)	NS
	Diabetes Treatment			
	Diet Alone	19.7 (180)	14.7 (22)	
	Oral Hypoglycaemics	64.5 (591)	66.0 (99)	NS
	Insulin ± Oral Hypoglycaemics	15.8 (145)	19.3 (29)	
Blood Pressure	Systolic BP (mmHg)	133.2 (16.4)	133.9 (17.1)	NS
	Diastolic BP(mmHg)	69.1 (9.0)	69.1 (9.2)	NS
Blood Lipids	Total Cholesterol (mmol/l)	4.31 (0.89)	4.31 (0.95)	NS
	HDL Cholesterol (mmol/l)	1.29 (0.35)	1.30 (0.43)	NS
Smoking	Current smoker	13.0 (119)	22.4 (33)	
	Ex smoker	46.9 (430)	48.3 (71)	0.002**
	Never smoker	40.1 (367)	29.3 (43)	
Obesity Index	BMI (kg/m^2)	31.3 (5.7)	32.1 (5.7)	NS

BP=blood pressure, HDL=high density lipoprotein, BMI=body mass index, NS=not significant ($p>0.05$)
Values are mean (SD), median (Interquartile range) or % (n) ** significant $p<0.01$

Other Variables

At year 1, there were differing degrees of missing data for each variable (table 4-4). Age and BMI were complete for all subjects. Duration of diabetes (years), systolic BP, diastolic BP, ABI, eGFR and IL-6 had missing data of less than 1%. HbA1c, total cholesterol, HDL cholesterol, CRP and NTproBNP had a missing data level between 1% and 2%. Continuous variables with higher missing data levels were albumin creatinine ratio (58.5%, n=550) and mean and maximum plaque thickness (5.4%, n=51 for both). Categorical variables used in this thesis demonstrated less

missing data than continuous variables. Sex, SIMD quintile, diabetes treatment, antihypertensive use and lipid lower medication use were complete. Smoking status had <1% missing data.

Table 4-4 Missing data in study population used in this thesis

		Total in group	N missing	% missing
Demographics	Age (years)	939	0	0.0
	Sex (male)	939	0	0.0
	SIMD	939	0	0.0
Diabetes	Duration of diabetes (years)	939	7	0.8
	HbA1c (%Hb)	939	10	1.1
	Diabetes Treatment	939	0	0.0
Blood Pressure	Systolic BP (mmHg)	939	5	0.5
	Diastolic BP (mmHg)	939	5	0.5
	Antihypertensive use (yes)	939	0	0.0
Lipids	Total Cholesterol (mmol/l)	939	10	1.1
	HDL Cholesterol (mmol/l)	939	10	1.1
	Lipid Lowering Medication (yes)	939	0	0.0
Smoking	Smoking status	939	1	0.1
Obesity Index	BMI (kg/m^2)	939	0	0.0
CVD Risk Marker	ABI	939	6	0.6
Renal Function	eGFR (ml/min/1.73m2)	939	7	0.7
	ACR (mg/mmol)	939	550	58.5
Plasma Biomarkers	CRP (mg/l)	939	16	1.7
	IL-6 (pg/ml)	939	2	0.2
	NTproBNP (pg/ml)	939	9	1.0
Carotid Ultrasound	Mean cIMT (mm)	939	23	2.4
	Max cIMT (mm)	939	23	2.4
	Max Mean cIMT (mm)	939	23	2.4
	Mean Max cIMT (mm)	939	23	2.4
	Mean Plaque Thickness (mm)	939	54	5.4
	Max Plaque Thickness (mm)	939	54	5.4
	Plaque Score	939	34	3.6
	Plaque Morphology	939	2	0.2

SIMD = Scottish Index of Multiple Deprivation, BP = blood pressure, HDL = high density lipoprotein, ABPI = ankle brachial pressure index, BMI = body mass index, eGFR = estimated glomerular filtration rate, ACR = albumin creatinine ratio, CRP = C reactive protein, IL-6 = interleukin 6, NTproBNP = N terminal pro-brain natriuretic peptide, cIMT = carotid intima media thickness

4.2.2 Characteristics of study populations at year 1

The characteristics of the study population at year 1 are presented in (table 4-5). At year 1, subjects in the IMT analysis study population had a mean age of 68.9 ± 4.2

years, and 51.7% (474) were male. There was a similar proportion of the sample in each of the quintiles of SIMD, with the exception of the most affluent quintile, which was over represented in the sample (quintile 5, 34.4% (315) vs quintile 1, 11.6% (106)). The median duration of diabetes was 7.0 (8.0) years, with a mean HbA1c of 7.18 ± 1.07%. A high percentage of the sample (64.5% (591)) was using oral hypoglycaemic medications alone to manage their diabetes while fewer participants were treating their diabetes with diet modification alone (19.7% (180)) or with subcutaneous insulin (alone or in combination with oral hypoglycaemics) (15.8% (145)). The mean systolic blood pressure was 138.1 ± 18.4 mmHg, with a mean diastolic pressure of 74.1 ± 9.5 mmHg. 85.7% (785) of the sample reported using antihypertensive medication. Mean total cholesterol was 4.15 ± 0.80 mmol/l and mean HDL cholesterol 1.23 ± 0.34 mmol/l. 84.3% (772) of participants reported use of lipid lowering medication. 13.0% (119) of the sample reported being current smokers, while 46.9% (430) reported that they were ex-smokers and 40.1% (367) reported that they had never smoked. The mean ankle brachial index was 0.99 ± 0.21 and the mean eGFR was 77.3 ± 18.6 ml/min/1.73m^2. Median ACR value was 1.80 (3.0) mg/mmol, median IL-6 was 2.82 (2.42) pg/ml, median CRP 1.76 (3.27) mg/l and NTProBNP had a median value of 74 (133) ng/ml.

The corresponding values for the plaque analysis group were almost identical to those of the IMT group (table 4-5).

Table 4-5 Demographic variables, cardiometabolic factors and biomarkers of the study population at Year 1 ET2DS follow up

		Subjects with valid IMT at year 1 (n=916)	Subjects with valid plaque assessment ay year 1 (n=905)
Demographics	Mean Follow up (years)	3.53 (0.27)	3.53 (0.27)
	Age (years)	68.9 (4.2)	69.0 (4.2)
	Sex (male)	474 (51.7)	465 (51.4)
	SIMD		
	Quintile 1	106 (11.6)	104 (11.5)
	Quintile 2	169 (18.4)	170 (18.8)
	Quintile 3	160 (17.5)	158 (17.5)
	Quintile 4	166 (18.1)	167 (18.5)
	Quintile 5	315 (34.4)	305 (33.7)
Diabetes	Duration of Diabetes (years)	7.0 (8.0)	7.0 (8.0)
	HbA1C (% Hb)	7.18 (1.07)	7.19 (1.08)
	Diabetes Medication		
	Diet Alone	180 (19.7)	172 (19.0)
	Oral Hypoglycaemics	591 (64.5)	590 (65.3)
	Insulin ± oral hypoglycaemics	145 (15.8)	142 (15.7)
Blood Pressure	Systolic Blood Pressure (mmHg)	138.1 (18.4)	138.0 (18.5)
	Diastolic Blood Pressure (mmHg)	74.1 (9.5)	74.0 (9.5)
	On antihypertensive (yes)	785 (85.7)	775 (85.7)
Lipids	Total Cholesterol (mmol/l)	4.15 (0.80)	4.15 (0.80)
	HDL Cholesterol (mmol/l)	1.23 (0.34)	1.23 (0.34)
	Lipid lowering meds (yes)	772 (84.3)	762 (84.3)
Smoking	Smoking status		
	Current smoker	119 (13.0)	115 (12.7)
	Ex smoker	430 (46.9)	425 (47.0)
	Never smoker	367 (40.1)	364 (40.3)
Obesity Index	BMI (kg/m^2)	31.3 (5.7)	31.3 (5.7)
CVD Risk Marker	Ankle Brachial Index	0.99 (0.21)	0.99 (0.21)
Renal Function	eGFR ($ml/min/1.73m^2$)	77.3 (18.6)	77.0 (18.2)
	ACR (mg/mmol)	1.80 (3.0)	1.8 (3.0)
Plasma Biomarkers	IL6 (pg/ml)	2.82 (2.42)	2.82 (2.43)
	CRP (mg/l)	1.76 (3.27)	1.76 (3.29)
	NTProBNP (pg/ml)	74 (133)	74 (133)

Values are mean (SD), median (Interquartile range) or %(n) SIMD=Scottish Index of Multiple Deprivation, HDL=high density lipoprotein, BMI=body mass index, eGFR=estimated glomerular filtration rate, ACR=albumin creatinine ratio, IL-6=interleukin 6, CRP=C reactive protein, NTProBNP=N terminal pro-brain natriuretic peptide,

4.3 Carotid intima media thickness

This section describes the results of the cIMT measurement validation study and a description of cIMT distribution and change in the study sample.

4.3.1 cIMT validation study at year 4 follow up

Because of the well documented issues regarding the reliability and repeatability of ultrasound measurements of cIMT, an assessment was made of the measurements performed by the ET2DS sonographer. This was carried out at the year 4 follow up clinics, at which time 52 subjects returned for repeat cIMT measurement. As there was only 1 sonographer, only intra-observer variability could be assessed. Data was assessed for normality using Kolmogorov-Smirnov tests at a 99% confidence level and found to be normal.

Mean values for the mean cIMT recorded at each attendance are reported in table 4-6. Mean cIMT at the first attendance was 0.89mm and at the repeat attendance 0.90mm. The difference between the means was not statistically significant ($p=0.537$). Pearson correlation coefficient between the means of the first and second sessions was 0.948 ($p<0.001$). Intra-class correlation was also determined using a two way random model with absolute agreement and was found to be 0.947 (0.910-0.969) ($p<0.001$). These analyses were repeated on two subgroups defined by time between the first and second reading (less than or more than 4 months apart). There were no significant differences in the results for each group, suggesting that the time between readings did not affect cIMT measurement.

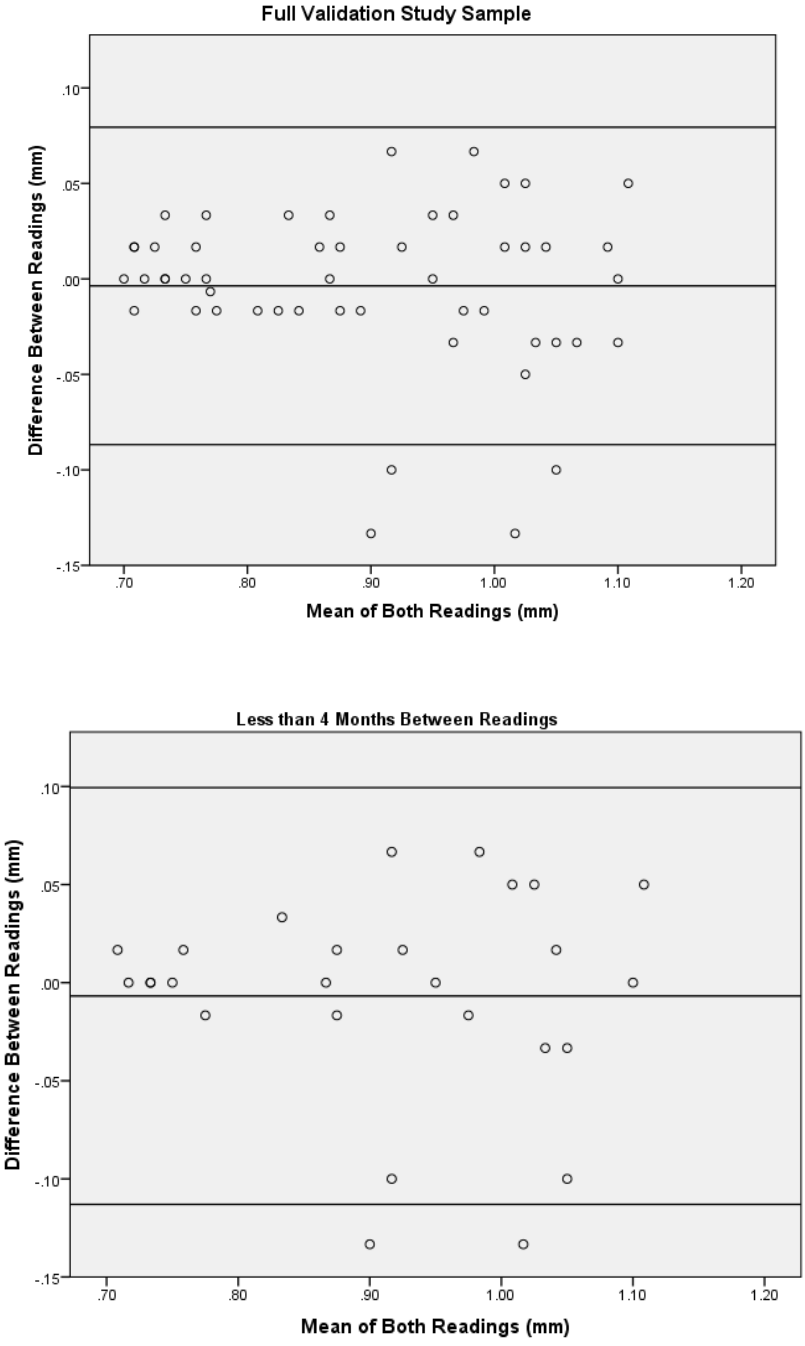
Table 4-6 Correlation between cIMT measurements in the validation study

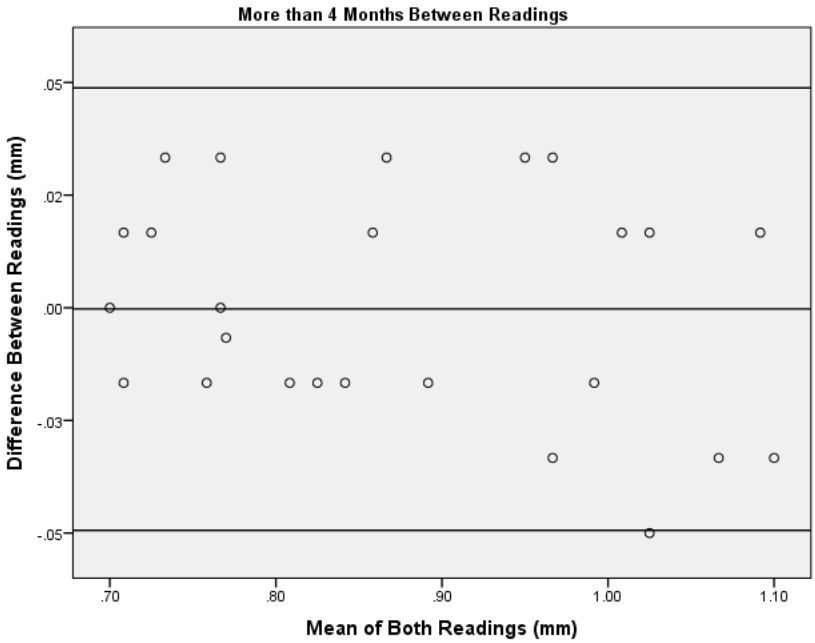
	N	1st Attendance (mm)	2nd Attendance (mm)	P value	Pearson Correlation Coefficient	Intraclass Correlation Coefficient (95%CI)
Overall	52	0.89	0.90	0.537	0.948***	0.947 (0.910-0.969)***
<4 months apart	27	0.93	0.91	0.520	0.912***	0.954 (0.898-0.979)***
>4 months apart	25	0.88	0.88	0.958	0.983***	0.991 (0.980-0.996)***

*** significant at $p<0.001$. Values for each attendance are mean cIMT.

Bland Altman plots were then created to visually evaluate agreement. 95% limits of agreement were calculated and plotted for the whole validation sample and the sub groups based on time between readings. No clinically significant bias was seen in any of the measurements.

Figure 4-1 Bland Altman Plots for cIMT Validation Study





4.3.2 Description of cIMT in the study population

Mean values for the various cIMT measurements derived from year 1 recordings are described in table 4-7. Year 1 mean cIMT was 0.94 ± 0.14mm, and year 1 maximum cIMT 1.06 ± 0.19mm. Year 1 maximum mean cIMT and mean maximum cIMT were 0.99 ± 0.17mm and 0.99 ± 0.16mm respectively. All measures of cIMT were higher in men than in women, and also higher in older participants than younger participants (table 4-8). Additionally, mean cIMT was higher in the left carotid artery than the right (for both men and women in all age categories) – (table 4-8).

Table 4-7 cIMT measured at year 1 and year 4 of the ET2DS

	Year 1 (n=916)	Year 4 (n=781)	Change (n=765)	P for change
Mean cIMT (mm)	0.94 (0.14)	0.92 (0.15)	-0.02 (0.11)	0.001**
Max cIMT (mm)	1.06 (0.19)	1.04 (0.22)	-0.02 (0.19)	0.001**
Max Mean cIMT (mm)	0.99 (0.17)	0.97 (0.17)	-0.02 (0.17)	0.052
Mean Max cIMT (mm)	0.99 (0.16)	0.99 (0.20)	-0.02 (0.14)	<0.001***
Mean Right cIMT (mm)	0.92 (0.15)	0.90 (0.16)	-0.02 (0.14)	0.001**
Mean Left cIMT (mm)	0.96 (0.17)	0.94 (0.20)	-0.02 (0.13)	<0.001***

Values are mean (SD), cIMT=carotid Intima Media Thickness ** significant at o<0.01, *** significant at p<0.001

Table 4-8 cIMT in five year age bands for men and women, including left and right measurements for mean cIMT

		Mean cIMT			Max cIMT	Max Mean cIMT	Mean Max cIMT
	Age	Mean	R	L			
Men	**61-66**	0.93	0.90	0.96	1.05	1.00	0.99
	66-71	0.97	0.96	0.99	1.10	1.03	1.03
	71-76	0.99	0.96	1.02	1.13	1.06	1.05
Women	**61-66**	0.88	0.87	0.89	0.99	0.93	0.93
	66-71	0.91	0.90	0.91	1.01	0.95	0.96
	71-76	0.93	0.91	0.95	1.04	0.98	0.98

Values are mean (mm). Age categories are in years. R=right, L=left

After follow up, year 4 mean cIMT was 0.92 ± 0.15mm and maximum cIMT 1.04 ± 0.22mm. Maximum mean cIMT and mean maximum cIMT were 0.97 ± 0.17mm and 0.99 ± 0.20mm respectively. Seven hundred and sixty five individuals had cIMT measurements at both year 1 and year 4. Overall, there was a small negative change in IMT between these time points, which was statistically significant for all measures except maximum mean cIMT (table 4-7). Average change in mean cIMT was -0.02 ±0.11mm and for maximum cIMT was -0.02 ± 0.19mm. Max mean cIMT and mean max cIMT also demonstrated a negative change (-0.02 ±0.17mm and -0.02 ±

0.13mm respectively). Because all the measures of cIMT demonstrated a similar change with time, only change in mean cIMT was further analysed. Because of the potential effect of some medications on cIMT progression, change in mean cIMT was evaluated in those who were and were not taking antihypertensive medication and lipid lowering medication. No statistically significant difference was noted in either group, although it is important to note that the direction of the change was different for those taking and not taking lipid lowering medication (taking -0.016mm vs not taking 0.002mm, p=0.111).

4.4 Carotid plaque

This section describes the frequency and distribution of carotid plaque in the study population. Table 4-9 describes the frequency and distribution of carotid plaque in the ET2DS.

4.4.1 Description of carotid plaque thickness in the study population

Plaque variables measured at year 1 and year 4 of the ET2DS are summarised in table 4-9. The prevalence of carotid plaque at year 1 in the ET2DS was high – 97.8% (884) of participants had at least 1 plaque (of any size and type) present on carotid ultrasound. Plaque was most common in the carotid bifurcations (right 90.7% and left 92.7%) and the internal carotids (right 59.8% and left 61.9%); and least common in the common carotids (right 32.0% and left 42.4%) and the external carotids (right 28.9% and left 26.2%). In most divisions of the carotid artery, plaque was more common on the left than right, with the exception of the internal carotids. Mean values for mean plaque thickness and maximum plaque thickness at year 1 were 2.44 ± 0.90 mm and 2.81± 1.11 mm respectively.

At year 4, the prevalence of plaque remained similar, with 99.6% (751) of participants demonstrating at least 1 plaque. Plaque remained most common in the carotid bifurcations (96.7% right and 97.2% left) and internal carotids (70.7% right and 71.1% left) and least common in the common carotids (55.0% right and 65.0%

left) and external carotids (42.5% right and 36.8% left). Plaque was most common on the right side than the left side, with the exception of the bifurcation, in contrast to the findings at year 1. Mean values for mean plaque thickness and maximum plaque thickness at year 4 were 2.81 ± 1.07mm and 3.26 ± 1.32 mm respectively.

The percentage of individuals with at least 1 plaque was similar at both year 1 and year 4 (97.8% and 99.6% respectively). However, the percentage of individuals with plaque present at each division of the carotid artery was significantly higher at year 4 than at year 1. Mean plaque thickness increased significantly from Year 1 to Year 4 by 0.40 ± 0.67 mm, $p<0.001$, which is in contrast to cIMT measures. Maximum plaque thickness also demonstrated a significant increase between the two time points (0.47 ± 0.67mm, $p<0.001$).

4.4.2 Plaque score

Because of the method by which plaque was assessed (thickness of only 1 plaque on each side measured) and limitations set by the ultrasound machine used in this study, it was not possible to calculate plaque burden by more traditional methods such as total plaque volume or plaque area. As such, a proxy for plaque burden was created using the presence of plaque in each of the segments of the carotid tree. A plaque score was calculated by totaling the number of areas of the right and left carotid arteries in which plaque was present. Values ranged from 0 (no plaque in any area) to 8 (presence of at least 1 plaque in all 8 areas).

At year 1, the majority of individuals (79.7% (720)) had a plaque score of between 2 and 6. 15.2% of individuals had a score of 7 or 8 and 5.1% had a score of 1 or 0. Mean plaque score was 4.39 (1.9). This can be summarized more clearly by exploring the percentage of participants who had a high plaque score (greater than four) - 45.2% of participants had a plaque score >4 at year 1.

At year 4, 81.8% of individuals had a plaque score of between 4 and 8, while 19.2% had a score of 3 or less. Again, by exploring the percentage of participants with a plaque score great than or less than 4, it can be seen that 65.0% had a plaque score >4 at year 4. The difference in percentage of participants with a plaque score >4 at

year 1 and year 4 was significant ($p<0.001$), suggesting that plaque burden may have increased in this time period.

4.4.3 Plaque morphology

At year 1, heterogeneous plaque (plaque where >20% of the plaque echogenicity differs from the rest of the plaque) was present in 44.8% (404) of participants (at least 1 heterogeneous plaque) and echolucent plaque in 37.0% (334) (at least 1 echolucent plaque). At year 4, 70.4% (529) participants had at least 1 heterogeneous plaque and 77.1% (579) had at least 1 echolucent plaque.

When these results are broken down further, at year 1 12.5% of participants had only echolucent plaque, 20.3% had only heterogeneous plaque, 24.5% had both echolucent and heterogeneous plaque and 42.7% had neither type of plaque (which means that they had predominantly echogenic plaque or no plaque). By summarizing plaque morphology as high risk (either echolucent or heterogeneous plaque) or low risk (no plaque or echogenic plaque), it can be seen that 57.3% of participants had high risk plaque at year 1.

At year 4, 20.4% had echolucent plaque only, 13.7% had heterogeneous plaque only, 56.7% had both echolucent and heterogeneous plaque and 9.2% had echogenic plaques. A clear comparison can be made with year 1 by examining high risk versus low risk. At year 1, 56.1% of participants had "high risk" plaque, while at year 4, this had risen to 89.8%. This was significantly higher.

Table 4-9 Carotid plaque measured at year 1 and year 4 of the ET2DS

	Year 1 (N max=904)	Year 4 (N max=751)	Change in mm	P value (N max=761)
Mean Plaque Thickness (mm)	2.44 (0.90)	2.81 (1.07)	0.40 (0.67)	<0.001***
Max Plaque Thickness (mm)	2.81 (1.11)	3.26 (1.32)	0.47 (0.91)	<0.001***
At least 1 plaque in any area of carotids	97.8 (884)	99.6 (748)	-	0.138
Plaque Present in:				
Right Common Carotid	32.0 (289)	55.0 (413)	-	<0.001***
Left Common Carotid	42.4 (383)	65.0 (488)	-	<0.001***
Right Internal Carotid	59.8 (541)	70.7 (531)	-	<0.001***
Left Internal Carotid	61.9 (560)	71.1 (534)	-	<0.001***
Right External Carotid	28.9 (261)	42.5 (319)	-	<0.001***
Left External Carotid	26.2 (237)	36.8 (276)	-	<0.001***
Right Bifurcation	90.7 (820)	96.7 (726)	-	0.064
Left Bifurcation	92.7 (838)	97.2 (730)	-	0.003***
Plaque Score:				
0	2.1 (19)	0.4 (3)	-	
1	3.0 (27)	1.1 (8)	-	
2	14.5 (131)	8.1 (61)	-	
3	16.3 (147)	8.5 (64)	-	
4	18.6 (168)	16.9 (127)	-	<0.001***
5	16.5 (149)	15.4 (116)	-	
6	13.8 (125)	16.5 (124)	-	
7	7.2 (65)	14.9 (112)	-	
8	8.0 (72)	18.1 (136)	-	
Plaque Score Category				
≤4	54.5 (492)	35.0 (263)	-	<0.001***
>4	45.2 (411)	65.0 (488)	-	
Plaque Morphology				
Echolucent plaque only	12.5 (113)	20.4 (153)	-	
Heterogeneous plaque only	20.3 (183)	13.7 (103)	-	<0.001***
Both Types of Plaque	24.5 (221)	56.7 (426)	-	
Neither type of plaque	42.7 (386)	9.2 (69)	-	
Plaque Risk Type				
Low risk	42.7 (386)	9.2 (69)	-	<0.001***
High risk	57.3 (517)	89.8 (682)	-	

Values are mean (SD) or % yes (n) *** significant at p<0.0001

4.5 Inter-correlation of cIMT and carotid plaque parameters at Year 1

The associations of cIMT and plaque variables with each other are described in tables 4-10 – 4-12. cIMT variables were highly correlated with one another (range: r=0.911-0.977). Right and left cIMT correlated moderately with each other (r=0.533, p<0.001) as did right and left plaque thickness (r=0.488, p<0.001). Summary

measures of cIMT correlated substantially with one another (r=0.911-0.977). They also correlated (albeit more modestly), with the plaque thickness measurements (range of r=0.223 - 0.278). Mean cIMT and mean plaque thickness were more moderately correlated (r=0.268, $p<0.001$), as were maximum cIMT and maximum plaque thickness (r=0.254, $p<0.001$).

Individuals with a higher plaque score (>4) tended to have a higher cIMT than those without (mean cIMT in those with high score 0.99 (0.15)mm versus those with low score 0.89 (0.12)mm). Individuals with a higher plaque score also had more high risk plaque than those with a low score (76.9% versus 40.7% respectively). Individuals with high risk plaque also tended to have a higher cIMT (mean cIMT 0.97mm) than those without (mean cIMT 0.89mm), as well as a higher plaque score (61.2% had plaque score >4 compared with those with low risk plaque (24.6%).

Table 4-10 Association of continuous cIMT with continuous carotid plaque thickness measurements at year 1 of the ET2DS

	Left cIMT	Mean cIMT	Max cIMT	Max Mean cIMT	Mean Max cIMT	Right Plaque Thickness	Left Plaque Thickness	Mean Plaque Thickness	Max Plaque Thickness
Right cIMT	0.533***	0.869***	0.727***	0.745***	0.844***	0.204***	0.138***	0.206***	0.188***
Left cIMT	-	0.877***	0.876***	0.916***	0.871***	0.207***	0.253***	0.270***	0.260***
Mean cIMT	-	-	0.911***	0.939***	0.977***	0.229***	0.223***	0.268***	0.254***
Max cIMT	-	-	-	0.964***	0.949***	0.227***	0.244***	0.278***	0.262***
Max Mean cIMT	-	-	-	-	0.936***	0.224***	0.238***	0.273***	0.260***
Mean Max cIMT	-	-	-	-	-	0.237***	0.231***	0.276***	0.258***
Right Plaque Thickness	-	-	-	-	-	-	0.488***	0.874***	0.839***
Left Plaque Thickness	-	-	-	-	-	-	-	0.859***	0.823***
Mean Plaque Thickness	-	-	-	-	-	-	-	-	0.959***

Values are Pearson correlation coefficients. ** *significant at level p<0.001 cIMT=carotid intima media thickness.

Table 4-11 Association of plaque score with cIMT, plaque thickness and plaque type

	Plaque score<=4 (n=479)	Plaque score >4 (n=402)	P value
Mean cIMT (mm)	0.89 (0.12)	0.99 (0.15)	<0.001***
Max cIMT (mm)	1.00 (0.16)	1.14 (0.20)	<0.001***
Max Mean cIMT (mm)	0.94 (0.14)	1.07 (0.18)	<0.001***
Mean Max cIMT (mm)	0.94 (0.13)	1.06 (0.16)	<0.001***
Mean Plaque Thickness (mm)	2.05 (0.72)	2.89 (0.88)	<0.001***
Max Plaque Thickness (mm)	2.36 (0.89)	3.33 (1.12)	<0.001***
Plaque morphology			
No or echogenic plaque	59.3 (291)	23.1 (95)	
Echolucent plaque only	14.1 (69)	10.7 (44)	<0.001***
Heterogeneous plaque only	15.5 (76)	26.0 (107)	
Both Types	11.2 (55)	40.1 (165)	
Plaque morphology category			
Low risk	59.3 (291)	23.1 (95)	<0.001***
High risk	40.7 (200)	76.9 (316)	

*** significant at p<0.001

Table 4-12 Association of plaque type with cIMT, plaque thickness and plaque score

		Low Risk (n=386)	High Risk (n max=516)	P value
Mean cIMT (mm)		0.89 (0.13)	0.97 (0.14)	<0.001***
Max cIMT (mm)		1.00 (0.17)	1.10 (0.19)	<0.001***
Max Mean cIMT (mm)		0.94 (0.15)	1.03 (0.19)	<0.001***
Mean Max cIMT (mm)		0.95 (0.14)	1.02 (0.16)	<0.001***
Mean Plaque Thickness (mm)		2.05 (0.79)	2.72 (0.87)	<0.001***
Max Plaque Thickness (mm)		2.34 (0.97)	3.15 (1.08)	<0.001***
Plaque Score				
	0	4.7 (18)	0.0 (0)	
	1	6.7 (26)	0.2 (1)	
	2	24.6 (95)	7.0 (36)	
	3	22.5 (87)	11.6 (60)	
	4	16.8 (65)	20.0 (103)	<0.001***
	5	12.2 (47)	19.8 (102)	
	6	7.3 (28)	18.8 (97)	
	7	3.4 (13)	10.1 (52)	
	8	1.8 (7)	12.6 (65)	
Plaque score category				
	<=4	75.4 (291)	38.8 (200)	<0.001***
	>4	24.6 (95)	61.2 (316)	

*** significant at p<0.001

4.6 Serial cIMT measurements

cIMT measurements at year 1 were repeated in 235 participants using a computer aided method for measuring cIMT. These mean values for these measurements are reported in table 4-13, in addition to the corresponding sonographer value. The minimum number of measurements taken for any individual image was 10 and the

maximum was 60, with an average number of measurements per participant of 192. For the sonographer measurements, the average number of measurements was 6 per participants (3 left and 3 right). The mean serial cIMT was significantly lower than mean sonographer cIMT in the sample (0.91vs0.92mm, $p<0.001$ respectively). The correlations between mean serial cIMT and mean cIMT were strong ($r=0.811$, $p<0.001$).

Table 4-13 Comparison of cIMT measurements made by sonographer with computer aided method

	Sub-sample (n=235)
Serial cIMT	
Mean number of measurements per participant	192 (10.6)
Mean serial cIMT (mm)	0.91 (0.14)
Sonographer cIMT	
Mean number of measurements of participant	6 (0)
Mean sonographer cIMT (mm)	0.92 (0.14)
P value for difference in mean cIMT measurements	0.034*

Values are mean (SD), * significant at $p<0.05$

4.7 Chapter summary

This chapter describes the socio-demographic characteristics and representativeness of the ET2DS study population and the sub-population used for analyses presented in this thesis as well as descriptive statistics of the ET2DS study sample. It further describes the results of carotid ultrasound measurements in the study. Participants in the ET2DS are generally representative of older adults with Type 2 diabetes living in Edinburgh and the Lothians and those who attended for year 1 cIMT measurements were representative of the cohort as a whole. cIMT was normally distributed in the sample and was higher in men, as well as older participants. cIMT was also higher in the left carotid artery than the right (for both men and women). Change in cIMT between year 1 and year 4 follow up of the ET2DS showed a small, but significant regression. cIMT measurement in the ET2DS was validated and found to be highly repeatable. In comparison with computer aided measurements, the sonographer measurements were on average, slightly higher than the computer measurements but they displayed a high degree of correlation.

There was a high prevalence of carotid plaque in the ET2DS. High risk plaques were particularly prevalent in the study sample, affecting 57% of participants and plaque was widely distributed in the carotid artery, as measured by plaque score. Continuous measurements of cIMT were highly correlated with one another, but less so with measures of plaque thickness. Similarly, measures of plaque thickness correlated highly with one another. Individuals with a high plaque score tended to have a high cIMT and more high risk plaque than those with a low plaque score. Similarly, those with a high risk plaque tended to have a higher cIMT and a higher plaque score.

Chapter 5: Results 2 - Cross Sectional Relationships between Carotid Ultrasound Parameters and Cardiovascular Disease in the ET2DS

This chapter presents the results of cross sectional analyses of year 1 cIMT and plaque measurements, assessing the associations with traditional cardiovascular risk factors (including those in the UKPDS risk score) and with the more novel biomarkers of cardiovascular risk (ABI, eGFR, ACR, IL-6, CRP and NTproBNP), as well as prevalent cardiovascular disease.

5.1 Association of cIMT with vascular risk factors and biomarkers

Unadjusted Pearson correlation coefficients and p values for associations of cIMT with continuous vascular risk factors and biomarkers, and ANOVA parameter estimates coefficients for categorical variables are presented in table 5-1. Age and sex adjusted values are presented in table 5-2. The ANOVA statistic quoted quantifies the difference in the mean value of the variable between the test group and the reference category.

5.1.1 cIMT and vascular risk factors

There were significant unadjusted associations between cIMT and traditional vascular risk factors. Increasing mean cIMT was associated with increasing age ($r=0.164$, $p<0.001$), higher systolic BP ($r=0.075$, $p=0.024$) and lower HDL cholesterol ($r=-0.101$, $p<0.001$). Men were more likely to have a higher cIMT than women and those using lipid lowering medication tended to have a higher cIMT than those who did not. Similarly, current and ex-smokers had significantly higher mean cIMT than non-smokers ($p<0.001$). Following adjustment for age and sex, the associations were attenuated somewhat and some associations were lost. Only a

higher systolic BP (r=0.075, p<0.001) remained associated with a higher mean cIMT, as did use of lipid lowering medication.

All other measures of cIMT were significantly associated with age, sex, systolic blood pressure, HDL cholesterol, lipid lowering medication and smoking. The only differing associations were the association of maximum cIMT and mean maximum cIMT with lower total cholesterol (r=-0.081 and r=-0.076 respectively)) and maximum mean cIMT with lower diastolic blood pressure (r=-0.070). After age and sex adjustment, all other measures of cIMT, except maximum cIMT remained associated with increased systolic blood pressure and the use of lipid lowering medication. In addition, they all retained a positive association with lower HDL cholesterol and both maximum cIMT and maximum mean cIMT remained associated with a lower diastolic blood pressure.

The key difference between the age and sex adjusted associations of mean cIMT and other measures with risk factors, was the association of the other measures with HDL cholesterol, where no association existed for mean cIMT.

5.1.2 cIMT and novel biomarkers of cardiovascular risk

Increasing mean cIMT was significantly associated with lower ABI (r=-0.099, p<0.001), increased IL-6 (r=0.069, p<0.001) and increased NTproBNP (r=0.126, p<0.001). Following adjustment for age and sex, these significant associations remained - IL-6 (r=0.068, p<0.001) and NTproBNP (r=0.107, p<0.001) remained moderately positively correlated with mean cIMT while ABI retained its negative association (r=-0.136, p<0.001)

All other measures of cIMT were associated with ABI and NTproBNP. In addition, mean maximum cIMT was associated with increasing IL6 and ACR. These associations remained after adjustment for age and sex, with the exception of the association of mean maximum cIMT with ACR, which lost statistical significance.

Table 5-1 Unadjusted correlation of cIMT with traditional cardiovascular risk factors and with novel biomarkers of cardiovascular risk

		Mean cIMT		Max cIMT		MaxMean cIMT		Mean Max cIMT	
		Correlation	Anova*	Correlation	Anova*	Correlation	Anova*	Correlation	Anova*
Demographics	Age	0.164***	-	0.141***	-	0.147***	-	0.149***	-
	Sex (male)	-	0.063***	-	0.087***	-	0.075***	-	0.072***
	SIMD								
	Quintile 1 (Most Deprived)	-	-0.001	-	0.005	-	-0.005	-	0.006
	Quintile 2	-	0.016	-	0.024	-	0.020	-	0.018
	Quintile 3	-	0.009	-	0.018	-	0.019	-	0.014
	Quintile 4	-	0.014	-	0.018	-	0.016	-	0.016
	Quintile 5 (Least Deprived)	-	-	-	-	-	-	-	-
Diabetes	Duration of DM	0.031	-	0.017	-	0.013	-	0.028	-
	HbA1C	0.009	-	0.007	-	0.013	-	0.008	-
	T2DM Meds								
	Diet Alone	-	-	-	-	-	-	-	-
	Oral	-	-0.024	-	-0.036	-	-0.027	-	-0.032
	Insulin ± oral	-	-0.015	-	-0.025	-	-0.016	-	-0.022
Blood Pressure	Systolic BP	0.075*	-	0.061	-	0.069*	-	0.067*	-
	Diastolic BP	-0.057	-	-0.064	-	-0.070*	-	-0.057	-
	Antihypertensive (yes)	-	0.009	-	0.008	-	0.013	-	0.009
Blood Lipids	Total Cholesterol	-0.065	-	-0.081*	-	-0.064	-	-0.076*	-
	HDL Cholesterol	-0.101**	-	-0.134***	-	-0.114**	-	-0.121***	-
	Cholesterol med (yes)	-	0.029*	-	0.043*	-	0.037*	-	0.032*
Smoking	Smoking status								
	Current smoker	-	0.029*	-	0.043*	-	0.042*	-	0.034*
	Ex smoker	-	0.040***	-	0.049***	-	0.043***	-	0.044***
	Never smoker	-	-	-	-	-	-	-	-
Obesity Index	BMI	-0.054	-	-0.055	-	-0.060	-	-0.051	-
CVD Risk Marker	Ankle Brachial Index	-0.099**	-	-0.092**	-	-0.100**	-	-0.098**	-
Renal Function	eGFR	-0.047	-	-0.033	-	-0.038	-	-0.044	-
	ACR	0.093	-	0.090	-	0.081	-	0.105*	-
Plasma Biomarkers	IL6	0.069*	-	0.048	-	0.051	-	0.067*	-
	CRP	-0.007	-	-0.019	-	-0.018	-	0.003	-
	NTProBNP	0.126***	-	0.105**	-	0.114**	-	0.119***	-

For continuous variables, Pearson correlation coefficients and corresponding p values are quoted. *For categorical variables, the ANOVA statistic reported quantifies the difference in mean or maximum plaque thickness for the given categorical variable compared with the reference level (reference levels are female sex, least deprived SIMD quintile, diet alone treatment for diabetes, no lipid lowering medication, no anti-hypertensive medication, never smoker)SIMD=Scottish Index of Multiple Deprivation, HDL=high density lipoprotein, BMI=body mass index, eGFR=estimated glomerular filtration rate, ACR=albumin creatinine ratio, IL-6=interleukin 6, CRP=C reactive protein, NTProBNP=N terminal pro-brain natriuretic peptide, Pack years = geometric mean.* significant at p<0.05, ** significant at p<0.01, *** significant at p<0.001

Table 5-2 Age and sex adjusted association of cIMT with traditional cardiovascular risk factors and with novel biomarkers of cardiovascular risk

		Mean cIMT		Max cIMT		Max Mean cIMT		Mean Max cIMT	
		Correlation	Anova*	Correlation	Anova*	Correlation	Anova*	Correlation	Anova*
Demographics	SIMD								
	Quintile 1 (Most Deprived)	-	0.012	-	0.022	-	0.011	-	0.021
	Quintile 2	-	0.021	-	0.031	-	0.026	-	0.024
	Quintile 3	-	0.009	-	0.018	-	0.018	-	0.013
	Quintile 4	-	0.014	-	0.018	-	0.016	-	0.016
	Quintile 5 (Least Deprived)	-	-	-	-	-	-	-	-
Diabetes	Duration of DM	0.023	-	0.009	-	0.014	-	0.027	-
	HbA1C	0.033	-	0.028	-	0.034	-	0.030	-
	Diabetes Medication								
	Diet Alone	-	-	-	-	-	-	-	
	Oral Hypoglycaemics	-	-0.021	-	-0.032	-	-0.024	-	-0.029
	Insulin ± oral hypoglycaemics	-	-0.007	-	-0.016	-	-0.008	-	-0.014
Blood Pressure	Systolic BP	0.075*	-	0.064	-	0.070*	-	0.069*	-
	Diastolic BP	-0.058	-	-0.068*	-	-0.074*	-	-0.061	-
	On antihypertensives (yes)	-	0.013	-	0.013	-	0.017	-	0.013*
Blood Lipids	Total Cholesterol	-0.010	-	-0.026	-	-0.011	-	-0.021	-
	HDL Cholesterol	-0.064	-	-0.095**	-	-0.076*	-	-0.081*	-
	Cholesterol lowering meds (yes)	-	0.024*	-	0.036*	-	0.032*	-	0.027
Smoking	Smoking status								
	Current smoker	-	0.022	-	0.032	-	0.034	-	0.025
	Ex-smoker	-	0.024*	-	0.026	-	0.024	-	0.026*
	Never smoker	-	-	-	-	-		-	-
Obesity Index	BMI	0.019	-	0.014	-	0.008	-	0.020	-
CVD Risk Marker	Ankle Brachial Index	-0.136***	-	-0.128***	-	-0.135***	-	-0.135***	-
Renal Function	eGFR	-0.011	-	-0.003	-	-0.005	-	-0.012	-
	ACR	0.088	-	0.086	-	0.079	-	0.100	-
Plasma Biomarkers	IL6	0.068*	-	0.048	-	0.050	-	0.068*	-
	CRP	0.047	-	0.035	-	0.034	-	0.059	-
	NTProBNP	0.107**	-	0.089**	-	0.097**	-	0.103**	-

For continuous variables, Pearson correlation coefficients and corresponding p values are quoted. . *For categorical variables, the ANOVA statistic reported quantifies the difference in mean or maximum plaque thickness for the given categorical variable compared with the reference level (reference levels are female sex, least deprived SIMD quintile, diet alone treatment for diabetes, no lipid lowering medication, no anti-hypertensive medication, never smoker) SIMD=Scottish Index of Multiple Deprivation, HDL=high density lipoprotein, BMI=body mass index, eGFR=estimated glomerular filtration rate, ACR=albumin creatinine ratio, IL-6=interleukin 6, CRP=C reactive protein, NTProBNP=N terminal pro-brain natriuretic peptide, Pack years = geometric mean.* significant at p<0.05, ** significant at p<0.01, *** significant at p<0.001

5.2 Association of plaque thickness with vascular risk factors and biomarkers

Unadjusted Pearson correlation coefficients and p values for associations of plaque thickness with continuous vascular risk factors and biomarkers, and ANOVA coefficients for associations with categorical variables are presented in table 5-3. Age and sex adjusted values are presented in table 5-4.

5.2.1 Plaque thickness and vascular risk factors

A variety of risk factors demonstrated a cross sectional association with mean plaque thickness. A higher mean plaque thickness was significantly associated with increasing age (r=0.197) and male sex, as well as longer duration of diabetes (r=0.101), increased systolic blood pressure (r=0.124), lower BMI (r=-0.075), use of antihypertensive medication and cigarette smoking. Following adjustment for age and sex, mean plaque thickness remained significantly associated with a longer duration of diabetes (r=0.089), systolic BP (r=0.124), the use of antihypertensive medication and cigarette smoking. Maximum plaque thickness demonstrated the same age and sex adjusted associations.

5.2.2 Plaque thickness and novel biomarkers of cardiovascular risk

Mean plaque thickness was also associated with novel marker of cardiovascular risk. Increasing plaque thickness was significantly associated with a lower ABI (r=-0.170), reduced eGFR (r=-0.114), increased ACR (r=0.102), increased IL6 (r=0.111), and higher NTproBNP (r=0.185). Following age and sex adjustment, these associations persisted with limited attenuation of the magnitude of association, with the exception of the association with ACR which lost significance. Maximum plaque thickness demonstrated the same pattern of association.

Effect Size

It is important to note that the effect sizes described in this section are not large. After age and sex adjustment the effect sizes are small (<0.1). The strongest of these

associations were ABI and NTproBNP (>0.1). Therefore, despite their significant p values, these associations may not be clinically significant.

Table 5-3 Unadjusted association of plaque thickness parameters with traditional cardiovascular risk factors and with novel biomarkers of cardiovascular risk

		Mean Plaque thickness (mm)		Max Plaque Thickness (mm)	
		Correlation	ANOVA*	Correlation	ANOVA*
Demographics	Age	0.197***	-	0.197***	-
	Sex (male)	-	0.376***	-	0.444***
	SIMD				
	Quintile 1(Most Deprived)	-	0.107	-	0.119
	Quintile 2	-	0.130	-	0.094
	Quintile 3	-	0.079	-	0.056
	Quintile 4	-	-0.011	-	0.005
	Quintile 5 (Least Deprived)	-	-	-	-
Diabetes	Duration of DM	0.101**	-	0.092**	-
	HbA1C	0.011	-	-0.001	-
	T2DM Meds				
	Diet Alone	-	-	-	-
	Oral	-	-0.043	-	-0.025
	Insulin ± oral	-	0.041	-	0.015
Blood Pressure	Systolic BP	0.124***	-	0.101**	-
	Diastolic BP	-0.043	-	-0.049	-
	Antihypertensives (yes)	-	0.199*	-	0.251*
Blood Lipids	Total Cholesterol	-0.060	-	-0.056	-
	HDL Cholesterol	-0.067	-	-0.058	-
	Cholesterol med (yes)	-	0.157	-	0.174
Smoking	Smoking status				
	Current smoker	-	0.647***	-	0.751***
	Ex smoker	-	0.377***	-	0.437***
	Never smoker	-	-	-	-
Obesity Index	BMI	-0.075*	-	-0.078*	-
CVD Risk Marker	Ankle Brachial Index	-0.170***	-	-0.153***	-
Renal Function	eGFR	-0.114**	-	-0.112**	-
	ACR	0.102*	-	0.105*	-
Plasma Biomarkers	IL6	0.111**	-	0.091**	-
	CRP	0.058	-	0.056	-
	NTProBNP	0.185***	-	0.182***	-

For continuous variables, Pearson correlation coefficients and corresponding p values are quoted. . *For categorical variables, the ANOVA statistic reported quantifies the difference in mean or maximum plaque thickness for the given categorical variable compared with the reference level (reference levels are female sex, least deprived SIMD quintile, diet alone treatment for diabetes, no lipid lowering medication, no anti-hypertensive medication, never smoker) SIMD=Scottish Index of Multiple Deprivation, HDL=high density lipoprotein, BMI=body mass index, eGFR=estimated glomerular filtration rate, ACR=albumin creatinine ratio, IL-6=interleukin 6, CRP=C reactive protein, NTProBNP=N terminal pro-brain natriuretic peptide, Pack years = geometric mean.* significant at p<0.05, ** significant at p<0.01, *** significant at p<0.001

Table 5-4 Age and sex adjusted associations of plaque thickness parameters with traditional cardiovascular risk factors and with novel biomarkers of cardiovascular risk

		Mean Plaque Thickness (mm)		Max Plaque Thickness (mm)	
		Correlation	ANOVA*	Correlation	ANOVA*
Demographics	SIMD				
	Quintile 1(most deprived)	-	0.192	-	0.221
	Quintile 2	-	0.165*	-	0.136
	Quintile 3	-	0.078	-	0.054
	Quintile 4	-	-0.018	-	-0.004
	Quintile 5 (least deprived)	-	-	-	-
Diabetes	Duration of DM	0.089**	-	0.080*	-
	HbA1C	0.036	-	0.024	-
	T2DM Meds				
	Diet Alone	-	-	-	-
	Oral	-	-0.023	-	0.000
	Insulin ± oral	-	0.092	-	0.075
Blood Pressure	Systolic BP	0.124***	-	0.099**	-
	Diastolic BP	-0.033	-	-0.038	-
	Antihypertensives (yes)	-	0.176*	-	0.223*
Blood Lipids	Total Cholesterol	-0.011	-	-0.008	-
	HDL Cholesterol	-0.032	-	-0.025	-
	Cholesterol med (yes)	-	0.134	-	0.149
Smoking	Smoking status				
	Current smoker	-	0.630***	-	0.733***
	Ex smoker	-	0.299***	-	0.345***
	Never smoker	-	-	-	-
Obesity Index	BMI	-0.002	-	-0.006	-
CVD Risk Marker	Ankle Brachial Index	-0.209***	-	-0.189***	-
Renal Function	eGFR	-0.069*	-	-0.068*	-
	ACR	0.087	-	0.091	-
Plasma Biomarkers	IL6	0.117**	-	0.086**	-
	CRP	0.119***	-	0.113**	-
	NTProBNP	0.162***	-	0.157***	-

For continuous variables, Pearson correlation coefficients and corresponding p values are quoted. . *For categorical variables, the ANOVA statistic reported quantifies the difference in mean or maximum plaque thickness for the given categorical variable compared with the reference level (reference levels are female sex, least deprived SIMD quintile, diet alone treatment for diabetes, no lipid lowering medication, no anti-hypertensive medication, never smoker)SIMD=Scottish Index of Multiple Deprivation, HDL=high density lipoprotein, BMI=body mass index, eGFR=estimated glomerular filtration rate, ACR=albumin creatinine ratio, IL-6=interleukin 6, CRP=C reactive protein, NTProBNP=N terminal pro-brain natriuretic peptide, Pack years = geometric mean.
* significant at $p<0.05$, ** significant at $p<0.01$, *** significant at $p<0.001$

5.2.3 Association of plaque score with vascular risk factors and biomarkers

Associations of plaque score (≤4 or >4) with vascular risk factors are presented in table 5-5. A further, more detailed analysis of the association between individual score values and risk factors is presented in table 5-6.

Individuals with a higher plaque score (>4) were on average almost a year older (69.5 vs 68.5 years respectively) than those with a low score and were more likely to be male. They tended to have a lower diastolic blood pressure, were more likely to have a history of cigarette smoking and had a lower BMI (all $p<0.01$). Those with a higher plaque score also had a higher prevalence of previous CVD (45% vs 27%, $p<0.001$). Several novel biomarkers were also associated with higher plaque score – lower ABI, reduced eGFR and increased NTproBNP ($p<0.01$).

Upon inspection of the individual score values, men tended to have a higher plaque score than women. A higher percentage of those with a plaque score of 8 were male (64.8%) compared with those with a plaque score of 0 (47.4%), however the trend across the scores was not strictly linear. There was a linear increase in age from group 0 to group 8 (66.8 ± 4.6 years). An overall negative trend in diastolic blood pressure across the groups was noted, however this was not linear. There were significantly more current and ex-smokers and fewer never smokers in group 8 (22.5%, 59.2% and 18.3% respectively) compared with group 0 (5.3%, 31.6% and 63.2% respectively). Baseline BMI had an unexpected negative relationship with plaque score. Those in group 0 had a higher BMI than those in group 8 (BMI 33.5 ± 7.8 and 30.2 ± 4.9 respectively. The overall trend for ABI across the groups was downwards (group 0 ABI 1.01 ± 0.14 vs group 8 0.92 ± 0.24) although again this was not a liner relationship. There was a significant negative linear trend between eGFR and plaque score, with mean eGFR 85.4 ± 13.9 ml/min/1.73m^2 in those with a plaque score of 0 and mean eGFR of 70.31 ± 22.3 ml/min/1.73m^2). NT proBNP was also higher in those with a higher plaque score (group 0 67.0 ± 125.0 pg/ml vs group 8 118.5 ± 213 pg/ml) and this trend was linear.

Table 5-5 Comparison of cardiovascular risk factors and novel risk markers in those with plaque score ≤4 or >4

		Score ≤4 (n=386)	Score>4 (n=517)	P value
Demographics	Age (years)	68.5 (4.2)	69.5 (4.08)	0.001**
	Sex (male)	46.3 (228)	57.4 (236)	0.001**
	SIMD			
	Quintile 1(most deprived)	11.6 (57)	11.4 (47)	
	Quintile 2	17.7 (87)	20.0 (82)	
	Quintile 3	17.9 (88)	17.0 (70)	0.787
	Quintile 4	19.7 (97)	17.0 (70)	
	Quintile 5 (least deprived)	33.1 (163)	34.5 (142)	
Diabetes	Duration of Diabetes (years)	7.0 (8.0)	8.0 (7.00)	0.117
	HbA1C (% haemoglobin)	7.17 (1.07)	7.23 (1.08)	0.405
	Diabetes Medication			
	Diet Alone	20.7 (102)	17.0 (70)	
	Oral Hypoglycaemics	65.4 (322)	65.2 (268)	0.147
	Insulin ± oral hypoglycaemics	13.8 (68)	17.8 (73)	
Blood Pressures	Systolic Blood Pressure (mmHg)	137.0 (17.0)	139.3 (20.1)	0.069
	Diastolic Blood Pressure (mmHg)	75.1 (9.2)	72.8 (9.6)	<0.001***
	On antihypertensives (% yes)	84.6 (416)	87.1 (358)	0.160
Blood Lipids	Total Cholesterol (mmol/l)	4.17 (0.79)	4.11 (0.81)	0.262
	HDL Cholesterol (mmol/l)	1.25 (0.35)	1.21 (0.33)	0.068
	Lipid lowering meds (% yes)	823.1 (409)	85.6 (352)	0.173
Smoking	Smoking status			
	Current smoker	9.3 (46)	16.8 (69)	
	Ex smoker	42.1 (207)	52.8 (217)	<0.001**
	Never smoker	48.6 (239)	30.4 (125)	
Obesity Index	BMI (kg/m^2)	32.0 (6.0)	30.5 (5.1)	<0.001***
CVD Risk Marker	Ankle Brachial Index	1.01 (0.18)	0.96 (0.24)	<0.001***
Renal Function	eGFR ($ml/min/1.73m^2$)	78.9 (18.0)	74.8 (19.5)	0.001**
	ACR (mg/mmol)	1.6.5 (2.6)	1.9 (3.6)	0.088
Plasma Biomarkers	IL6	2.72 (2.40)	3.01 (2.62)	0.174
	CRP (mg/l)	1.69 (3.12)	1.92 (3.34)	0.167
	NTProBNP (pg/ml)	66.0 (114)	82.0 (172)	<0.001***
CVD History	Previous CVD	27.8 (137)	44.8 (184)	<0.001***

SIMD=Scottish Index of Multiple Deprivation, HDL=high density lipoprotein, BMI=body mass index, eGFR=estimated glomerular filtration rate, ACR=albumin creatinine ratio, IL-6=interleukin 6, CRP=C reactive protein, NTProBNP=N terminal pro-brain natriuretic peptide, Pack years = geometric mean. * significant at $p<0.05$, ** significant at $p<0.01$, *** significant at $p<0.001$

Table 5-6 Comparison of individuals carotid plaque score with traditional cardiovascular risk factors and novel biomarkers of cardiovascular risk

	Plaque Score								
	0	1	2	3	4	5	6	7	8
Age (years)	66.8 (4.6)	67.6 (4.3)	67.7 (3.9)	68.7 (4.18)	69.3 (4.16)	69.1 (4.0)	69.2 (4.1)	69.4 (4.3)	70.8 (3.8)
Sex (male)	47.4 (9)	42.3 (11)	33.1 (42)	49.3 (71)	53.7 (88)	53.7 (79)	57.7 (71)	55.7 (34)	64.8 (46)
SIMD									
Quintile 1(most deprived)	10.5 (2)	7.7 (2)	12.6 (16)	10.4 (15)	11.6 (19)	7.5 (11)	12.2 (15)	19.7 (12)	12.7 (9)
Quintile 2	10.5 (2)	15.4 (4)	14.2 (18)	22.9 (33)	16.5 (27)	22.4 (33)	15.4 (19)	21.3 (13)	19.7 (14)
Quintile 3	21.1 (4)	19.2 (5)	19.7 (25)	17.4 (25)	17.7 (29)	19.0 (28)	17.9 (22)	9.8 (6.0)	18.3 (13)
Quintile 4	15.8 (3)	23.1 (6)	18.1 (23)	24.3 (35)	16.5 (27)	15.6 (23)	15.4 (19)	21.3 (13)	19.7 (14)
Quintile 5 (least deprived)	42.1 (8)	34.6 (9)	35.4 (45)	25.0 (36)	37.8 (62)	35.4 (5)	39.0 (48)	27.9 (17)	29.6 (21)
Duration of Diabetes (years)	6.0 (6.0)	6.5 (6.25)	7.0 (7.0)	6.0 (7.0)	8.0 (7.0)	7.0 (7.0)	8.0 (9.0)	8.0 (9.0)	8.5 (9.0)
HbA1C (% haemoglobin)	7.4 (1.0)	7.6 (1.1)	7.3 (1.2)	7.0 (0.9)	7.1 (1.1)	7.3 (1.2)	7.3 (1.0)	7.1 (0.9)	7.1 (1.2)
Diabetes Medication									
Diet Alone	21.1 (4)	11.5 (3)	18.1 (23)	22.9 (33)	22.6 (37)	20.4 (30)	16.3 (20)	19.7 (12)	11.3 (8)
Oral Hypoglycaemics	57.9 (11)	69.2 (18)	66.9 (85)	68.1 (98)	60.4 (99)	63.9 (94)	64.2 (79)	62.3 (38)	70.4 (50)
Insulin ± oral hypoglycaemics	21.1 (4)	19.2 (5)	15.0 (19)	9.0 (13)	17.1 (28)	15.6 (23)	19.5 (24)	18.0 (11)	18.3 (13)
Systolic Blood Pressure (mmHg)	135.4 (15.2)	134.2 (19.5)	132.9 (15.6)	137.0 (17.2)	138.8 (17.6)	138.7 (19.0)	140.6 (20.3)	139.2 (20.6)	137.1 (21.0)
Diastolic Blood Pressure (mmHg)	74.5 (10.7)	74.5 (9.3)	76.15 (9.6)	73.8 (9.2)	75.3 (8.7)	73.0 (8.2)	72.6 (9.0)	72.6 (11.6)	72.7 (10.7)
On antihypertensives (% yes)	68.4 (13)	80.8 (21)	79.5 (101)	87.5 (126)	87.2 (143)	85.0 (125)	87.0 (107)	88.5 (54)	91.5 (65)
Total Cholesterol (mmol/l)	4.08 (0.89)	4.29 (0.98)	4.14 (0.80)	4.21 (0.79)	4.15 (0.75)	4.08 (0.82)	4.19 (0.78)	4.17 (0.80)	4.02 (0.83)
HDL Cholesterol (mmol/l)	1.22 (0.39)	1.31 (0.29)	1.24 (0.38)	1.28 (0.33)	1.23 (0.35)	1.22 (0.34)	1.24 (0.32)	1.22 (0.33)	1.17 (0.32)
Lipid lowering meds (% yes)	73.7 (14)	69.2 (18)	79.5 (101)	81.9 (118)	89.6 (147)	84.4 (124)	88.6 (109)	82.0 (50)	85.9 (61)
Smoking status									
Current smoker	5.3 (1)	11.5 (3)	5.5 (7)	10.4 (15)	12.2 (20)	14.3 (21)	12.2 (15)	24.6 (15)	22.5 (16)
Ex smoker	31.6 (6)	38.5 (10)	43.3 (55)	41.7 (60)	41.5 (68)	47.6 (70)	52.8 (65)	59.0 (36)	59.2 (42)
Never smoker	63.2 (12)	50 (13)	51.2 (65)	47.9 (69)	46.3 (76)	38.1 (56)	35.0 (43)	16.4 (10)	18.3 (13)
BMI (kg/m^2)	33.5 (7.8)	33.2 (7.5)	33.0 (6.1)	32.1 (6.1)	30.8 (5.3)	31.2 (5.5)	30.1 (5.0)	30.4 (4.6)	30.2 (4.9)
Ankle Brachial Index	1.01 (0.14)	1.02 (0.12)	1.02 (0.16)	0.99 (0.19)	1.03 (0.18)	0.99 (0.20)	0.95 (0.22)	0.94 (0.35)	0.92 (0.24)
eGFR ($ml/min/1.73m^2$)	85.4 (13.9)	83.15 (16.2)	78.25 (17.66)	77.56 (19.06)	79.15 (17.84)	77.24 (17.76)	74.44 (19.7)	75.08 (17.23)	70.31 (22.3)
ACR (mg/mmol)	1.3 (0.0)	1.75 (3.8)	2.30 (3.7)	1.40 (1.9)	1.75 (2.8)	1.3 (2.5)	2.30 (5.3)	1.95 (2.8)	3.00 (6.2)
IL6	1.8 (2.06)	2.96 (3.80)	2.73 (2.30)	2.70 (2.09)	2.90 (2.52)	2.60 (2.12)	2.78 (2.34)	3.23 (3.13)	3.67 (3.28)
CRP (mg/l)	1.45 (2.18)	1.77 (4.11)	1.7 (3.56)	1.82 (3.08)	1.57 (3.39)	1.69 (2.51)	1.76 (2.90)	2.69 (6.03)	2.65 (5.36)
NTProBNP (pg/ml)	67.00 (125)	74.00 (137)	60.00 (119)	64.50 (99)	74.00 (126)	70.00 (111)	85.00 (233)	100 (236)	118.5 (213)

SIMD=Scottish Index of Multiple Deprivation, HDL=high density lipoprotein, BMI=body mass index, eGFR=estimated glomerular filtration rate, ACR=albumin creatinine ratio, IL-6=interleukin 6, CRP=C reactive protein, NTProBNP=N terminal pro-brain natriuretic peptide, Pack years = geometric mean.. a=No SD as n is 1

* significant at $p<0.05$, ** significant at $p<0.01$, *** significant at $p<0.001$

5.2.4 Association of plaque morphology with vascular risk factors and biomarkers

Associations of plaque morphology (low risk or high risk) with vascular risk factors are presented in table 5-7 and table 5-8.

Individuals with high risk plaque (echolucent or heterogeneous) were significantly older than those with low risk plaque (69.2 vs 68.6 years), however they were no more likely to be male than female. They were more likely to be using lipid lowering medications and had a lower BMI. They were also more likely to have history of CVD. There were no differences in blood pressure or blood lipids between the groups. Several novel biomarkers were associated with the presence of high risk plaques. Those with high risk plaques tended to have reduced eGFR (75.8 vs 78.5ml/min/1.73m^2, p=0.041), and increased NTproBNP (106.0 vs 69.0 pg/ml, p<0.001).

If one examines the individual categories of plaque morphology mean age was highest in those with both types of plaque (69.8 ± 4.3 years) and lowest in those with echogenic or no plaque (68.5 ± 4.3 years). ABI was lowest in those with both types of plaque (0.94 ± 0.23). eGFR was significantly lower in the group with both types of plaque (72.7 ± 19.5 ml/min/1.72m^2) and ranged from 77.7-79.0 in the other 3 groups. NT pro BNP showed a linear increase across the groups of plaque, and was highest in those with both types of plaque (95.0 ± 219).

Table 5-7 Association of plaque type with cardiovascular risk factors and novel risk markers

		Low Risk (n=386)	High Risk (n=517)	P value
Demographics	Age (years)	68.6 (4.23)	69.2 (4.08)	0.029**
	Sex (male)	50.0 (193)	52.6 (272)	0.239
	SIMD			
	Quintile 1(most deprived)	10.76 (41)	12.2 (63)	
	Quintile 2	17.6 (68)	19.7 (102)	
	Quintile 3	18.1 (70)	17.0 (88)	0.838
	Quintile 4	18.9 (73)	18.2 (94)	
	Quintile 5 (least deprived)	34.7 (134)	32.9 (170)	
Diabetes	Duration of Diabetes (years)	7.0 (6.0)	8.0 (8.0)	0.217
	HbA1C (% haemoglobin)	7.2 (1.01)	7.2 (1.13)	0.858
	Diabetes Medication			
	Diet Alone	19.9 (77)	18.4 (95)	
	Oral Hypoglycaemics	65.8 (254)	64.8 (335)	0.534
	Insulin ± oral hypoglycaemics	14.2 (55)	16.8 (87)	
Blood Pressure	Systolic Blood Pressure (mmHg)	136.9 (17.6)	138.9 (19.1)	0.105
	Diastolic Blood Pressure (mmHg)	74.6 (9.5)	73.6 (9.4)	0.108
	On antihypertensives (% yes)	85.2 (329)	86.1 (445)	0.396
Blood Lipids	Total Cholesterol (mmol/l)	4.17 (0.81)	4.13 (0.79)	0.397
	HDL Cholesterol (mmol/l)	1.24 (0.33)	1.23 (0.34)	0.895
	Lipid lowering meds (% yes)	81.6 (315)	86.3 (446)	0.035*
Smoking	Smoking status			
	Current smoker	10.6 (41)	14.3 (74)	
	Ex smoker	45.9 (177)	48.0 (248)	0.109
	Never smoker	43.5 (168)	37.7 (195)	
Obesity Index	BMI (kg/m^2)	31.9 (6.1)	30.9 (5.3)	0.005**
CVD Risk Marker	Ankle Brachial Index	1.00 (0.18)	0.98 (0.23)	0092
Renal Function	eGFR ($ml/min/1.73m^2$)	78.5 (18.7)	75.8 (18.8)	0.041*
	ACR (mg/mmol)	1.7 (2.6)	1.9 (3.3)	0.176
Plasma Biomarkers	IL6	2.75 (2.37)	2.85 (2.59)	0.322
	CRP (mg/l)	1.76 (2.98)	1.76 (3.41)	0.452
	NTProBNP (pg/ml)	66.0 (113)	82 153)	0.008**
CVD History	Previous CVD	28.8 (111)	40.8 (211)	<0.001***

SIMD=Scottish Index of Multiple Deprivation, HDL=high density lipoprotein, BMI=body mass index, eGFR=estimated glomerular filtration rate, ACR=albumin creatinine ratio, IL-6=interleukin 6, CRP=C reactive protein, NTProBNP=N terminal pro-brain natriuretic peptide, Pack years = geometric mean.

* significant at $p<0.05$, ** significant at $p<0.01$, *** significant at $p<0.001$

Table 5-8 Association of individual carotid plaque morphology categories with traditional cardiovascular risk factors and novel biomarkers

	Echogenic or no Plaque	Echolucent Plaque Only	Heterogeneous Plaque Only	Both
Age	68.5 (43)	68.8 (3.8)	68.8 (3.9)	69.8 (4.3)
Sex (male)	50.9 (204)	33.0 (37)	61.1 (110)	55.7 (123)
SIMD				
Quintile 1	10.7 (43)	15.2 (17)	8.9 (16)	13.6 (30)
Quintile 2	17.2 (69)	19.6 (22)	20.0 (36)	19.0 (42)
Quintile 3	17.7 (71)	15.2 (17)	20.0 (36)	15.8 (35)
Quintile 4	18.0 (72)	18.8 (21)	16.1 (29)	19.9 (44)
Quintile 5	36.4 (146)	31.3 (35)	35.0 (63)	31.7 (70)
Duration of DM (years)	7.0 (7.0)	6.0 (7.0)	8.0 (8.0)	8.0 (9.0)
HbA1C	7.20 (1.0)	7.13 (1.2)	7.20 (1.1)	7.23 (1.2)
T2DM Meds				
Diet Alone	21.2 (85)	14.3 (16)	20.0 (36)	19.0 (42)
Oral	64.3 (258)	70.5 (79)	63.9 (115)	62.4 (138)
Insulin ± oral	14.5 (58)	15.2 (17)	16.1 (29)	18.6 (41)
Systolic BP (mmHg)	137.1 (17.4)	137.7 (14.3)	136.6 (18.2)	141.0 (21.6)
Diastolic BP (mmHg)	74.75 (9.7)	74.9 (8.3)	72.8 (9.7)	73.5 (9.5)
Antihypertensives (n, % yes)	85.3 (342)	81.3 (91)	86.7 (156)	87.8 (194)
Total Cholesterol	4.18 (0.8)	4.24 (0.8)	4.06 (0.8)	4.12 (0.8)
HDL Cholesterol	1.23 (0.33)	1.27 (0.4)	1.22 (0.3)	1.22 (0.4)
Cholesterol med (n, % yes)	81.5 (327)	83.9 (94)	90.0 (162)	84.6 (187)
Smoking status (%)				
Current smoker	11.2 (45)	15.2 (17)	13.3 (24)	14.9 (33)
Ex smoker	45.9 (184)	43.8 (49)	45.6 (82)	52.0 (115)
Never smoker	42.9 (172)	41.1 (46)	41.1 (74)	33.0 (73)
BMI	31.9 (6.1)	31.9 (5.3)	31.4 (5.6)	30.0 (5.0)
Ankle Brachial Index	0.99 (0.18)	1.00 (0.19)	1.00 (0.25)	0.94 (0.2)
eGFR	79.0 (18.2)	79.0 (17.6)	77.7 (18.4)	72.7 (19.5)
ACR	1.7 (2.7)	1.45 (3.2)	1.5 (2.5)	2.25 (4.0)
IL6	2.73 (2.32)	2.81 (2.7)	2.51 (2.4)	3.07 (2.9)
CRP	1.76 (3.1)	2.16 (3.9)	1.42 (2.7)	2.25 (3.7)
NTProBNP	66.5 (117)	59.0 (106)	78.0 (162)	95.0 (219)

SIMD=Scottish Index of Multiple Deprivation, HDL=high density lipoprotein, BMI=body mass index, eGFR=estimated glomerular filtration rate, ACR=albumin creatinine ratio, IL-6=interleukin 6, CRP=C reactive protein, NTProBNP=N terminal pro-brain natriuretic peptide, Pack years = geometric mean.

* significant at $p<0.05$, ** significant at $p<0.01$, *** significant at $p<0.001$

5.3 Multifactorial associations of cIMT and carotid plaque

Linear regression models were used to explore the multivariable association of risk factors with cIMT and plaque thickness. Logistic regression was used to explore the same associations with plaque score and plaque morphology. Two models were used to explore these relationships. The first model examines only traditional cardiovascular risk factors (model 1). The second models incorporate traditional cardiovascular risk factors and the more novel biomarkers (model 2). Results for linear regression models, logistic regression models and the explained variance of each model are detailed in tables 5-9 – 5-13.

5.3.1 cIMT

Traditional cardiovascular risk factors were entered as covariates into a linear regression model (model 1) for mean cIMT, maximum cIMT, maximum mean cIMT, mean maximum cIMT (table 5-9). Age, male sex and systolic blood pressure showed a positive independent association with each cIMT variable. In addition to these factors, maximum cIMT was associated with HDL cholesterol. Interestingly, diastolic pressure demonstrated a negative association with each variable, which is not the expected direction of association. This may be a reflection of the high percentage of individuals in the ET2DS that are taking antihypertensive medication.

In the second model (table 5-10), both traditional cardiovascular risk factors and novel biomarkers of cardiovascular risk were entered into a linear regression model for each cIMT variable. In this model, ABI and NTproBNP were associated with all measures of cIMT after accounting for traditional risk factors.

For mean cIMT, maximum cIMT, maximum mean cIMT and mean maximum cIMT, these models containing traditional cardiovascular risk factors explained 10.8%, 10.6%, 10.4% and 10.8% of variance respectively, suggesting that these factors do not completely explain the variance seen in cIMT (table 5-11). When novel cardiovascular risk factors were added to the traditional risk factors, these new models explained 16.7%, 14.2%, 14.8% and 16.4% of variance for mean cIMT, maximum cIMT, maximum mean cIMT, and mean maximum cIMT respectively.

5.3.2 Carotid plaque thickness

On entry of covariates to model 1, age, male sex, systolic and diastolic BP, and smoking status had a significant independent association with both mean plaque thickness and maximum plaque thickness. All the associations were positive, with the exception of diastolic pressure which, as for cIMT, had a negative association with both mean and maximum plaque thickness.

Addition of novel risk factors to the model containing traditional cardiovascular risk factors revealed that ABI and NTproBNP had significant independent associations

with mean plaque thickness. For maximum plaque thickness, only NTproBNP was independently associated following multifactorial adjustment.

Model 1, containing only traditional risk factors explained 15.7% of the variance of mean plaque thickness and 14.0% of maximum plaque thickness. Adding novel risk factors to the model increased variance to 18.9% and 17.6% respectively.

5.3.3 Plaque score and plaque morphology

Logistic regression models for the association of plaque score (<=4 or >4) are presented in table 5-12. After multifactorial adjustment, risk factors that were independently associated with a higher plaque score were increasing age, increasing systolic BP and diastolic BP, cigarette smoking and a low BMI.

Logistic regression models for the association of plaque morphology (high risk or low risk) are presented in table 5-13. After multifactorial adjustment, risk factors that were independently associated with high risk plaque were increasing systolic BP and diastolic BP, and low BMI.

Table 5-9 Multifactorial associations of cIMT and plaque thickness with traditional cardiovascular risk factors (Model 1)

	Mean cIMT		Max cIMT		Max Mean cIMT		Mean Max cIMT		Mean Plaque Thickness		Max Plaque Thickness	
	B coeff	P value	B coeff	P value	B coeff	P value	B Coeff	P value	B coeff	P value	B Coeff	P Value
Age (years)	0.142	<0.001***	0.116	0.001	0.121	<0.001***	0.128	<0.001***	0.176	<0.001***	0.178	<0.001***
Sex (male)	-0.218	<0.001***	-0.208	<0.001***	-0.206	<0.001***	-0.216	<0.001***	-0.155	<0.001***	-0.146	<0.001***
SIMD	-0.022	0.513	-0.025	0.459	-0.016	0.609	-0.030	0.345	-0.046	0.154	-0.030	0.362
Duration of diabetes	0.022	0.536	0.010	0.782	-0.003	0.943	0.025	0.480	0.082	0.021*	0.075	0.035*
HbA1c	0.032	0.366	0.032	0.373	0.037	0.299	0.032	0.363	0.025	0.473	0.020	0.563
Diabetes Medication	-0.048	0.196	-0.057	0.125	-0.039	0.290	-0.062	0.096	-0.037	0.300	-0.040	0.270
Systolic BP (mm Hg)	0.149	<0.001***	0.143	<0.001***	0.157	<0.001***	0.145	<0.001***	0.168	<0.001***	0.139	0.001**
Diastolic BP (mm Hg)	-0.144	<0.001***	-0.146	<0.001***	-0.163	<0.001***	-0.141	<0.001***	-0.099	0.011*	-0.092	0.019*
Antihypertensive Use	-0.058	0.086	-0.053	0.115	-0.061	0.068	-0.057	0.093	0.050	0.131	0.058	0.080
Total Cholesterol	0.022	0.463	0.017	0.636	0.028	0.441	0.017	0.649	0.037	0.302	0.039	0.284
HDL Cholesterol	-0.051	0.144	-0.081	0.021*	-0.066	0.055	-0.067	0.051	-0.017	0.619	-0.012	0.733
Lipid lowering medication	0.060	0.073	0.062	0.068	0.065	0.056	0.056	0.096	0.035	0.286	0.033	0.326
Smoking status	-0.051	0.125	-0.054	0.107	-0.059	0.080	-0.054	0.108	-0.230	<0.001***	-0.222	<0.001***
BMI	0.015	0.660	0.001	0.971	0.005	0.890	0.011	0.751	-0.031	0.416	-0.032	0.367

SIMD=Scottish Index of Multiple Deprivation, HDL=high density lipoprotein, BMI=body mass index, eGFR=estimated glomerular filtration rate, ACR=albumin creatinine ratio, IL-6=interleukin 6, CRP=C reactive protein, NTProBNP=N terminal pro-brain natriuretic peptide, Pack years = geometric mean.

* significant at p<0.05, ** significant at p<0.01, *** significant at p<0.001

Table 5-10 Multifactorial associations of cIMT and plaque thickness with novel risk factors after adjustment for traditional risk factors (Model 2)

	Mean cIMT		Max cIMT		Max Mean cIMT		Mean Max cIMT		Mean Plaque Thickness		Max Plaque Thickness	
	B coeff	P value	B coeff	P value	B coeff	P value	B Coeff	P value	B coeff	P value	B Coeff	P value
ABI	-0.161	0.004**	-0.145	0.011**	-0.146	0.010*	-0.148	0.008**	-0.139	0.011*	-0.106	0.070
eGFR	0.066	0.283	0.086	0.168	0.083	0.186	0.079	0.200	0.024	0.688	0.020	0.747
ACR	0.025	0.656	0.027	0.483	0.019	0.740	0.037	0.515	0.008	0.885	0.028	0.615
IL-6	-0.081	0.196	-0.045	0.705	-0.072	0.256	-0.041	0.511	0.085	0.167	0.091	0.141
CRP	-0.001	0.989	-0.046	0.468	-0.017	0.788	-0.010	0.866	-0.029	0.630	-0.048	0.433
NTproBNP	0.241	<0.001***	0.199	0.002***	0.221	<0.001***	0.223	<0.001***	0.142	0.020*	0.155	0.012*

Adjusted for Age (years), Sex (male), SIMD, Duration of diabetes, HbA1c, Diabetes Medication, Systolic BP (mm Hg), Diastolic BP (mm Hg), Antihypertensive Use, Total Cholesterol, HDL Cholesterol, Lipid lowering medication, Smoking status, BMISIMD=Scottish Index of Multiple Deprivation, HDL=high density lipoprotein, BMI=body mass index, eGFR=estimated glomerular filtration rate, ACR=albumin creatinine ratio, IL-6=interleukin 6, CRP=C reactive protein, NTProBNP=N terminal pro-brain natriuretic peptide, Pack years = geometric mean* significant at p<0.05, ** significant at p<0.01, *** significant at p<0.001

Table 5-11 Explained variance for linear regression models of continuous ultrasound parameters (R^2)

	Mean cIMT	Max cIMT	Max Mean cIMT	Mean Max CIMT	Mean Plaque Thickness	Max Plaque Thickness
Model 1	0.108	0.106	0.104	0.108	0.157	0.140
Model 2	0.167	0.142	0.148	0.164	0.189	0.176

Model 1 – traditional cardiovascular risk factors
Model 2 – traditional cardiovascular risk factors and novel biomarkers

Table 5-12 Multifactorial associations of plaque score and plaque morphology with traditional cardiovascular risk factors (Model 1)

	Plaque score >4		High risk plaque	
	Exp (B)	P value	Exp (B)	P value
Age (years)	1.04	0.020*	1.03	0.129
Sex (male)	1.24	0.186	1.02	0.912
SIMD	1.11	0.689	0.77	0.287
Duration of diabetes	0.87	0.614	1.05	0.842
HbA1c	1.07	0.400	0.993	0.921
Diabetes Medication	1.42	0.219	1.25	0.416
Systolic BP (mm Hg)	1.02	<0.001**	1.01	0.012*
Diastolic BP (mm Hg)	0.96	<0.001***	0.98	0.029*
Antihypertensive Use	1.19	0.429	1..08	0.720
Total Cholesterol	1.04	0.685	1.00	0.967
HDL Cholesterol	0.74	0.202	0.98	0.945
Lipid lowering medication	1.05	0.819	1.36	0.0.129
Smoking status	1.95	<0.001***	1.17	0.310
BMI	0.95	0.001**	0.97	0.012*

SIMD=Scottish Index of Multiple Deprivation, HDL=high density lipoprotein, BMI=body mass index, eGFR=estimated glomerular filtration rate, ACR=albumin creatinine ratio, IL-6=interleukin 6, CRP=C reactive protein, NTProBNP=N terminal pro-brain natriuretic peptide, Pack years = geometric mean.* significant at p<0.05, ** significant at p<0.01, *** significant at p<0.001

Table 5-13 Multifactorial associations of plaque score and plaque morphology with novel risk factors after adjustment for traditional risk factors (Model 3)

	Plaque score >4		High risk plaque	
	B coeff	P value	B coeff	P value
ABI	0.19	0.014*	0.62	0.450
eGFR	1.00	0.788	0.99	0.434
ACR	0.77	0.351	1.07	0.814
IL-6	0.57	0.270	0.99	0.977
CRP	1.40	0.278	1.11	0.726
NTproBNP	2.38	0.003**	2.10	0.007**

Adjusted for Age (years), Sex (male), SIMD, Duration of diabetes, HbA1c, Diabetes Medication, Systolic BP (mm Hg), Diastolic BP (mm Hg), Antihypertensive Use, Total Cholesterol, HDL Cholesterol, Lipid lowering medication, Smoking status, BMI, SIMD=Scottish Index of Multiple Deprivation, HDL=high density lipoprotein, BMI=body mass index, eGFR=estimated glomerular filtration rate, ACR=albumin creatinine ratio, IL-6=interleukin 6, CRP=C reactive protein, NTProBNP=N terminal pro-brain natriuretic peptide, Pack years = geometric mean* significant at p<0.05, ** significant at p<0.01, *** significant at p<0.001

5.5 Change in cIMT and vascular risk factors

A univariate correlation analysis (table 5-14) of change in cIMT between year 1 and year 4 follow up with risk factors revealed that only baseline cIMT was correlated with change in cIMT (r=-0.300, p<0.001). Multifactorial regression modelling (table 5-15) of change in mean cIMT revealed the independent predictors of change in cIMT were baseline mean cIMT (B -0.300, p<0.001) and BMI (-0.184, p=0.005).

Table 5-14 Univariate association of risk factors and baseline cIMT with change in mean cIMT

		Change mean cIMT	
		Correlation	**Anova**
Demographics	Age	-0.024	-
	Sex (male)	-	0.004
	SIMD		
	Quintile 1 (Most Deprived)	-	0.012
	Quintile 2	-	0.013
	Quintile 3	-	-0.007
	Quintile 4	-	-0.004
	Quintile 5 (Least Deprived)	-	-
Diabetes	Duration of DM (years)	-0.046	-
	HbA1C	-0.026	-
	T2DM Meds		
	Diet Alone	-	-0.009
	Oral	-	-0.004
	Insulin ± oral	-	-
Blood Pressure	Systolic BP (mmHg)	0.068	-
	Diastolic BP (mmHg)	0.080	-
	Antihypertensives (n, % yes)	-	0.004
Blood Lipids	Total Cholesterol	0.008	-
	HDL Cholesterol	-0.064	-
	Cholesterol med (n, % yes)	-	-0.018
Smoking	Smoking status (%)		
	Current smoker	-	0.035
	Ex smoker	-	0.006
	Never smoker	-	-
Obesity Index	BMI	-0.050	-
CVD Risk Marker	ABI	-0.020	-
Renal Function	eGFR	-0.012	-
	ACR	-0.109	-
Plasma Biomarkers	IL6	0.008	-
	CRP	0.036	-
	NTProBNP	-0.061	-
Carotid Ultrasound	Baseline cIMT	-0.300***	-

For continuous variables, Pearson correlation coefficients and corresponding p values are quoted. For categorical variables, ANOVAs quantify the difference in mean and maximum plaque thickness for the given categorical variable compared with the reference level (reference levels are female sex, least deprived SIMD quintile, diet alone treatment for diabetes, no lipid lowering medication, no anti-hypertensive medication, never smoker) SIMD=Scottish Index of Multiple Deprivation, HDL=high density lipoprotein, BMI=body mass index, ABI =ankle brachial index, eGFR=estimated glomerular filtration rate, ACR=albumin creatinine ratio, IL-6=interleukin 6, CRP=C reactive protein, NTProBNP=N terminal pro-brain natriuretic peptide, Pack years = geometric mean. *** significant at p<0.001

Table 5-15 Multifactorial associations of change in mean cIMT with cardiovascular risk factors and baseline cIMT

	Change mean cIMT	
	B coeff	**P value**
Age (years)	-0.023	0.721
Sex (male)	-0.052	0.479
SIMD	0.061	0.320
Duration of diabetes	0.011	0.875
HbA1c	-0.018	0.771
Diabetes Medication	0.114	0.082
Systolic BP (mm Hg)	0.115	0.116
Diastolic BP (mm Hg)	0.099	0.198
Antihypertensive Use	0.027	0.668
Total Cholesterol	-0.043	0.507
HDL Cholesterol	-0.114	0.089
Lipid lowering medication	-0.038	0.531
Smoking status	-0.054	0.390
BMI	-0.183	0.005**
ABI	-0.090	0.157
eGFR	-0.110	0.109
ACR	-0.083	0.186
IL-6	-0.013	0.965
CRP	-0.003	0.845
NTproBNP	-0.061	0.389
Baseline cIMT	-0.327	<0.001***

SIMD=Scottish Index of Multiple Deprivation, HDL=high density lipoprotein, BMI=body mass index, eGFR=estimated glomerular filtration rate, ACR=albumin creatinine ratio, IL-6=interleukin 6, CRP=C reactive protein, NTProBNP=N terminal pro-brain natriuretic peptide, Pack years = geometric mean.** significant at p<0.01 *** significant at p<0.001

5.6 Prevalent vascular disease

Prevalence of cardiovascular disease is reported in table 5-16. At year 1, previous myocardial infarction was recorded for 129 (14.1%) participants, a diagnosis of angina for 250 (27.3%), previous stroke for 50 (5.5%) and previous TIA for 29 (3.2%). When vascular diseases were categorized into broader categories, 280 (30.6%) had a history of any coronary artery disease (MI and/or angina), 79 (8.6%) had a history of any cerebrovascular disease (stroke and/or TIA) and 317 (34.6%) had a history of any cardiovascular disease (MI, angina, stroke or TIA).

Table 5-16 Prevalence of Vascular Disease in the ET2DS year 1 study population

	% participants (n)
Myocardial Infarction	14.1 (129)
Angina	27.3 (250)
Stroke	5.5 (50)
Transient Ischaemic Attack	3.2 (29)
Any Cardiovascular Disease	34.6 (317)
Any Coronary Artery Disease	30.6 (280)
Any Cerebrovascular Disease	8.6 (79)

The groups reported are not mutually exclusive. Prevalence is reported for individuals who have ever had any of the above diagnoses, regardless of whether they had also been diagnosed with one of the other diagnoses ie a participant who has had both an MI and a TIA is counted in both groups, and so the percentages do no total 100. For the amalgamated groups, the group 'any cardiovascular disease' has more participants because participants could be in both any CAD and any Cerebrovascular disease but only be counted once in the any CVD group.

5.6.1 Association of prevalent cardiovascular disease with vascular risk factors and plasma biomarkers

Distribution of cardiovascular risk factors and novel plasma biomarkers in those with grouped CVD outcomes are detailed in table 5-17.

Any CVD

Individuals with any CVD were older (69.35 vs 68.55 years, p=0.016), were more likely to be male (61.5% vs 46.6%, p<0.001) and more likely to be from less affluent social groups (p<0.001) than those with no history of CVD. They had a longer duration of diabetes (8.0 vs 7.0 years, p=0.018) but there was no significant difference in HbA1c between the groups. Slightly more individuals with a history of CVD used insulin in addition to oral medications compared to those without disease (19.9% vs 13.7%, p=0.045).

Systolic blood pressure was broadly similar between the groups (139.26 vs 137.42 mmHg, p=0.151) however diastolic blood pressure was significantly lower in those with CVD (72.763 vs 74.75 mmHg, p<0.001), a surprising finding which may be accounted for by the increased use of antihypertensive medication in this group (96.5% vs 80.0%, p<0.001). Total cholesterol was lower in those with CVD (4.054

vs 4.198 mmol/l, p=0.010), as was HDL cholesterol (1.145 vs 1.276 mmol/l, p<0.001), while use of lipid lowering medications was higher (88.0 vs 82.3%, p=0.024). While one might expect to see lower HDL cholesterol in those with CVD, while total cholesterol tends to be higher. The unexpected finding of lower total cholesterol might be accounted for by the increased use of lipid lowering medication in the disease group. A higher percentage of those with CVD were current or ex-smokers compared to those without disease (67.5 vs 55.9%, p=0.003). BMI was similar in both groups (31.8 vs 31.1, p=0.095).

A lower ABI was also noted in those with disease (0.961 vs 1.001, p=0.006). Individuals who experienced any cardiovascular disease had poorer renal function than those who did not, with lower mean eGFR (72.97 vs 79.53 ml/min/1,73m^2, p<0.001, respectively) and higher ACR (2.5 vs 1.5 mg/mol, p<0.001 respectively). Inflammatory markers were also higher in those with CVD (IL6 3.29 vs 2.59 pg/ml, p<0.001; CRP 2.19 vs 1.56 mg/l, p<0.001). NTproBNP was also higher (144.5 vs 59.0 pg/ml, p<0.001).

Coronary Artery Disease

Differences in risk factors and biomarkers in those with and without coronary artery disease were broadly similar to those seen for individuals with any cardiovascular disease, except that in the CAD group, there was no significant difference in the type of treatment for diabetes.

Cerebrovascular Disease

Differences in risk factors and plasma biomarkers between those with and without cerebrovascular disease were not as pronounced as in those with any cardiovascular disease or any CAD. Those with cerebrovascular disease tended to be older and male, with a longer duration of diabetes and increased frequency of insulin use, than those without cerebrovascular disease. They also had lower HDL cholesterol and a greater prevalence of current smoking. They also tended to have a lower ABI and poorer renal function. CRP was the only inflammatory marker that was significantly

higher in those with cerebrovascular disease than those without. Again, NTproBNP was higher in the disease group than those without disease.

5.6.2 Association of prevalent cardiovascular disease with carotid ultrasound parameters

Distribution of cardiovascular risk factors and novel plasma biomarkers in those with grouped CVD outcomes are detailed in table 5-18.

Any CVD

In subjects with any cardiovascular disease, mean cIMT was higher (0.99 vs 0.92 mm, p<0.001) as were maximum cIMT (1.15 vs 1.04mm, p=0.001), maximum mean cIMT (1.06 vs 0.98mm, p=0.001) and mean maximum cIMT (1.07 vs 0.98, p<0.001). There was no significant difference in change in mean cIMT.

Mean plaque thickness was also higher (2.75 vs 2.28mm, p<0.001). Individuals with CVD were more likely to have a higher plaque score. When plaque score and plaque morphology were considered as dichotomous variables (plaque score: ≤4 or >4 and plaque type: high risk/low risk respectively), those with disease tended to have a higher plaque score (57.3 vs 39.0%, p<0.001) and more high risk plaque (65.5 vs 52.7%, p<0.001).

Coronary Artery Disease

Continuous carotid ultrasound variables were also significantly higher in individuals with CAD (mean cIMT 0.96 vs 0.93 mm, p=0.001; max cIMT 1.09 vs 1.05 mm, p=0.001, max mean cIMT 1.02 vs 0.98 mm, p=0.001; mean plaque thickness 2.78 vs 2.29 mm p<0.001). There was no significant difference in change in mean cIMT. Plaque score tended to be higher in those with CAD (>4 58.2 vs <=4 39.8%, p<0.001) and individuals with CAD tended to have more high risk plaque when compared with individuals without CAD (67.5 vs 52.6% respectively, p<0.001).

Cerebrovascular Disease

Mean cIMT was higher in those with cerebrovascular disease (0.96 vs 0.93 mm, $p<0.001$) as was max cIMT (1.09 vs 1.05 mm, $p<0.001$), max mean cIMT (1.02 vs 0.99, $p<0.001$) and mean plaque thickness (2.85 vs 2.41, $p<0.001$). There was no significant difference in change in mean cIMT. Individuals with a history cerebrovascular disease were more likely to have a plaque score >4 when compared to those without disease (62.5 vs 43.9% respectively, $p=0.002$) but there was no significant difference in presence of high risk plaque between the groups ($p=0.191$).

Table 5-17 Prevalent Cardiovascular Disease, Cardiovascular Risk Factors and Novel plasma biomarkers

	Any CVD	No CVD	P value	Any CAD	No CAD	P value	Any Cerebro	No Cerebro	P value
Age (years)	69.35 (4.01)	68.66 (4.08)	0.016	69.5 (4.4)	68.64 (4.05)	0.003	68.9 (4.05)	68.89 (4.18)	0.893
Sex (male)	61.5 (195)	46.6 (279)	<0.001	61.8 (173)	47.3 (301)	<0.001	67.1 (53)	50.3 (421)	0.004
SIMD									
Quintile 1	14.8 (47)	9.8 (59)		15.0 (42)	10.1 (64)		19.0 (15)	10.9 (91)	
Quintile 2	23.7 (75)	15.7 (94)		23.6 (66)	16.2 (103)		24.1 (19)	17.9 (15)	
Quintile 3	19.6 (62)	16.4 (98)	<0.001	18.6 (52)	17.0 (108)	0.002	24.1 (19)	16.8 (141)	0.006
Quintile 4	15.4 (46)	20.0 (120)		15.4 (43)	19.3 (123)		8.9 (7)	19.3 (159)	
Quintile 5	27.4 (87)	38.1 (228)		27.5 (77)	37.4 (27.5)		24.1 (19)	35.4 (296)	
Duration of Diabetes (years)	8.0 (7.0)	7.0 (8.0)	0.018	8.00 (7.00)	7.0 (8.0)	0.037	9.00 (8.25)	7.0 (8.0)	0.013
HbA1C (% haemoglobin)	7.239 (1.158)	7.154 (1.023)	0.257	7.199 (1.092)	7.176 (1.064)	0.764	7.372 (1.364)	7.165 (1.039)	0.101
Diabetes Medication									
Diet Alone	19.6 (62)	19.7 (118)		18.9 (53)	20.0 (127)		21.5 (17)	19.5 (163)	
Oral Hypoglycaemics	60.6 (192)	66.6 (399)	0.045	62.1 (174)	65.6 (417)	0.234	53.2 (42)	65.6 (549)	0.033
Insulin ± oral hypoglycaemics	19.9 (63)	13.7 (82)		18.9 (53)	14.5 (92)		25.3 (20)	14.9 (125)	
Systolic BP (mmHg)	139.26 (20.248)	137.42 (17.310)	0.151	139.19 (20.591)	137.55 (17.321)	0.216	140.29 (19.011)	137.85 (18.331)	0.261
Diastolic BP (mmHg)	72.73 (10.30)	74.75 (8.99)	0.002	72.56 (10.384)	74.71 (9.023)	0.002	72.40 (10.037)	74.21 (9.449)	0.108
On antihypertensives (% yes)	96.5 (306)	80.0 (479)	<0.001	98.2 (275)	80.2 (510)	<0.001	92.4 (73)	85.1 (712)	0.075
Total Cholesterol (mmol/l)	4.054 (0.817)	4.198 (0.794)	0.010	4.035 (0.822)	4.197 (0.792)	0.005	4.071 (0.765)	4.155 (0.808)	0.380
HDL Cholesterol (mmol/l)	1.145 (0.283)	1.276 (0.354)	<0.001	1.138 (0.274)	1.271 (0.354)	<0.001	1.128 (0.300)	1.240 (0.339)	0.005
Lipid lowering meds (% yes)	88.0 (279)	82.3 (493)	0.024	89.6 (251)	81.9 (521)	0.003	81.0 (15)	84.6 (708)	0.404
Smoking status									
Current smoker	14.5 (46)	12.2 (73)		14.3 (40)	12.4 (79)		16.5 (13)	12.7 (106)	
Ex smoker	53.0 (168)	43.7 (168)	0.003	52.9 (148)	44.3 (282)	0.013	57.0 (45)	46.0 (385)	0.037
Never smoker	32.5 (103)	44.1 (264)		32.9 (92)	43.2 (275)		26.6 (21)	41.3 (346)	
Ankle Brachial Index	0.961 (0.242)	1.001 (0.188)	0.006	0.966 (0.249)	0.997 (0.189)	0.039	0.891 (0.246)	0.996 (0.204)	<0.001
BMI (kg/m^2)	31.76 (5.61)	31.10 (5.72)	0.095	31.71 (5.61)	31.16 (5.71)	0.176	31.55 (5.54)	31.30 (5.70)	0.709
eGFR ($ml/min/1.73m^2$)	72.97 (20.26)	79.53 (17.307)	<0.001	72.65 (20.43)	79.29 (17.42)	<0.001	71.64 (22.15)	77.81 (18.19)	0.005
ACR (mg/mmol)	2.5 (4.2)	1.5 (2.2)	<0.001	2.5 (4.3)	1.5 (2.3)	<0.001	2.85 (4.5)	1.7 (3.0)	0.012
IL6	3.29 (2.81)	2.59 (2.24)	<0.001	3.31 (2.87)	2.66 (2.25)	<0.001	3.28 (2.30)	2.76 (2.43)	0.083
CRP (mg/l)	2.19 (4.24)	1.56 (3.03)	<0.001	2.18 (4.23)	1.6 (3.08)	0.002	2.26 (4.62)	1.67 (3.15)	0.011
NTProBNP (pg/ml)	144.5 (269)	59.0 (83)	<0.001	164.5 (285)	59.0 (82)	<0.001	93.5 (232)	72.0 (129)	0.004

SIMD=Scottish Index of Multiple Deprivation, HDL=high density lipoprotein, BMI=body mass index, eGFR=estimated glomerular filtration rate, ACR=albumin creatinine ratio, IL-6=interleukin 6, CRP=C reactive protein, NTProBNP=N terminal pro-brain natriuretic peptide, Pack years = geometric mean. * significant at p<0.05, ** significant at p<0.01, *** significant at p<0.001

Table 5-18 Prevalent cardiovascular disease, carotid intima media thickness and carotid plaque

	Any CVD	No CVD	P value	Any CAD	No CAD	P value	Any Cerebro	No Cerebro	P value
Mean cIMT (mm)	0.99 (0.15)	0.92 (0.14)	<0.001	0.96 (0.14)	0.93 (0.14)	0.001	0.96 (0.14)	0.93 (0.14)	<0.001
Max cIMT (mm)	1.15 (0.22)	1.04 (0.19)	0.001	1.09 (0.19)	1.05 (0.19)	0.001	1.09 (0.19)	1.05 (0.19)	<0.001
Max Mean cIMT (mm)	1.06 (0.20)	0.98 (0.17)	0.001	1.02 (0.17)	0.98 (1.02)	0.001	1.02 (0.17)	0.99 (0.17)	<0.001
Mean Max cIMT (mm)	1.07 (0.17)	0.98 (0.15)	<0.001	1.02 (0.16)	0.98 (0.16)	<0.001	1.02 (0.17)	0.99 (0.15)	<0.001
Mean Plaque Thickness (mm)	2.75 (0.94)	2.28 (0.84)	<0.001	2.78 (0.93)	2.29 (0.84)	<0.001	2.85 (1.15)	2.41 (0.86)	<0.001
Max Plaque Thickness (mm)	3.15 (1.15)	2.62 (1.04)	<0.001	3.20 (1.16)	2.64 (1.04)	<0.001	3.33 (1.46)	2.76 (1.06)	0.001
Change in mean cIMT	-0.01 (0.12)	-0.01 (0.11)	0.774	-0.01 (0.11)	-0.01 (0.11)	0.940	0.01 (0.15)	-0.02 (0.11)	0.096
Plaque Score									
0	1.2 (4)	2.6 (15)		0.7 (2)	2.7 (17)		2.5 (2)	2.1 (17)	
1	0.9 (3)	4.1 (24)		1.1 (3)	3.9 (24)		1.3 (1)	3.2 (26)	
2	9.3 (30)	17.4 (101)		8.5 (24)	17.2 (107)		10.0 (8)	14.9 (123)	
3	14.3 (46)	17.4 (101)		13.8 (39)	17.4 (108)		12.5 (10)	16.6 (137)	
4	16.8 (54)	19.6 (114)	<0.001	17.7 (49)	19.0 (118)	<0.001	11.3 (9)	19.3 (159)	0.043
5	18.7 (60)	15.3 (89)		17.4 (49)	16.1 (100)		17.5 (14)	16.4 (135)	
6	16.5 (53)	12.4 (72)		18.8 (53)	11.6 (72)		17.5 (14)	13.5 (111)	
7	9.0 (29)	6.2 (36)		8.9 (25)	6.4 (40)		11.3 (9)	6.8 (56)	
8	13.1 (42)	5.2 (30)		13.1 (37)	5.6 (35)		16.3 (13)	7.2 (59)	
Plaque Score									
≤4	42.7 (137)	61.0 (355)	<0.001	41.8 (118)	60.2 (374)	<0.001	37.5 (30)	56.1 (462)	0.001
>4	57.3 (184)	39.0 (227)		58.2 (164)	39.8 (247)		62.5 (50)	43.9 (361)	
Plaque Morphology									
Echogenic/no plaque	34.5 (111)	47.3 (275)		32.5 (92)	47.4 (294)		37.5 (30)	43.3 (356)	
Echolucent only	9.6 (31)	14.1 (82)	<0.001	10.2 (29)	13.5 (84)	<0.001	6.3 (5)	13.1 (107)	0.002
Heterogenous only	22.7 (73)	18.9 (110)		23.7 (67)	18.7 (116)		15.0 (12)	20.8 (171)	
Both types	33.2 (107)	19.6 (114)		33.6 (95)	20.3 (126)		37.5 (33)	22.8 (188)	
Plaque Morphology									
Low Risk	34.5 (111)	47.3 (275)	<0.001	32.5 (92)	47.4 (294)	<0.001	37.5 (30)	43.3 (356)	0.191
High Risk	65.5 (211)	52.7 (306)		67.5 (191)	52.6 (326)		62.5 (50)	55.7 (467)	

* significant at $p<0.05$, ** significant at $p<0.01$, *** significant at $p<0.001$

5.7 Logistic regression analyses for cardiovascular events

Logistic regression analyses were performed to assess the association of cIMT and carotid plaque with prevalent cardiovascular events before and after adjustment for traditional cardiovascular risk factors and the more novel biomarkers. Results are reported as odds ratios for 1 SD change in the factor being measured in tables 5-19, 5-20 and 5-21.

cIMT (table 5-19)

In the unadjusted models (model 1), a 1-SD increase in mean cIMT had an odds ratio of 1.26 (1.10-1.45), p<0.001 for any prevalent CVD. Age and sex adjustment (model 2) attenuated the odds ratio slightly (OR 1.16 (1.01-1.34), p=0.036), while after additional adjustment for traditional cardiovascular risk factors (model 3) and for novel biomarkers and SIMD (model 4) the odds ratios became non-significant (p>0.05). Odds ratios for any coronary artery disease and any cerebrovascular disease were similar to those for any CVD and were again attenuated and became non-significant once traditional risk factors were adjusted for (see table 5-12, column 1).

Max cIMT had an unadjusted odds ratio of 1.25 (1.10-1.43), p=0.001, and an age and sex adjusted OR 1.16 (1.01-1.33), p=0.039. As was seen for mean cIMT, the odds ratios were attenuated in models 3 and 4, and subsequently fell out of statistical significance. A similar pattern was seen for coronary artery disease and cerebrovascular disease (see table 5-13, column 2).

For maximum mean cIMT, the unadjusted and age and sex adjusted odds ratios for any cardiovascular disease were OR 1.24 (1.08-1.42), p=0.002, and OR 1.15 (0.99-1.32), p=0.052 respectively. Further adjustment for CV risk factors caused the odds ratios to fall out of significance. The same was seen for coronary artery disease and cerebrovascular disease. (see table 5-13, column 3).

For mean max cIMT, unadjusted and age and sex adjusted odds ratios for any cardiovascular disease were OR 1.29 (1.13-1.49), p<0.001 and OR 1.19 (1.03-1.38),

p=0.018 respectively. Again, further adjustment for traditional and then novel risk factor attenuated the odds ratios and cause them to fall out of statistical significance (see table 5-12, column 4). The same pattern was seen for CAD and cerebrovascular disease.

Plaque Thickness (table 5-20)

Both mean and maximum plaque thickness demonstrated a more robust association with prevalent cardiovascular disease than cIMT.

The unadjusted odds ratio of mean plaque thickness for cardiovascular disease was 1.70 (1.47-1.97), p<0.001. Adjusting for age and sex attenuated the odds ratio slightly to OR 1.60 (1.38-1.86), p<0.001. However, unlike for cIMT variables, mean plaque thickness retained significant odds ratios upon additional adjustment for cardiovascular and novel risk factors (OR 1.50 (1.27-1.77), p<0.001 and OR 1.50 (1.11-2.01), p=0.009 respectively). Similar odds ratios were seen for coronary artery disease and cerebrovascular disease and they remained significant for both following adjustment for cardiovascular risk factors, although mean plaque thickness lost significance for cerebrovascular disease once novel factors were accounted for (OR 1.28 (0.86-1.91), p=0.0.224) (see table 5-13, column 1).

Max plaque thickness had unadjusted odds ratio of 1.62 (1.40-1.88) p<0.001 and an age and sex adjusted OR of 1.52 (1.31-1.77), p<0.001 for any cardiovascular disease. Similarly to mean plaque thickness, further adjustment for both traditional and novel risk factors did not attenuated the odds ratio significantly (OR 1.45 (1.22-1.71), p<0.001 and OR 1.38 (1.03-1.83), p=0.030) (see table 5-13, column 2). Similar odds ratios were seen for CAD and cerebrovascular disease, although, again, once novel risk factors were accounted for, max plaque thickness lost its association with cerebrovascular disease.

Plaque score and plaque morphology (table 5-21)

Similarly to the measures of plaque thickness, plaque score and plaque morphology were also strongly associated with prevalent cardiovascular disease and survived full

adjustment for risk factors (OR 1.74, p=0.049 and HR 1.74, p=0.043 respectively). Plaque score and plaque morphology were also associated with prevalent CAD, although plaque score lost its association once novel biomarkers were adjusted for. There was no relationship between cerebrovascular disease and either plaque score or plaque morphology once traditional risk factors were accounted for.

Table 5-19 Multifactorial adjusted associations between prevalent cardiovascular disease and cIMT

	Mean cIMT			Max cIMT			Max Mean cIMT			Mean Max cIMT		
Outcome	**Odds Ratio**	**CI**	**P value**	**Odds Ratio**	**CI**	**P value**	**Odds Ratio**	**CI**	**P value**	**Odds Ratio**	**CI**	**P value**
Any CVD												
Model 1	**1.26**	**1.10-1.45**	**0.001**	**1.25**	**1.10-1.43**	**0.001**	**1.24**	**1.08-1.42**	**0.002**	**1.29**	**1.13-1.49**	**<0.001**
Model 2	**1.16**	**1.01-1.34**	**0.036**	**1.16**	**1.01-1.33**	**0.039**	1.15	0.99-1.32	0.052	**1.19**	**1.03-1.38**	**0.018**
Model 3	1.06	0.91-1.25	0.442	1.05	0.90-1.23	0.556	1.05	0.90-1.23	0.559	1.08	0.92-1.27	0.351
Model 4	0.99	0.75-1.30	0.938	0.97	0.75-1.26	0.833	0.97	0.75-1.25	0.805	0.99	0.75-1.31	0.964
Coronary Artery Disease												
Model 1	**1.28**	**1.11-1.47**	**0.001**	**1.27**	**1.10-1.45**	**0.001**	**1.25**	**1.09-1.44**	**0.001**	**1.29**	**1.13-1.50**	**<0.001**
Model 2	**1.17**	**1.01-1.35**	**0.036**	**1.16**	**1.01-1.34**	**0.038**	**1.15**	**1.00-1.33**	**0.049**	**1.19**	**1.03-1.38**	**0.022**
Model 3	1.08	0.92-1.27	0.353	1.08	0.92-1.27	0.376	1.07	0.91-1.26	0.411	1.10	0.93-1.30	0.279
Model 4	0.98	0.73-1.32	0.898	0.99	0.75-1.31	0.972	0.99	0.75-1.30	0.930	0.99	0.73-1.33	0.940
Cerebrovascular Disease												
Model 1	**1.46**	**1.18-1.81**	**0.001**	**1.51**	**1.22-1.86**	**<0.001**	**1.43**	**1.16-1.76**	**0.001**	**1.56**	**1.25-1.95**	**<0.001**
Model 2	**1.41**	**1.12-1.76**	**0.003**	**1.45**	**1.17-1.81**	**0.001**	**1.37**	**1.11-1.70**	**0.004**	**1.51**	**1.20-1.89**	**<0.001**
Model 3	1.22	0.95-1.56	0.122	1.25	0.98-1.59	0.074	1.19	0.94-1.51	0.185	1.28	0.99-1.65	0.055
Model 4	1.35	0.91-2.00	0.134	1.27	0.89-1.83	0.191	1.21	0.85-1.72	0.292	1.40	0.94-2.08	0.099

model 1 unadjusted
model 2 age and sex
model 3 Age, sex,SIMD, duration of DM, HbA1C, diabetes treatment, systolic blood pressure, diastolic blood pressure, Antihypertensive use, total cholesterol, HDL cholesterol, lipid lowering medication use, smoking, BMI
model 4 Age, sex, SIMD,duration of DM, HbA1C, diabetes treatment, systolic blood pressure, diastolic blood pressure, Antihypertensive use, total cholesterol, HDL cholesterol, lipid lowering medication use, smoking, BMI, ankle brachial index, albumin creatinine ratio, interleukin 6, c reactive protein, NTProBNP
SIMD=Scottish Index of Multiple Deprivation, HDL=high density lipoprotein, BMI=body mass index, eGFR=estimated glomerular filtration rate, ACR=albumin creatinine ratio, IL-6=interleukin 6, CRP=C reactive protein, NTProBNP=N terminal pro-brain natriuretic peptide, Pack years = geometric mean.
* significant at $p<0.05$, ** significant at $p<0.01$, *** significant at $p<0.001$

Table 5-20 Multifactorial adjusted associations between prevalent cardiovascular disease and carotid plaque thickness

	Mean Plaque Thickness			Max Plaque Thickness		
Outcome	**Odds Ratio**	**CI**	**P value**	**Odds Ratio**	**CI**	**P value**
Any CVD						
Model 1	**1.70**	**1.47-1.97**	**<0.001**	**1.62**	**1.40-1.88**	**<0.001**
Model 2	**1.60**	**1.38-1.86**	**<0.001**	**1.52**	**1.31-1.77**	**<0.001**
Model 3	**1.50**	**1.27-1.77**	**<0.001**	**1.45**	**1.22-1.71**	**<0.001**
Model 4	**1.50**	**1.11-2.01**	**0.007**	**1.38**	**1.03-1.83**	**0.030**
Coronary Artery Disease						
Model 1	**1.72**	**1.48-1.99**	**<0.001**	**1.66**	**1.43-1.92**	**<0.001**
Model 2	**1.61**	**1.38-1.87**	**<0.001**	**1.55**	**1.33-1.81**	**<0.001**
Model 3	**1.56**	**1.31-1.85**	**<0.001**	**1.52**	**1.27-1.80**	**<0.001**
Model 4	**1.57**	**1.15-2.14**	**0.004**	**1.48**	**1.09-2.01**	**0.011**
Cerebrovascular Disease						
Model 1	**1.55**	**1.25-1.90**	**<0.001**	**1.56**	**1.27-1.92**	**<0.001**
Model 2	**1.49**	**1.19-1.85**	**<0.001**	**1.50**	**1.21-1.87**	**<0.001**
Model 3	**1.32**	**1.03-1.70**	**0.028**	**1.37**	**1.07-1.75**	**0.012**
Model 4	1.28	0.86-1.91	0.224	1.28	0.85-1.91	0.234

model 1 unadjusted
model 2 age and sex
model 3 Age, sex,SIMD, duration of DM, HbA1C, diabetes treatment, systolic blood pressure, diastolic blood pressure, Antihypertensive use, total cholesterol, HDL cholesterol, lipid lowering medication use, smoking, BMI
model 4 Age, sex, SIMD,duration of DM, HbA1C, diabetes treatment, systolic blood pressure, diastolic blood pressure, Antihypertensive use, total cholesterol, HDL cholesterol, lipid lowering medication use, smoking, BMI, ankle brachial index, eGFR, albumin creatinine ratio, interleukin 6, c reactive protein, NTProBNP
SIMD=Scottish Index of Multiple Deprivation, HDL=high density lipoprotein, BMI=body mass index, eGFR=estimated glomerular filtration rate, ACR=albumin creatinine ratio, IL-6=interleukin 6, CRP=C reactive protein, NTProBNP=N terminal pro-brain natriuretic peptide.
* significant at $p<0.05$, ** significant at $p<0.01$, *** significant at $p<0.001$

Table 5-21 Multifactorial adjusted associations between prevalent cardiovascular disease and carotid plaque score and morphology

	Plaque Score >4			High Risk Plaque Type		
Outcome	**Odds Ratio**	**CI**	**P value**	**Odds Ratio**	**CI**	**P value**
Any CVD						
Model 1	**2.10**	**1.59-2.77**	**<0.001**	**1.71**	**1.29-2.26**	**<0.001**
Model 2	**1.93**	**1.46-2.57**	**<0.001**	**1.66**	**1.25-2.21**	**<0.001**
Model 3	**1.64**	**1.20-2.26**	**0.001**	**1.61**	**1.17-2.20**	**0.003**
Model 4	**1.74**	**1.00-3.04**	**0.049**	**1.74**	**1.02-2.98**	**0.043**
Coronary Artery Disease						
Model 1	**2.10**	**1.58-2.80**	**<0.001**	**1.87**	**1.40-2.51**	**<0.001**
Model 2	**1.92**	**1.44-2.57**	**<0.001**	**1.82**	**1.35-2.45**	**<0.001**
Model 3	**1.65**	**1.19-2.30**	**0.003**	**1.81**	**1.30-2.52**	**<0.001**
Model 4	1.71	0.94-3.09	0.078	**2.64**	**146-4.76**	**0.001**
Cerebrovascular Disease						
Model 1	**2.13**	**1.33-3.42**	**0.002**	1.27	0.79-2.04	0.354
Model 2	**2.01**	**1.24-3.24**	**0.004**	1.25	0.77-2.01	0.370
Model 3	1.53	0.91-2.58	0.112	1.17	0.70-1.97	0.555
Model 4	1.37	0.60-3.14	0.454	0.65	0.29-1.48	0.305

model 1 unadjusted
model 2 age and sex
model 3 Age, sex,SIMD, duration of DM, HbA1C, diabetes treatment, systolic blood pressure, diastolic blood pressure, Antihypertensive use, total cholesterol, HDL cholesterol, lipid lowering medication use, smoking, BMI
model 4 Age, sex, SIMD,duration of DM, HbA1C, diabetes treatment, systolic blood pressure, diastolic blood pressure, Antihypertensive use, total cholesterol, HDL cholesterol, lipid lowering medication use, smoking, BMI, ankle brachial index,eGFR, albumin creatinine ratio, interleukin 6, c reactive protein, NTProBNP
SIMD=Scottish Index of Multiple Deprivation, HDL=high density lipoprotein, BMI=body mass index, eGFR=estimated glomerular filtration rate, ACR=albumin creatinine ratio, IL-6=interleukin 6, CRP=C reactive protein, NTProBNP=N terminal pro-brain natriuretic peptide.
* significant at $p<0.05$, ** significant at $p<0.01$, *** significant at $p<0.001$

5.8 Chapter summary

This chapter describes the cross sectional relationship between cIMT, carotid plaque and vascular risk factors (both traditional and novel), as well as the relationship between cIMT and carotid plaque and prevalent vascular disease. cIMT was predominantly associated with increasing age, male sex and increased systolic blood pressure, although these were not strong associations with some factors having only small effect sizes. These associations persisted following multivariable linear regression. In addition, increased cIMT was associated with adverse levels the novel biomarkers ABI and NTproBNP following multivariable regression. Further, change in cIMT between year 1 and year 4 demonstrated a regression. The main risk factors associated with change in cIMT were BMI and baseline cIMT following multifactorial adjustment.

Plaque thickness demonstrated more extensive associations with traditional risk factors, including with increasing age, male sex, longer duration of diabetes, increased systolic blood pressure and cigarette smoking, all of which survived multifactorial modelling but which again like cIMT, had only small effect sizes suggesting only modest associations. It was also associated with ABI and NTproBNP in multifactorial models. The relationship between plaque score and cardiovascular risk factors was more complex, although several key risk factors that were associated with increased plaque score were increased age, increased systolic and diastolic blood pressure, cigarette smoking and a lower BMI. Reduced ABI and increased NTproBNP were also associated with plaque score. Plaque morphology was associated with systolic and diastolic blood pressure and lower BMI. It also associated with lower ABI and increased NTproBNP.

Vascular disease had a prevalence of 34.6% (MI, angina, stroke or TIA) in the ET2DS at year 1 and was associated with increased values of both cIMT and carotid plaque. Age and sex adjusted logistic regression models revealed that all measures of cIMT and plaque were associated with prevalent cardiovascular disease and CAD. However, for cIMT, these relationships lost statistical significance after full adjustment for traditional cardiovascular vascular risk factors, whereas for mean and

maximum plaque thickness and plaque score and plaque morphology, they persisted following full adjustment including both traditional risk factors and novel biomarkers. Neither cIMT, plaque score or plaque morphology were as strongly associated with cerebrovascular disease once full adjustment was performed.

These results suggest that carotid plaque may be more strongly associated with prevalent cardiovascular disease than cIMT and thus may have more potential to predict incident cardiovascular events.

Chapter 6: Results 3 – Longitudinal Analysis of Carotid Ultrasound Markers and Incident Cardiovascular Events

This chapter reports the results of longitudinal analyses of the relationship between carotid ultrasound parameters and incident cardiovascular events in the Edinburgh Type 2 diabetes study. Incidence rates are described, as well as the cardiovascular risk factor profile of those who had an event. Finally, survival analyses of cIMT and carotid plaque parameters for cardiovascular events are described.

6.1 Incident cardiovascular events in the ET2DS

In the period between cIMT measurement at year 1, and the end of year 4 follow up of the ET2DS (31st August 2011), there were 74 individuals in the cIMT analysis group with fatal or non-fatal incident cardiovascular events in the study population of this thesis (only first events were recorded), which translates to an incidence rate for any cardiovascular disease of 8.1%. Of the fatal events, three were confirmed myocardial infarction, two were confirmed stroke and eleven were cases of other ischaemic heart disease (not specified). Of the non-fatal events, fifteen were confirmed myocardial infarction, eighteen were new diagnoses of angina, twenty were confirmed non-fatal stroke, and five were confirmed transient ischaemic attack. Because these were first events, the individuals groups are mutually exclusive. For the plaque group, the number of overall CVD events was 75.

As the number of events in each individual category was small, for the purposes of analysis, the events were categorized into four composite endpoints to maximize the possibility of detecting associations. These categories were: Any CVD (Any cardiovascular event), CAD (Fatal MI, Non-Fatal MI and Angina), Cerebrovascular (Fatal Stroke, Non-fatal Stroke and TIA) and Fatal CVD (Fatal MI, Fatal Stoke and Other Fatal IHD). The number of participants in each group is detailed in table 6-1. These groups are not mutually exclusive, for example, individuals who experienced a

fatal MI appear in both any CAD and fatal CVD. This also applies to those who had a fatal stroke. In addition, because the group of fatal events includes diagnoses recorded as "fatal ischaemic heart disease", these individuals were not included in the any CAD group because they could not be confirmed as MI or angina (the definition of any CAD used in this thesis).

Table 6-1 Incident Cardiovascular Events in IMT group in the period between year 1 follow up and August 2011 in the ET2DS

Outcome	Number of events	% of sample
Fatal MI	3	4.1
Non-Fatal MI	15	20.3
Angina	18	24.3
Fatal Stoke	2	2.7
Non-Fatal Stroke	20	27.0
TIA	5	6.8
Other Fatal IHD	11	14.9
Total	74	100.0
Composite Outcomes		
Any CVD	74	8.1
CAD	36	3.9
Cerebrovascular	27	2.9
Fatal CVD	16	1.7

MI=myocardial infarction, TIA=transient ischaemic attack, IHD=ischaemic heart disease
The individual events listed in the first part of the table are mutually exclusive because each event listed is a first event. For the second part of the table, an event could belong to more than one composite group. Therefore, no totals are listed for this section.

Absolute risk of MI in the year 1 IMT group was 5.6 events per 1000 patient years. If we compare equivalent rates in other groups with Type 2 diabetes, Mulnier et al found (in data derived from a GP data base of 1739 people with Type 2 diabetes and almost 4000 without, a rate of 19.4 events per 1000 patient years for MI in adults with Type 2 diabetes aged 65-74 years (similar to the ET2DS), which is considerably higher than that seen in the ET2DS (Mulnier, Seaman et al. 2008). This same study estimated a rate for a similarly aged non-diabetic population of 7.03 per 1000 patient years.

6.2 Demographics, vascular risk factors and incident cardiovascular events

Demographic variables and traditional cardiovascular risk factors were compared in those participants with IMT measurements who had an incident cardiovascular event and those who did not (table 6-2). Individuals who had an incident event were older and more likely to be male, but there was no difference in sociodemographic status between the groups. Those with incident vascular events had a poorer diabetic profile than those with no event, with a longer median duration of diabetes, higher HbA1c and a higher percentage of individuals using insulin ± hypoglycaemics. When cardiovascular risk factors were compared between the groups, there was no significant difference in either systolic or diastolic blood pressure, but there was more antihypertensive medication use among those who had an event, which could explain why blood pressure was similar in each group. There was no difference in total cholesterol levels between the groups and while HDL cholesterol was slightly lower in the incident event group, the difference did not reach statistical significance. There was however a higher percentage of individuals using lipid lowering medication in the group who experienced an event. Smoking profiles did not vary between the groups, with no significant difference in the percentage of current, ex or never smokers in the groups. Obesity indices were examined and BMI was found to be similar between the groups. eGFR was also significantly lower in those who experienced an event, as was IL-6 and NTproBNP. There was no difference in ABI, ACR or CRP between the groups. Results were similar in the plaque analysis group.

The relationship between prevalent cardiovascular and incident vascular disease was also explored. Those individuals who experienced an incident event were more likely to have experienced a previous CVD event (51.4% previous CVD vs 34.0% no previous CVD, p=0.002).

Table 6-2 Demographics and Vascular Risk factors in Individuals with Incident Events and No events (IMT group)

	Incident Event (n=74)	No Event (n=842)	P Value
Age (years)	70.1 (3.9)	68.8 (4.2)	0.009**
Sex (male)	63.5 (47)	50.7 (427)	0.023*
SIMD			
Quintile 1(most deprived)	12.2 (9)	11.5 (97)	
Quintile 2	20.3 (15)	18.3 (154)	
Quintile 3	21.6 (16)	17.1 (144)	0.567
Quintile 4	20.3 (15)	17.9 (151)	
Quintile 5 (least deprived)	25.7 (19)	35.2 (296)	
Duration of Diabetes (years)	9.0 (11.0)	7.0 (8.0)	0.010*
HbA1C (% haemoglobin)	7.44 (1.19)	7.16 (1.06)	0.030*
Diabetes Medication			
Diet Alone	16.2 (12)	20.0 (168)	
Oral Hypoglycaemics	50.0 (37)	65.8 (554)	<0.001***
Insulin ± oral hypoglycaemics	33.8 (25)	14.3 (120)	
Systolic BP (mmHg)	140.6 (19.3)	137.8 (18.3)	0.208
Diastolic BP (mmHg)	72.4 (10.1)	74.2 (9.4)	0.120
On antihypertensives (% yes)	93.2 (69)	85.0 (716)	0.032*
Total Cholesterol (mmol/l)	4.14 (0.96)	4.15 (0.79)	0.883
HDL Cholesterol (mmol/l)	1.16 (0.34)	1.24 (0.34)	0.061
Lipid lowering meds (% yes)	91.9 (68)	83.6 (704)	0.037*
Smoking status			
Current smoker	14.9 (11)	12.8 (108)	
Ex smoker	50.0 (37)	46.7 (393)	0.648
Never smoker	35.1 (26)	40.5 (341)	
Pack Years	0.00 (0.00)	0.00 (0.00)	0.816
BMI (kg/m^2)	31.2 (5.2)	31.3 (5.7)	0.807
ABI	0.96 (0.32)	0.99 (0.20)	0.257
eGFR ($ml/min/1.73m^2$)	69.2 (20.4)	77.9 (18.3)	<0.001**
ACR	2.0 (8.)	1.7 (3.1)	0.147
IL6	3.64 (2.6)	2.75 (2.4)	0.047*
CRP	2.29 (3.3)	1.70 (3.3)	0.256
NTproBNP	137.5 (235)	70.0 (126)	<0.001***
Previous History of CVD at baseline	51.4 (38)	34.0 (286)	0.002**

SIMD=Scottish Index of Multiple Deprivation, HDL=high density lipoprotein, BMI=body mass index, eGFR=estimated glomerular filtration rate, CVD=cardiovascular disease *=p<0.05, **p<0.01, ***significant at p<0.001 Values are mean (SD) , median IQR or % (n)

6.3 IMT, plaque and incident vascular events

The distribution of cIMT and plaque in individuals with incident events was examined using the students T test and chi squared statistic. Further exploration through a series of Cox Proportional Hazards models examined the predictive ability of cIMT for incident CVD in people with diabetes.

6.3.1 Distribution of IMT and plaque in participants with incident cardiovascular events

All measures of cIMT were significantly higher in those who experienced an incident event when compared with those who did not (table 6-3). Mean and maximum

plaque thickness were also significantly higher in those who had an event although the absolute difference was not as great as that seen for cIMT. There was no significant difference in change in mean cIMT. Plaque score was considered as a dichotomous score of less than or greater than 4, revealing a significantly higher proportion of participants with a plaque score >4 in the group with incident events than without. Plaque morphology was significantly different between the groups. Participants with incident events were less likely to have echogenic/no plaque, echolucent plaque alone or heterogeneous plaque alone, and more likely to have both types of plaque (p=0.008) than individuals who did not have an event. However, when plaque morphology was categorized as high or low risk, there was no significant difference in plaque morphology between those with and without incident events. These results were repeated in the plaque analysis group.

Table 6-3 cIMT and Plaque Measurements in those with and without incident events

		Incident Event (n=74)	No Event (n=842)	P Value
Mean IMT (mm)		0.985 (0.14)	0.931 (0.14)	0.001**
Max IMT (mm)		1.112 (0.17)	1.054 (0.193)	0.013*
Max Mean IMT (mm)		1.047 (0.16)	0.990 (0.17)	0.006**
Mean Max IMT (mm)		1.048 (0.15)	0.990 (0.16)	0.002**
Mean Plaque Thickness (mm)		2.694 (0.91)	2.421 (0.89)	0.015*
Max Plaque Thickness (mm)		3.116 (1.16)	2.786 (1.10)	0.017*
Change in mean cIMT (mm)		-0.02 (0.15)	-0.01 (0.11)	0.753
Plaque Score				
	0	1.4 (1)	2.2 (18)	
	1	0.0 (0)	3.2 (26)	
	2	8.5 (6)	14.9 (121)	
	3	9.9 (7)	16.9 (137)	
	4	22.5 (16)	18.2 (148)	0.116
	5	16.9 (12)	16.6 (135)	
	6	15.5 (11)	13.8 (112)	
	7	12.7 (9)	6.4 (52)	
	8	12.7 (9)	7.6 (62)	
Plaque score ≤ 4		42.3 (30)	55.5 (450)	0.022*
Plaque Score >4		57.7 (41)	44.5 (361)	
Plaque Morphology				
	Echogenic/no plaque	35.1 (26)	44.6 (375)	
	Echolucent only	9.5 (7)	12.5 (105)	0.008**
	Heterogenous only	14.9 (11)	20.1 (169)	
	Both types	40.5 (30)	22.7 (191)	
High risk plaque (yes)		64.9 (48)	55.4 (465)	0.072

Variables are reported as mean (SD) or %,n P values determined by students T test, ANOVA or chi square

6.3.2 Cox regression models

In order to explore further the relationship between cIMT and incident cardiovascular disease in people with Type 2 diabetes, Cox proportional hazards models were

employed. Three models were created – Model A: unadjusted, Model B: age, sex and previous CVD adjusted and Model C: age, sex, previous CVD and UKPDS risk factor adjusted. By adjusting for these risk factors, it is possible to comment on the potential to use cIMT or carotid plaque over and above these risk factors in cardiovascular risk prediction. Hazard ratios for each of the models are presented in tables 6-4, 6-5 and 6-6.

Incident cardiovascular disease

Survival modelling for any cardiovascular disease revealed the most interesting results. All four cIMT measures were predictive of incident cardiovascular disease. In unadjusted models, for a 1-SD increase in both mean cIMT and mean maximum cIMT there was a 1.4 fold increase in risk for any cardiovascular disease, while maximum cIMT and mean maximum cIMT had a slightly lower risk (1.30 and 1.33 respectively). Further adjustment for age, sex and previous CVD caused maximum cIMT and maximum mean cIMT to lose statistical significance in the model. Mean cIMT and mean maximum cIMT, however, survived this additional adjustment, albeit with a modest reduction in risk (HR 1.29 (1.03-1.61) and 1.28 (1.01-1.60) respectively). Full adjustment for UKPDS risk factors caused mean maximum cIMT to lose significance in the model but mean cIMT survived full adjustment with a HR of 1.26 (1.00-1.58), p=0.046.

In unadjusted models, a 1-SD increase in both mean plaque thickness and maximum plaque thickness corresponded to a 1.26 fold increase in risk for incident vascular disease, however both measures lost significance once age, sex and previous CVD were adjusted for. Plaque score showed the same pattern of association as plaque thickness in the models, while plaque morphology did not achieve any significance in even the unadjusted model.

Incident coronary artery disease

In unadjusted models, all measures of cIMT were predictive of incident coronary artery disease. For a 1-SD increase in mean cIMT and mean maximum cIMT, there was a 1.5 fold increase in risk for coronary artery disease, while estimates for maximum cIMT and maximum mean cIMT were slightly lower with HR 1.36 and

1.37 respectively. Adjustment for age, sex and previous CAD attenuated the risk modestly for all measures of cIMT and at this point, max cIMT and max mean cIMT fell out of significance in the models. Both mean cIMT and mean max cIMT however, survived further adjustment for the risk factors included in the UKPDS risk score (HR 1.49 for both measures).

Mean plaque thickness and maximum plaque thickness were entered first into the unadjusted Cox model but failed to achieve statistical significance. This was also the case for plaque score and plaque morphology.

Incident cerebrovascular disease

In unadjusted models for incident cerebrovascular disease, only plaque morphology demonstrated a significant association, which survived adjustment for age, sex and previous cerebrovascular disease. However, full adjustment for the UKPDS risk factors caused the hazard ratios to lose statistical significance.

Incident fatal cardiovascular disease

On entry to the unadjusted model for fatal CVD, none of the carotid ultrasound markers achieved a statistical association with risk. This is likely due to the low number of events in this group.

Table 6-4 Cox regression models for incident cardiovascular events and cIMT

		Mean cIMT (mm)			Max cIMT (mm)			Max Mean cIMT (mm)			Mean Max cIMT (mm)		
	Events (n)	HR	95% CI	P value	HR	95% CI	P value	HR	95% CI	P value	HR	95% CI	P value
Any CVD Event	*74*												
Model A		1.41	1.14-1.74	0.001**	1.30	1.06-1.60	0.013*	1.33	1.08-1.63	0.006**	1.41	1.13-1.75	0.002**
Model B		1.29	1.03-1.61	0.029*	1.18	0.95-1.47	0.141	1.22	0.98-1.51	0.074	1.28	1.01-1.60	0.038*
Model C		1.26	1.00-1.58	0.046*	1.16	0.93-1.45	0.181	1.20	0.96-1.49	0.108	1.25	0.99-1.57	0.062
CAD Events	*36*												
Model A		1.52	1.12-2.04	0.006**	1.36	1.01-1.82	0.041*	1.37	1.02-1.83	0.034*	1.53	1.13-2.07	0.007**
Model B		1.47	1.08-2.01	0.015*	1.31	0.96-1.79	0.087	1.33	0.98-1.80	0.070	1.48	1.09-2.04	0.016*
Model C		1.49	1.08-2.06	0.016*	-	-	-	-	-	-	1.49	1.07-2.07	0.017*
Cerebrovascular Events	*27*												
Model A		1.38	0.97-1.97	0.070	1.29	0.92-1.82	0.145	1.31	0.94-1.84	0.117	1.39	0.97-1.99	0.079
Model B		-	-	-				-	-	-			
Model C		-	-	-				-	-	-			
Fatal Events	*16*												
Model A		1.09	0.68-1.76	0.724	1.06	0.66-1.71	0.802	1.12	0.70-1.78	0.647	1.06	0.64-1.73	0.833
Model B		-	-	-	-	-	-	-	-	-	-	-	-
Model C		-	-	-									

Model A Unadjusted
Model B Age, Sex and Previous corresponding vascular events adjusted (except fatal events – previous any CVD used)
Model C Age, Sex, Previous CVD and additional UKPDS risk factor adjusted (duration diabetes, HbA1c, systolic BP, cholesterol, HDL cholesterol, smoking)
HR for 1SD change in each variable

Table 6-5 Cox regression models for incident cardiovascular events and carotid plaque thickness

		Mean Plaque Thickness (mm)			Maximum Plaque Thickness (mm)		
	Number of Events	HR	95% CI	P value	HR	95% CI	P value
Any CVD Event	*74*						
Model A		1.26	1.03-1.55	0.025*	1.26	1.02-1.54	0.029*
Model B		1.10	0.88-1.38	0.403	1.10	0.88-1.38	0385
Model C		-	-	-	-	-	-
CAD Events	*36*						
Model A		1.12	0.82-1.54	0.462	1.13	0.83-1.54	0.434
Model B		-	-	-	-	-	-
Model C		-	-	-	-	-	-
Cerebrovascular Events	*26*						
Model A		1.37	0.98-1.91	0.069	1.35	0.97-1.89	0.076
Model B		-	-	-			
Model C		-	-	-			
Fatal Events	*16*						
Model A		1.33	0.86-2.04	0.201	1.32	0.86-2.03	0.202
Model B		-	-	-	-	-	-
Model C							

Model A Unadjusted
Model B Age, Sex and Previous corresponding vascular events adjusted (except fatal events – previous any CVD used)
Model C Age, Sex, Previous CVD and additional UKPDS risk factor adjusted (duration diabetes, HbA1c, systolic BP, cholesterol, HDL cholesterol, smoking)
HR for 1SD change in each variable

Table 6-6 Cox regression models for incident cardiovascular events and carotid plaque score and morphology

	Plaque Score >4				Plaque Morphology Dichotomous			
	Number of Events	HR	95% CI	P value	Number of Events	HR	95% CI	P value
Any CVD Event	*75*				75			
Model A		1.77	1.12-2.81	0.015*		1.40	0.87-2.24	0.161
Model B		1.44	0.90-2.31	0.129		-	-	-
Model C		-	-	-		-	-	-
CAD Events	*36*				36			
Model A		1.75	0.90-3.40	0.0.098		0.89	0.46-1.72	0.731
Model B		-	-	-		-	-	-
Model C		-	-	-		-	-	-
Cerebrovascular Events	*27*				27			
Model A		1.82	0.85-3.93	0.126		2.76	1.11-6.85	**0.029***
Model B		-	-	-		2.53	1.02-6.30	**0.046***
Model C		-	-	-		2.28	0.91-5.71	0.079
Fatal Events	*16*				16			
Model A		1.24	0.464-3.30	0.670		1.31	0.47-3.60	0.605
Model B		-	-	-		-	-	-
Model C		-	-	-		-	-	-

Model A Unadjusted
Model B Age, Sex and Previous corresponding vascular events adjusted (except fatal events – previous any CVD used)
Model C Age, Sex, Previous CVD and additional UKPDS risk factor adjusted (duration diabetes, HbA1c, systolic BP, cholesterol, HDL cholesterol, smoking)
HR are quoted for the plaque score>4 versus plaque score less than 4, and for high risk plaque versus low risk plaque

6.3.3 Assessment of model

Mean cIMT survived adjustment for UKPDS risk factors in the prediction of global CVD and CAD, and as did mean maximum cIMT for CAD. In order to assess the impact of cIMT on risk classification, only the models for mean cIMT were explored, for two reasons. Firstly, only mean cIMT was chosen because the hazard ratios produced for both mean cIMT and mean maximum cIMT were almost identical in the models and so to avoid repetition, only mean cIMT was analysed. And secondly, the number of events in the CAD model was almost half that of the global CVD model and because reclassification is calculated in events and non-events, the low number of events in the CAD group may not provide a meaningful measure of change.

The model containing both cIMT and UKPDS risk factors was compared with the model containing UKPDS risk factors only, in order to explore any improvement in risk prediction brought by cIMT. Area under the ROC curve was initially used to assess model improvement upon addition of cIMT, after which net reclassification index (NRI) was determined to assess the reclassification of predicted risk.

ROC curve analysis

ROC curves were created using the predicted risk (X beta) provided by the Cox regression model in section 6.3.2 (figure 6-1). Area under the curve was compared for the model containing UKPDS risk factors alone and the model containing both risk factors and mean cIMT (table 6-7). The area under the curve for the model containing only traditional risk factors was found to be 0.691 (85% CI 0.629-0.753). When cIMT was added to the model, there was a small improvement in AUC to 0.704 (95% CI 0.645-0.763), suggesting that cIMT may contribute additional, albeit modest, information to current risk classification models.

Figure 6-1 ROC curves of predicted risk in global CVD model containing risk factors only and model containing mean cIMT and traditional risk factors

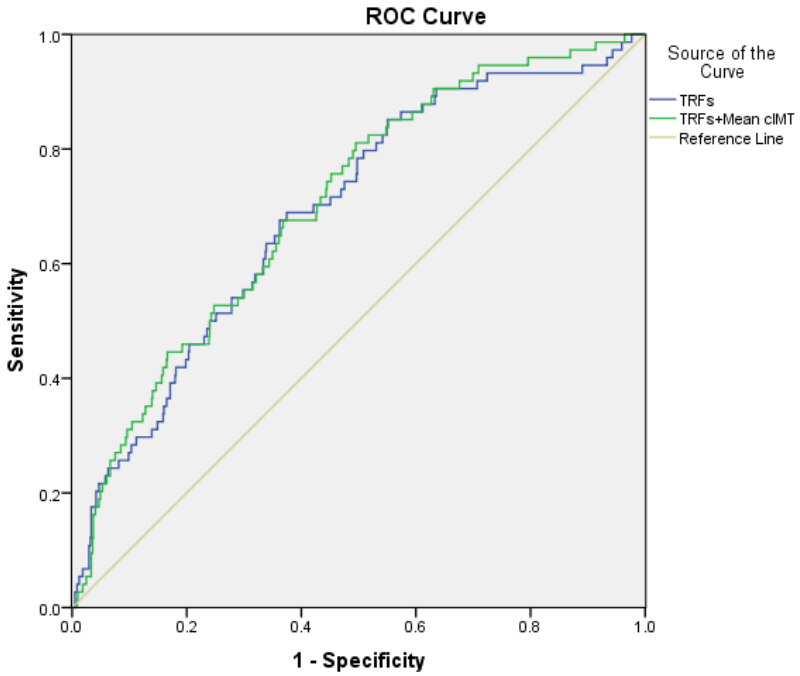
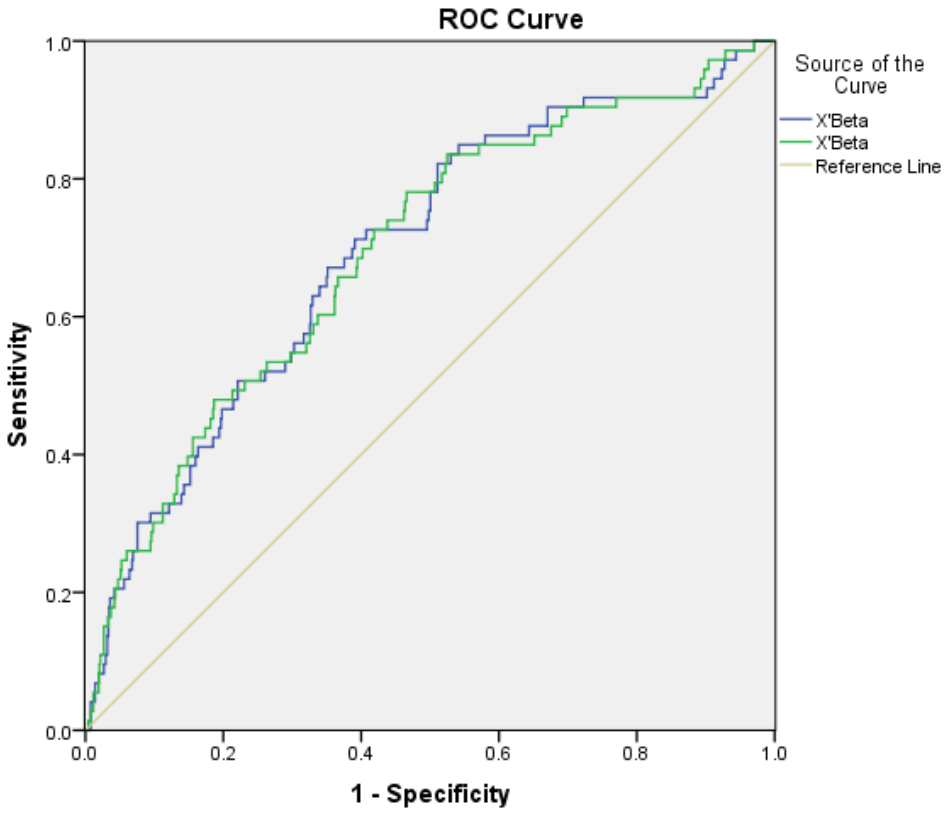


Table 6-7 AUC for models containing risk factors only, and cIMT in addition to risk factors

		Area Under the Curve	95% Confidence Interval	P value
Global CVD	Model 1 TRF	0.691	0.629 - 0.753	<0.001
	Model 2 TRF + mean cIMT	0.704	0.645 – 0.763	<0.001

TRF=traditional risk factors

Figure 6-2 ROC curves of predicted risk in global CVD model containing risk factors only and model containing mean plaque thickness and risk factors



When the ROC curve for mean plaque thickness versus traditional risk factors was plotted, the AUC was 0.695 for both he TRF model only and the TRF and plaque model, confirming what was demonstrated in the Cox regression – that adding plaque to traditional risk factors does not improve prediction of cardiovascular events.

Net Reclassification Index

To explore the effect of adding cIMT to the reclassification of risk category for global CVD, net reclassification index was determined for the addition of mean cIMT to each model (table 6-8). Risk was calculated from arbitrary tertiles of predicted risk produced by the models and categorized at low (1st tertile), intermediate and high risk (3rd tertile) (de Ruijter W, Westendorp RGJ et al. 2009). For global CVD, in the event group, 5 individuals had their risk category reclassified upwards, and 5 were reclassified down. In the non-event group, 77 individuals had

their risk category reclassified upwards and 81 were reclassified down, producing a net reclassification index of 0.25%.

Table 6-8 Reclassification of incident cardiovascular events when cIMT is added to the model containing cardiovascular risk factors

			Model with TRF and cIMT		
			Low	Intermediate	High
Event Group	Model with TRF	Low	5	2	0
		Intermediate	2	21	3
		High	0	3	38
			Model with TRF and cIMT		
			Low	Intermediate	High
Non-Event Group	Model with TRF	Low	248	38	2
		Intermediate	40	191	39
		High	0	41	213

TRF=traditional risk factors

6.4 Chapter summary

This chapter aimed to explore the relationship between cIMT, carotid plaque and incident cardiovascular disease in older adults with Type 2 diabetes. Incident cardiovascular events in the study population were associated with increased age, male sex, longer duration of diabetes, increased HbA1c and reduced eGFR, as well as a previous history of cardiovascular disease. Those individuals who experienced an incident event had significantly higher cIMT and plaque thickness, as well as a higher plaque score and a tendency to have high risk plaque present in the carotid arteries.

In unadjusted Cox regression models for global cardiovascular disease, both cIMT and carotid plaque (thickness, score and morphology) were predictive of incident events; however only mean cIMT survived full adjustment for cardiovascular risk factors used in the UKPDS cardiovascular risk score. Mean cIMT and mean maximum cIMT were also predictive of incident CAD. No measures of cIMT or

plaque were predictive of cerebrovascular disease once traditional risk factors were adjusted for.

Further analysis of the global CVD model revealed that while there was an increase in the area under the curve when cIMT was added to the model containing traditional cardiovascular risk factors, there was only a small improvement in risk classification. Therefore, the usefulness of cIMT in predicting cardiovascular events over and above traditional risk factors may only be limited.

Chapter 7: Discussion

Introduction

Year on year, cardiovascular disease continues to be the number one cause of death globally. Identifying individuals at high risk of cardiovascular events is particularly important for the targeting of preventive therapies, even in high risk individuals such as people with Type 2 diabetes. While cardiovascular risk scores based on conventional cardiovascular risk factors are the primary means by which cardiovascular risk is estimated in the general population, exploration of more novel factors, such as cIMT and carotid plaque, as markers of cardiovascular risk has received considerable attention, although there is still no global consensus as to their use in predicting risk in this group. The evidence for the use of cIMT in individuals with Type 2 diabetes is particularly sparse and has been identified as an important area for exploration by the recent USPSTF statement (United States Preventative Services Task Force 2009). In addition, the use of cIMT as a surrogate end point in clinical trials of drugs used in the treatment of diabetes are based primarily on evidence from the general population of the association between cIMT and cardiovascular risk. There is little evidence from studies of people with diabetes to directly support this use.

This thesis aimed to explore the relationship of carotid intima media thickness and carotid plaque with cardiovascular disease in older adults with Type 2 diabetes, using data from the Edinburgh Type 2 Diabetes Study cohort. Both cross sectional and longitudinal relationships of carotid intima media thickness and carotid plaque with cardiovascular disease outcomes were investigated using a variety of statistical methods. In addition, an exploration of measurement methods for cIMT was also undertaken. The current chapter discusses the results of these analyses in the context of current research and describes potential areas for future research. Methodological strengths and limitations of the thesis are also discussed.

7.1 Summary of main findings

In order to meet the primary aim of this thesis (to explore the association of cIMT and carotid plaque with cardiovascular risk in older people with Type 2 diabetes), a series of objectives was addressed. The first objective was to describe the frequency and distribution of cIMT and carotid plaque in older adults with Type 2 diabetes. Participants in the ET2DS were generally representative of older adults with Type 2 diabetes living in Edinburgh and the Lothians and those who attended for year 1 cIMT measurements were largely representative of the cohort as a whole. A validation study revealed that cIMT measurement in the study was highly repeatable and was comparable with computer aided measurements. cIMT was normally distributed in the sample and was higher in men, as well as older participants. cIMT was also higher in the left carotid artery than the right. There was a high prevalence of carotid plaque in the ET2DS, with at least 1 plaque present in approximately 97% of the study population. Continuous measurements of cIMT were correlated with one another but less so with measures of plaque thickness. Similarly, measures of plaque thickness correlated with one another. Surprisingly, the change in mean cIMT was negative between year 1 and year 4, suggesting there was an improvement in cIMT over the follow up period.

The second objective of this thesis was to describe the cross sectional relationship of cIMT and carotid plaque with vascular risk factors (both traditional and novel), as well as the relationship of cIMT and carotid plaque with prevalent vascular disease. Increased cIMT was predominantly associated with older age, male sex and higher systolic blood pressure and these associations persisted following multivariable linear regression, suggesting that these factors are independently associated with cIMT. In addition, increased cIMT was associated with adverse levels of the novel biomarkers ABI and NTproBNP following multivariable regression. However, it should be noted that the small effect sizes demonstrated were small, which may suggest that these were not strong relationships between cIMT and cardiovascular risk factors.

Increased plaque thickness demonstrated more extensive independent associations with traditional vascular risk factors than cIMT, namely older age, male sex, longer duration of diabetes, higher systolic blood pressure, and cigarette smoking. Plaque thickness was also positively associated with ABI and NTproBNP following multivariable adjustment. Again, in some cases, the effect size was noted to be small and may suggest only a weak association between plaque and risk factors. Plaque score was independently associated with increasing age, raised systolic and reduced diastolic blood pressure, smoking and lower BMI and was independently associated with reduced ABI and increased NTproBNP. Plaque morphology was independently associated with raised systolic blood pressure, lower diastolic blood pressure and low BMI, as well as ABI and NTproBNP.

There was a high prevalence of vascular disease (MI, angina, TIA, stroke) in the ET2DS at year 1 and the prevalence of vascular disease was associated with increased values of both cIMT and carotid plaque. Age and sex adjusted logistic regression models revealed that all measures of cIMT and plaque were associated with prevalent cardiovascular disease. However, for cIMT, these relationships lost statistical significance after adjustment for traditional cardiovascular risk factors, whereas for mean and maximum plaque thickness, they persisted following full adjustment including both traditional risk factors and novel biomarkers. Plaque score and plaque morphology remained associated with CVD and CAD following full adjustment. These results raise the possibility that carotid plaque may have more potential to predict incident cardiovascular events than cIMT.

Building on the findings of the cross sectional analysis, the third objective, addressed in chapter 6, was to explore the relationship of cIMT and carotid plaque and incident cardiovascular disease. Incident cardiovascular events in the study population were associated with older age, male sex, longer duration of diabetes, higher HbA1c and lower eGFR, as well as a previous history of cardiovascular disease. Those individuals who experienced an incident event had significantly higher cIMT and plaque thickness, as well as a higher plaque score and a tendency to have high risk (echolucent or heterogeneous plaque) present in the carotid arteries. In unadjusted cox regression models, both cIMT and carotid plaque measures were predictive of

incident cardiovascular events. However, only the association with mean cIMT survived full adjustment for cardiovascular risk factors used in the UKPDS cardiovascular risk score. Only mean cIMT and mean maximum cIMT were predictive of CAD after multifactorial analysis but neither cIMT nor plaque global was predictive of cerebrovascular disease. Further analysis of the model for any cardiovascular disease and mean cIMT revealed that while there was a small increase in the area under the curve for the model containing mean cIMT, there was a negligible improvement in risk classification, suggesting that while cIMT may be an independent predictor of cardiovascular risk and carotid plaque may not, the ultimate usefulness of cIMT in predicting cardiovascular events over and above traditional vascular risk factors may be limited.

7.2 Strengths of the ET2DS

The Edinburgh Type 2 Diabetes study is a longitudinal cohort study of individuals with Type 2 diabetes living in Edinburgh and the Lothians. This study design, with a representative risk population, is ideally suited to the evaluation of potential risk prediction markers (Hlatky MA, Greenland P et al. 2009). The study also possesses other factors highlighted by an American Heart Association Scientific Statement (Hlatky MA, Greenland P et al. 2009) as important in the evaluation of novel risk markers, including an accurate definition and ascertainment of outcome events, as well as robust reporting. Hlatky et al highlight the "Strengthening the Reporting of Observational Studies in Epidemiology" (STROBE) guidelines (von Elm E, Altman DG et al. 2007; Hlatky MA, Greenland P et al. 2009) as the standard to which studies should be reported. The reporting of the analyses presented in this thesis complies with the key areas in the STROBE guidelines.

Bias, Chance and Confounding

If one considers the key epidemiological concepts of bias, chance and confounding, the design of the ET2DS goes a long way to addressing each of these. Bias was minimised by having robust data collection protocols, including SOPs for the clinics,

which were adhered to by a small team of dedicated study staff. Generalizability was addressed by using a well-structured, random sampling frame which recruited a representative sample of individuals from the population of older people with Type 2 diabetes living in Edinburgh and the Lothians. Chance was addressed by the use of robust statistical testing. Finally, the wide ranging data collection performed in the study produced a well phenotyped cohort, allowing researchers to adequately address many possible sources of confounding through multiple risk factor adjustment in statistical models.

7.2.1 Study recruitment, response and representativeness

Following recruitment and attendance of 1066 participants at baseline, 939 participants returned to follow up at 1 year. While there was a range of reasons for non-attendance at this time point, for many of which little could be done (e.g. death, inability to complete examination), the majority of reasons given by participants were related to inability or unwillingness to attend an appointment. Vigorous attempts were made to encourage attendance by providing a choice of appointment time, as well as providing transport to and from the clinic if necessary. In addition, a further appointment was offered in the case of non-attendance or illness. Travel expenses were also offered on a case by case basis. While this took considerable time and effort, it did result in 88.0% of the baseline participants returning for follow-up at year 1.

Because of the time that had elapsed between year 1 and year 4 follow up, several attempts were made, prior to invitation to clinics, to determine current contact details for all ET2DS participants who were not already coded as withdrawn from the study, in order to maximize participation in the follow up study. An initial strategy of posting a study newsletter to the last known address for each participant managed to capture up-to-date contact details for the majority of participants and where it was not possible after further exploration to identify a current address (or in the absence of a notification of the participants death), it was accepted that these individuals had been lost to follow up (n=15). In order to further encourage as high an attendance as possible, a similar vigorous strategy for appointment making to that used at year 1

was adopted. This, combined with the robust approach to obtaining contact details, encouraged 77.9% of the original cohort to participate in year 4 follow up. Despite the reduction in the size of the cohort attending year 4, the follow up of incident events remained nearly complete for the full cohort due to ISD data linkage which allows for follow up even without clinic attendance. This is similar to follow up rates demonstrated by other studies of individuals with diabetes (Wang, Van Belle et al. 2004; Kramer CK, Muhlen D von et al. 2010).

In comparison to some other larger collaborative studies of cIMT in people with diabetes eg USE IMT (Den Ruijter HM, Peters SA et al. 2012) or large single cohort studies such as the Framingham Study in which subpopulations of people with diabetes are identified from within the larger study population, the population of the ET2DS was recruited directly from a representative sampling frame of all individuals in Edinburgh with Type 2 diabetes, in order to examine specific outcomes in individuals with diabetes. All participants were subject to the same entry criteria at recruitment, and measurement of cIMT and plaque was made using the same SOP and ultrasonographer, in comparison to larger studies that have drawn participants from multiple studies using different radiological SOPs and using different criteria to define diabetes (Den Ruijter, Peters et al. 2013). This will have gone some way to addressing the effect that these sources of variability will have exerted on the results of larger studies.

Representativeness

The ET2DS is a cohort of older adults with Type 2 diabetes living in Edinburgh and the Lothians. The representativeness analyses are presented in chapter 4 of this thesis and have been published previously (Marioni, Strachan et al. 2010). The variables used to compare the two groups were limited to relevant demographic and cardiometabolic variables available on the Lothian Diabetes Register in order to allow comparison between attenders and non-attenders. The analysis demonstrates that at baseline, those who agreed to participate in the ET2DS were broadly similar to those who did not participate, suggesting that the study sample is largely representative of the invited target population, although slightly more men participated in the study than did not, and individuals from the least deprived quintile

of SIMD were slightly overrepresented in the participant group, while those from more deprived quintiles slightly underrepresented. This suggests that those from the least deprived quintile may have been more likely to agree to take part than those from more deprived quintiles.

When this analysis was extended to compare baseline demographic and cardiometabolic characteristics of those that attended baseline, year 1 and year 4 follow up, a more extensive panel of variables was selected from the baseline data collection, as these would be available for all participants and allow for a fuller comparison. This was important to assess given the loss to follow up over the course of the study and the risk of introducing bias as a result. It was found that there were very few differences in baseline characteristics between those who attended each follow up, suggesting that at each wave of follow up, participants were broadly representative of the target population, and of each other. A slightly higher proportion of the follow up cohorts were non-smokers at baseline compared with the original baseline cohort. One explanation for this finding is the survivor effect (Arrighi HM and Hertz-Picciotto I 1994), whereby smokers might be more likely to have co-morbidities that could prevent attendance at follow up clinics, whereas non-smokers are more likely to suffer less ill health and consequently be available to return for follow up. The overall effect of the difference however is likely to be small given the small absolute difference in smoking prevalence between the groups.

Because not all participants in the study underwent cIMT measurement, additional comparisons were made of baseline variables in those who had valid cIMT measurements at year 1 and those who did not. There were no significant differences in major vascular risk factors, with the exception of smoking status, whereby those who did not have valid cIMT measurements were more likely to be smokers than those who did have cIMT measurements. One explanation for this may have been that it was perhaps more technically challenging to identify the carotid arteries in smokers or again, that the smokers may have had co-morbidities preventing re-attendance. As several of those without valid cIMT did in fact have measurements that were made, but that were made incorrectly and were therefore unrelated to the

individuals concerned, it is possible that the differences in smoking may be related purely to chance.

7.2.2 Completeness and accuracy of data collection

A key aspect of a longitudinal or indeed, any epidemiological study, is complete, accurate data collection. In particular for longitudinal studies, complete follow up data for identification of incident events is often the more difficult but most important data to obtain. This section discusses the completeness and accuracy of data collection at year 1 and year 4 of the ET2DS. Overall, missing data in the analysis population chosen for this thesis was low (<2%) for all variables with the exception of ACR, and some plaque measurements.

Self-completed questionnaires

In most cases at both year 1 and year 4, participants completed the questionnaire prior to attending the clinics. In cases where the questionnaire was not completed, assistance was given to participants by study staff at the clinics. For the small number of participants who were not withdrawn from the study but did not attend clinics, questionnaires were posted out either to individuals or to their GPs for completion. This strategy allowed for a high rate of completion of the questionnaires and maximized data completeness which was especially important for the determination of incident cardiovascular events.

Blood Sampling at Baseline and Year 1

Individuals in whom it was not possible to obtain a blood sample on the day of clinic attendance were invited to return for repeat venipuncture. Whilst some samples were processed by the NHS Lothian haematology and biochemistry labs, other samples were processed and stored by study staff for use at a later date (eg NTproBNP, inflammatory markers). Extensive training for study staff in the processing and storage of remaining blood samples encouraged a high rate of sample viability. Circumstances out with the control of the study included loss or damage of samples in transit or storage, or during processing in external labs.

Clinical Examination at Year 1

The data used from the clinical examination at year 1 was the systolic and diastolic blood pressure, which was obtained as part of the measurement of pulse wave analysis. Measurements were performed by only one examiner, which eliminated the potential effect of inter-observer variability in the measurements although no official assessment of this was made.

cIMT and Plaque Measurements

Carotid ultrasound scans were performed on each participant that attended the clinics at both year 1 and year 4, unless there was a medical reason for not performing the scan. A thorough scan was performed and every attempt made to visualize the carotid arteries, within the limits of the ultrasound SOP (appendix D) and recommended clinical guidelines. Nearly 100% of participants were scanned at year 1; however, on later visual inspection of individual scan results that were identified as outlying values, 23 individuals had their measurements excluded from the final dataset. In the case of readings that were lower than expected, this was mostly due to incorrect caliper placement. In the case of measurements that were higher than might be biologically plausible, incorrect readings were usually made in areas with plaque present. As these problems contradicted the SOP for measurement of cIMT and plaque in the study, these measurements were excluded from the dataset.

7.2.3 Cardiovascular Event Follow Up

In order to provide conditions essential for a robust analysis of the association between cIMT and cardiovascular disease in the ET2DS, complete follow up for incident cardiovascular events was necessary. In order to maximize the capture of events and reduce misdiagnosis, several sources were used to identify and corroborate potential cases (self-completed questionnaires, ECGs and ISD data linkage). A formal event identification pathway was created in order to ensure impartiality and uniformity for each case and in cases were a diagnosis was ambiguous or unclear, decisions were agreed upon by a panel of clinically qualified member of the study team. These methods resulted in a high degree of success in identifying potential events. ISD linkage data was analyzed in the first instance, as this captures all hospital in patient stays in Edinburgh and the Lothians between

prescribed dates. Patient self-reports and ECGs were then searched for potential events and the results combined with the data linkage.

7.2.4 IMT measurement methods

In order to minimize recording bias in cIMT and plaque measurements, all measurements were performed by only one sonographer, which eliminated inter-observer variability in the study. Nonetheless, it was important to assess the intra-observer variability of the measurements made in the ET2DS to ensure confidence in the readings. The validation study performed on year 4 measurements confirmed a high correlation between repeat measures of cIMT, suggesting that there was a good degree of repeatability in the measurements, which in turn increases confidence in accuracy of the cIMT measurements. Although only the year 1 rather than the year 4 cIMT measurements were used for the main analyses of this thesis, the sonographer at year 1 and year 4 was the same, suggesting that the accuracy of the readings at year 4 can be taken as a marker that the year 1 readings were performed to the same standard, although this must also be taken into consideration in the interpretation of results.

Alternative method for measuring cIMT

Limitations in the ultrasound technology and type of images saved at year 1 of the ET2DS prohibited the use of fully automated edge detection systems. These have been recommended by consensus statements on the measurement of cIMT (Stein JH, Korcarz CE et al. 2008; Touboul PJ, Hennerici MG et al. 2012) in which the use of edge detection systems is suggested to improve measurement accuracy and to reduce variability in cIMT measurements both within and between studies by reducing the bias introduced by using different sonographers and SOPs (human error), as well as a reduction in cost and time required to perform measurement. However, in an attempt to explore the effect of more extensive and perhaps accurate measurement of cIMT in the ET2DS, a partially-automated programme designed to take multiple measurements of cIMT along a user-defined section of cIMT was implemented. The advantages of such a programme over that of the ultrasonographer's measurements lies in the ability to take multiple measurements over a longer segment of the arterial wall, which may capture a more comprehensive measure of the cIMT of the vessel

wall and thus provide a measure which more truly reflects the relationship between cIMT and vascular risk. The major limitation of this approach lies in the continued dependence upon user identification (human error) of the artery wall interfaces. An additional limitation is the added time to process the images after the initial data collection. However, as this was to be considered an exploratory analysis only, it was felt that these limitations could be accepted.

Each individual who underwent cIMT measurement at year 1 by the study sonographer had a maximum of 6 (3 right and 3 left) carotid images stored. Due to time limitations, a sample of 233 individuals was processed using the semi-automated software. These images were processed by myself and two other study team members, identifying the area to measure allowing for multiple measurements to be taken along the length of wall identified. The distance between measurements was arbitrarily set as 10 pixels and the line of best fit was every 50 pixels. The minimum number of measurements made in any image was 10 and the maximum was 60. The mean number of measurements for any given individual was 192 measurements, which is considerably more extensive than the 6 measurements made by the sonographer.

A summary statistic of the serial cIMT measurements was calculated as the mean of all the measurements made for an individual (mean serial cIMT). While mean serial cIMT was statistically significantly lower than mean sonographer cIMT in the sample (0.91vs0.92mm, $p<0.001$), the correlations between mean serial cIMT and mean cIMT were strong ($r=0.811$). This, in combination with the small absolute difference between the measures (0.01mm) suggests that multiple measures along the vessel wall may not improve on sonographer cIMT measurement in the ET2DS. The ultimate method of testing whether taking more measurements of cIMT provides a more representative measure of cIMT in the ET2DS would be to examine the association of both measures of cIMT with risk factors. However, the small sample size of individuals with serial cIMT measurements in the ET2DS prohibits this at the time of writing, as the true direction and strength of any relationships may not be captured.

The serial, computer aided measurements made in this thesis are still considered as "manual" measurements, as the method by which the actual IMT boundaries are identified is by manual tracing by the reader. Several studies have compared similar "manual" measures with edge detection methods. A recent study by Peters et al compared manual and semi-automated measurements of cIMT of the far wall of the common carotid artery in the METEOR study (Peters SA, den Ruijter HM et al. 2011). They identified that the measurements made by each method were comparable. The authors reported that the automated measurements were lower than the "manual" measurements, with an absolute difference of 0.02mm between the manual and automated readings (for both treatment and control groups). In addition, they found the same associations with risk factors for each method. The MESA investigators also compared edge detected and manual measures of cIMT, although they report that edge detected measures were greater than manual readings, which contrasts with the findings of both the METEOR study and this thesis. However, they did find similar (although slightly weaker) associations between edge detected cIMT and risk factors as they found between manual measurements and risk factors. Taking the results of this thesis in the context of the results of these studies, they could suggest that the serial measures made in the ET2DS might be comparable with those that could be obtained by edge detection. Therefore, by extension, because of the good correlation between the serial measurements and the sonographer measurements, they may suggest that the sonographer measurements provide as good a measure of cIMT as might be obtained with edge detection methods. However, this conclusion does constitute quite a leap and cannot be considered a definite statement, but more a hypothesis.

Recommendations for future research would include the assessment of the full ET2DS cohort using the manual serial method to allow for a more meaningful comparison between the two methods.

Summarising cIMT and carotid plaque

In general, the method by which cIMT is summarised and reported in studies has not been formalised. Studies often report a variety of summaries of IMT and do not always create the variables in the same way. The way in which IMT is measured on

ultrasound plays a large part in how IMT can be summarised. Measurement of cIMT in the common carotid artery leads to less flexibility in the summary statistics than if IMT is measured in the internal carotid, external carotid and bifurcation also.

The Mannheim consensus provides brief guidance on the summarisation of cIMT measures. The options they discuss include the mean, maximum and composite measures from both sides and different arterial sites (CCA, bifurcation and ICA). They highlight that the mean IMT values are less susceptible to outliers. However the maximal IMT may reflect more advanced thickening, although care must be exercised with maximum cIMT as it may be more susceptible to measurement error. They also suggest that the values from both the right and left can be averaged, although it should be noted that the left cIMT is often higher than that of the right (Foerch, Buehler et al. 2003) and it may be that averaging the values could attenuate the associations between cIMT and vascular risk . They also suggest avoiding composite scores including both plaque and cIMT. In terms of plaque measurement they recommend the measurement of plaque location, thickness, area and number, scanned in longitudinal and cross-sections must be recorded. The ET2DS was able to record plaque presence, location (summarised as plaque score), thickness and morphology.

The cIMT parameters used in this thesis showed a high degree of inter-correlation (range of r=0.911-0.977). This may suggest that the choice of cIMT summary in future analyses could be flexible. The association of each cIMT measure with cardiovascular risk factors was examined to further explore this relationship. The multifactorial adjusted associations between the individual summary statistics and cardiovascular risk factors were broadly similar (age, sex, systolic and diastolic blood pressure). The same was seen for the novel markers ABI and NTproBNP. Whether or not this impacts upon the choice of summary statistic remains open for discussion. The strong overall correlation between the measures, and broad similarities in association with risk factors, add weight to the suggestion that none should be used in preference to the others. However, further brief consideration of the findings from the prospective analysis of cIMT and incident vascular disease found that the mean cIMT was most closely associated with future vascular risk.

When one considers carotid plaque thickness measures, there was a high degree of correlation between mean plaque thickness and maximum plaque thickness (r=0.959) and the relationship between mean and maximum plaque thickness and cardiovascular risk factors was identical, suggesting that again , neither need be used in preference to the other. Plaque score and plaque morphology were also associated with similar risk factors to plaque thickness and were also associated with prevalent vascular disease. However, given the negative findings of the association of carotid plaque with incident vascular disease, it is difficult to come to a firm conclusion of which measure of plaque thickness is best to use. Several consensus statements highlight that the evidence for which measure of plaque to use in risk prediction is not clear and indeed, further evidence is required before any firm recommendations can be made regarding these measures (Touboul PJ, Hennerici MG et al. 2012).

7.3 Study Limitations

One of the major limitations of the study is that cIMT was measured 1 year after the baseline risk factors, which may have attenuated the strength of the relationship between the cIMT parameters and measured vascular risk factors. The reason underlying the collection of cIMT at year 1 rather than baseline was funding considerations. Assessment of cohort representativeness found that the cohort at baseline and year 1 were very similar when baseline risk factors were compared. The time discrepancy was addressed by selecting variables from year 1 where possible and where variables were not available, substituting baseline variables. Whilst this was not ideal, all steps possible were taken to minimise the number of variables that were substituted. For some variables, the 1 year difference may only have had a small impact on the magnitude of any associations eg BMI, which may be expected to change only a small amount over the course of the year. In the ET2DS, mean BMI at baseline was 31.3, while at year 4, was 31.4, suggesting that mean BMI remained fairly stable across the course of the study follow-up. In addition, smoking variables were taken from baseline. It is unlikely (although not impossible) that

individuals in the study would have taken up smoking during the study, and so any change in smoking habits between baseline and year 1 is likely to have been a decline in current smokers and an increase in ex-smokers. The effect of this would be an underestimation of the association between carotid ultrasound parameters and smoking. Indeed, when smoking rates in year 1 and year 4 are assessed, there were fewer people reporting current smoking at year 4 than at year 1 (14.0% vs 9.4%).

Carotid ultrasound in the study did not provide as extensive a set of measures as some other studies of cIMT (O'Leary DH, Polak JF et al. 1999; Iglesias del Sol A, Moons KG et al. 2001; Iglesias del Sol A, Bots ML et al. 2002). cIMT was measured only in the CCA, with 6 measures taken in total. Other studies of cIMT have measured in several areas including CCA, internal carotids and the bifurcation and included a greater number of measurements (Iglesias del Sol A, Bots ML et al. 2002). However, there is evidence that suggests that while measurements in other areas of the carotids do not necessarily predict measures at other sites, the CCA is sufficient for use due to its greater ease of access (Touboul PJ, Hennerici MG et al. 2012). Carotid plaque information was also limited in comparison to some other studies. In terms of quantitative measures, only plaque thickness, and the presence or absence of plaque in different segments of the carotid arteries, were noted and it was not possible to quantify total plaque area or plaque volume. In addition, plaque morphology measurements were limited by the subjective qualification of morphology by the sonographer. More recent techniques such as gray scale median (GSM) (Irie, Katakami et al. 2013) were not used in this study due to limitations in the technology and quality of the images produced. This may have an effect on the magnitude of the relationships between carotid plaque and vascular disease in this thesis, and may underestimate the risk. In addition, the Mannheim consensus recommends measurement of the inter-adventitial diameter and the intraluminal diameter, as IMT is related to arterial diameter (Touboul PJ, Hennerici MG et al. 2012), however, these measures are not available in the ET2DS data.

The number of incident cardiovascular events recorded in the study was small relative to the number of participants in the study. The use of a composite endpoint allowed for a maximization of the numbers included in each analyses, increasing the

likelihood of finding the true association between carotid ultrasound parameters and incident vascular disease. Interestingly, the event rate for MI in the ET2DS was 5.6 events per 1000 patient years. If this is compared with figures published by Mulnier et al in 2008, for a similarly aged group they found an equivalent rate of 19.4 events per 1000 patient years (Mulnier, Seaman et al. 2008). The rate they found for those without diabetes was 7.3 events per 1000 patient years, highlighting that the rate in the ET2DS was low, even for a general population.

As this thesis was in the final stage of completion, the NICE guidelines were revised (NICE CG 181 2014). There was a change in their recommendations for cardiovascular risk prediction in people with Type 2 diabetes. As of 2014, they recommend the QRISK2 score for assessing cardiovascular risk rather than the UKPDS Score, which they recommended previously. The majority of risk factors included in QRISK2 are similar to those included in the UKPDS used in this thesis, with the exception of rheumatoid arthritis, atrial fibrillation, BP treatment, deprivation, family history. The impact of this change on the results of this thesis is unknown. Some of the risk factors mentioned above are not available in the ET2DS (AF and family history) but the others are potentially available. Therefore, recommendations for future research would include a recalibration of the models to include the additional risk factors in the QRISK2 score in order to produce up to date estimates for the association between cIMT and carotid plaque with incident vascular disease in the ET2DS.

The ET2DS specifically aimed to recruit individuals between the age of 60 and 75 years of age as the major outcomes of the study included cognition, liver disease and heart disease. Therefore, the study sample does not include younger adults with Type 2 diabetes. In addition, the majority of the study participants were white and there were only a few individuals of other ethnicities participating in the study. These two factors together impose limits upon the generalizability (external validity) of the results to other populations.

Summary

The ET2DS has much strength, including sample size, good external validity and high quality, complete data collection for both risk factors and cardiovascular events. Limitations include the time between cIMT measurement and risk factor measurement and the limitations discussed regarding cIMT measurement. However these issues have been addressed as far as possible and are likely to impact only in a small way on the results described in this thesis.

The following sections discuss the key results from the various phases of analysis (summarized in section 8.1) in more detail.

7.4 Frequency and distribution of cIMT and Carotid Plaque in People with Type 2 Diabetes

7.4.1 cIMT

Because of the similarity in the relationship between the different summary measures of cIMT and vascular risk factors (discussed in section 8.2.4), only mean cIMT will be discussed in any great detail in this section.

Mean cIMT was normally distributed in the ET2DS and the mean value was 0.94mm. A comparison of mean cIMT with both studies in general populations and studies of people diabetes is summarised in table 7-1. In general the ET2DS mean cIMT was comparable with mean cIMT values described in other studies of people with diabetes (range 0.83-1.21mm). In addition, it was higher than the range of cIMT reported in several studies from the general population (range 0.59-0.84mm). This is in keeping with the results of a 2006 systematic review that identified 21 studies of cIMT in people with diabetes and glucose intolerance (Brohall G, Odén A et al. 2006). Among the 24 111 individuals in the studies included in the review, 4019 had Type 2 diabetes. The authors identified that in 20 out of the 21 studies, individuals with diabetes had a higher cIMT than the healthy controls (Brohall G, Odén A et al. 2006). They performed a random effects meta-analysis of the results and found that on average, mean CCA cIMT in people with diabetes was 0.13mm higher than in the control groups. Mean CCA cIMT in individuals with diabetes

included in the meta-analysis ranged from 0.73-1.44mm. The mean CCA cIMT in ET2DS was comparable with this range. It was also comparable with the range for similar ethnic groups (Caucasian European range 0.798-1.44mm). This suggests that the mean cIMT in the ET2DS may be higher than comparable healthy individuals, although the nature of the ET2DS does not allow for firm conclusions on this topic as no controls were available for direct comparison (longitudinal cohort study).

Table 7-1 Summary of CCA cIMT values in a selection of epidemiological studies

	Reference	Total N	Population	Age	Mean IMT
ET2DS	n/a	916	Diabetes	67.9	0.94 ± 0.14
Insulin Resistance Atherosclerosis Study	(Wagenknecht, D'Agostino Jr et al. 1998)	1392	Diabetes	57 (established DM)	0.890 ± 0.02
				58 (new DM)	0.858 ± 0.016
Hoorn Study	(Henry RMA, Kostense PJ et al. 2004)	301	Diabetes	67.8	0.88 ± 0.17
n/a	(Sigurdardottir V, Fagerberg B et al. 2004)	262	Diabetes	61 (established DM)	0.87 ± 0.0281
				61 (new DM)	0.85 ± 0.0315
n/a	(Rajala U, Laakso M et al. 2002)	208	Diabetes	62	0.99 ± 0.04
n/a	(Niskanen L, Rauramaa R et al. 1996)	203	Diabetes	67	1.21 ± 0.04
n/a	(Geroulakos G, Ramaswami G et al. 1994)	194	Diabetes	49	0.83 ± 0.02
ARIC	(Chambless LE, Heiss G et al. 1997)	12841	General	53.7 (f)	0.60 ± 0.00
				54.3 (m)	0.66 ± 0.01
CHS	(O'Leary DH, Polak JF et al. 1999)	4476	General	72.5	-
Rotterdam	(Iglesias del Sol A, Bots ML et al. 2002)	2073	General	70.0 (No MI)	-
				72 (MI)	-
MDCS	(Rosvall M, Janzon L et al. 2005)	5077	General	57.4 (No CAD)	0.760 (n/a)
				60.5 (CAD)	0.840 (n/a)
CAPS	(Lorenz MW, von Kegler S et al. 2006)	5056	General	50.1	Left 0.71 ± 0.17
					Right 0.74 ± 0.20
MESA	(Folsom, Kronmal et al. 2008)	6698	General	45-84	-
Framingham Offspring	(Polak, Pencina et al. 2011)	2965	General	57.3 (no CVD)	0.59 ± 0.13
				62.9 (CVD)	0.69 ± 0.15
Improve	(Baldassarre D, Hamsten A et al. 2012)	3703	General	64.2	0.71 ± 0.65-0.8

All reported measures are for common carotid artery (CCA)

7.4.2 Plaque presence and thickness

There was a high prevalence of carotid plaque in the ET2DS at year 1 (97.6% of participants had at least 1 plaque). Plaque was most common at the carotid bifurcation. This is an area of altered intravascular dynamics and it is well documented that carotid plaque is more prevalent at the bifurcation (Imparato, Riles et al. 1979). The prevalence of carotid plaque reported in this thesis was considerably greater than both the general population and at risk populations prevalences of between 30% - 78% quoted by previous studies (Fabris F, Zanocchi M et al. 1994; Joakimsen O, Bønaa KH et al. 1999; Kwon TG, Kim KW et al. 2009; Sillesen H, Muntendam P et al. 2012). The mean age of participants with a plaque prevalence of 30% was 59.7 year, while the mean age for participants in the study with prevalence of 78% was 68.8 years, which is comparable with the ET2DS. However, the prevalence in the ET2DS was considerably higher, at 97.6%, suggesting that carotid plaque may be more prevalent in people with Type 2 diabetes than in the general population.

Published data concerning carotid plaque prevalence in people with Type 2 diabetes is limited. A Chinese study published in 2010 reported carotid plaque prevalence of 91% in persons who had Type 2 diabetes and suspected cerebrovascular disease (mean age 66 years) (He C, Yang Z et al. 2010). While the participants in this study were not directly comparable with the ET2DS in that they were all suspected of having cerebrovascular disease, the mean age of participants was similar. Another study from Macedonia in 2007 identified carotid plaque prevalence of just over 80% in a sample of 145 individuals with Type 2 diabetes and coronary artery disease. The mean age of the participants was 59 years (Bosevski, Borozanov et al. 2007), which was only slightly lower than that of the ET2DS. In 2005, Bernard et al reported a plaque prevalence of 50% in 229 individuals with Type 2 diabetes (Bernard S, Sérusclat A et al. 2005). Another Chinese study of 250 individuals with and without microalbuminuria had a plaque prevalence of approximately 53%, which is markedly lower than that of the ET2DS (Zhang YH, Gao Y et al. 2013). Participants in both this study and the Bernard study had a mean age of around 50 years, which was considerably younger than the ET2DS participants; this may go some way to explaining the differences in plaque prevalence.

Carotid plaque was assessed using several different methods in the ET2DS. Mean plaque thickness and maximum plaque thickness were continuous measurements that were made on carotid ultrasound. A prospective study by Irie et al examined carotid plaque in 287 individuals with Type 2 diabetes (Irie, Katakami et al. 2013). They authors reported a mean plaque thickness of 2.26 ± 0.86mm which was comparable with the mean plaque thickness of the ET2DS (2.44mm).

The correlation between plaque thickness and measures of cIMT was only moderate, suggesting that cIMT and carotid plaque are not interchangeable measures of atherosclerosis in the study. This finding supports the results of study of 98 Oji-Cree adults published in 2005 (Pollex RL, Spence JD et al. 2005). The authors found that the correlation between cIMT and total plaque volume was modest at 0.7 (which was higher than the correlation seen in the ET2DS). This may support the theory that cIMT and plaque represent different aspects and stages of carotid atherosclerosis.

7.4.3 Plaque score and plaque morphology

The plaque score in the ET2DS was based on a method used by Lee et al (Lee EJ, Kim HJ et al. 2007) in a study of carotid plaque and stroke in people with Type 2 diabetes. It reflects the presence of at least 1 plaque in each of the sections of the carotid artery assessed. This was created as a proxy measure of plaque burden as more extensive measures such as total plaque area or total plaque volume were not available in this study. In the ET2DS, plaque score ranged from 0-8 while in the Lee study, the range was 0-6. In the ET2DS, most participants (79.9%) had a plaque score between 2 and 6, while in the Lee study, 77.5% of participants had a score of only 0-3, suggesting that the plaque burden in the ET2DS was higher than that demonstrated by Lee et al. The association of high plaque score (>4) with increasing cIMT and the presence of high risk plaques suggests that it is a good proxy measure for plaque burden.

This thesis presents relatively unique data concerning carotid plaque morphology in people with Type 2 diabetes. As reported earlier, there was a high prevalence of carotid plaque in the ET2DS but different plaque morphologies are associated with different risks of CVD. Echolucent and heterogeneous plaques are thought to

represent a higher risk of rupture and thrombosis than fibrotic, echogenic and homogenous plaques, although the exact nature and strength of this risk remains uncertain. In the ET2DS, there was a high prevalence at year 1 of so called "high risk" plaques (56.1%) and at year 4 (91%). Reinforcing their status of "high risk", these plaques were associated with increased cIMT, increased plaque thickness and a higher plaque score. A study of plaque morphology measured by dual source CT angiography in 125 patients with type 2 diabetes describes the prevalence of carotid and cerebrovascular plaques (He C, Yang Z et al. 2010). The prevalence of non-calcified or mixed plaques (high risk plaques) in the extracranial arteries and the intracranial ICA in the study by He et al was 18.2% and 74.8% respectively. It is difficult to compare these results with the ET2DS because while the ET2DS measured plaque prevalence using ultrasound in the common carotid, internal carotid, bifurcation and external carotid, the study by He et al used CT to determine plaque presence in both the carotids and the cerebral vasculature. The also divided the entire arterial tree up in to 40 segments which are not easily compared with the ET2DS. An earlier study of 47 T2DM participants and 51 controls had found that individuals with T2DM had more echolucent plaque than non-diabetic individuals (Ostling G, Hedblad B et al. 2007). Again, it is difficult to directly compare the results of this study with those presented in this thesis as the authors quantify plaque echogenicity using GSM as a continuous measure, rather than describing simply presence of echolucent plaque as in the ET2DS. In the general population, Joakimson et al report the prevalence of high risk (soft) plaque in those with morphologically classifiable plaque as 37.7% (Joakimsen O, Bønaa KH et al. 1999).

7.4.4 Change in cIMT and plaque

In this thesis, analysis of cIMT over the course of follow up revealed a small but significant regression in mean cIMT over a mean follow up period of 3.5 years. Multifactorial analysis revealed the only independent predictors of change were baseline cIMT and BMI. The negative β coefficients suggest that change in cIMT is smaller in those who have a larger cIMT and in those with a larger BMI.

The lack of progression, and indeed regression of cIMT in the ET2DS is a somewhat unexpected result as the association of increasing cIMT with increasing age would suggest that cIMT would progress over the course of follow up. Indeed, Wagenknecht et al have reported that cIMT not only progresses in people with diabetes, but that progression is greater than that seen in people without diabetes (Wagenknecht, Zaccaro et al. 2003). Progression has also been reported in the 2000 study by Yamasaki et al, where the authors identified a progression in cIMT of 0.04mm/year in their study of 287 individuals with Type 2 diabetes over a mean follow up period of 3.1 years (Yamasaki Y, Kodama M et al. 2000). The authors identified that the independent predictors of cIMT change were baseline thickness and HbA1c, which is partly supported by the findings in this thesis. However, they noted a positive correlation between cIMT change and baseline cIMT whereas in the ET2DS, the correlation was negative. In the general population, the ARIC study has also examined the determinants of change of cIMT with time and found that baseline diabetes, smoking, HDL cholesterol, pulse pressure, white blood cell count and fibrinogen were associated with change in cIMT (Chambless, Folsom et al. 2002). Further risk factors identified by the Rotterdam study include age, BMI, male sex, current smoking, systolic BP and hypertension (van der Meer, Iglesias del Sol et al. 2003). It is not easy to explain these differences, although the high frequency of lipid lowering medication use in the ET2DS in comparison to these studies (84.3% vs 48.7% (Yamasaki) and 9.7% (Wagenknect)) may go some way to explaining this phenomenon, as lipid lowering medications have been show to slow or indeed reverse cIMT progression (Hedblad B, Zambanini A et al. 2007; Yu CM, Zhang Q et al. 2007; Kastelein JJ, Akdim F et al. 2008).

The lack of progression in cIMT in the ET2DS is puzzling and there are several potential explanations for this. Firstly, the lower mean cIMT identified at the year 4 follow up may reflect the loss to follow up of those with a higher cIMT (who may be at increased risk of morbidity and mortality and therefore less likely to reattend for measurement of cIMT). However, the absolute difference in year 1 mean cIMT in those who did and did not have a cIMT measurement at year 4 was small (no follow up 0.93 v follow up 0.94mm, p=0.775) and is unlikely to be the reason underlying

the lack of progression of cIMT in the ET2DS. A second potential explanation may be measurement error and the resultant variability in cIMT measurements. The validation study performed in the ET2DS at year 4 suggested that measurement of cIMT by the sonographer was reliable and was not influenced by time between readings. While a validation study was not performed to confirm this at year 1, these findings were extrapolated to year 1 because the same sonographer was used at both times points. Therefore, it is unlikely that measurement error would explain the change in cIMT.

Another potential explanation for the regression of cIMT at year 4 may be that it could reflect the ongoing influence of vascular risk factor modification by medications such as statins or antihypertensive medications. Clinical trials of drugs such as statins have demonstrated a slowing and even regression of cIMT in the treatment group (Fleg, Mete et al.). Further analysis of cIMT progression in those taking and not taking antihypertensives in the ET2DS revealed no statistically significant difference in progression between these groups and both groups showed a regression in cIMT. When this analysis was extended to lipid lowering medication, those who were not taking these medications did have a positive change in cIMT (0.002mm) while the group taking medication showed regression, however the difference was not statistically significant so little comment can be made upon the effect of statins on cIMT change. Therefore, it may be that the most likely explanation for the lack of progression in cIMT in the ET2DS is the short follow up time. The development of atherosclerosis and vessel wall changes reflects years of influence from cardiovascular risk factors (Lorenz, Polak et al. 2012), whereas the change as measured between two follow up clinics represents only a very short period of time in comparison. It may be that there is not sufficient change in the cIMT to be recorded between these two points, in addition to the modifying effect of drugs such as statins on the vessel wall and vascular risk factors such as hypertension that are known to impact on the cIMT.

With regards to the association of cIMT change with cardiovascular risk and its use as a surrogate cardiovascular endpoint, as highlighted in the literature review, change in cIMT was analysed in those with and without both prevalent and incident vascular

events. There was no significant difference in the change in mean cIMT between the groups, suggesting that change in cIMT is not related to future vascular risk in the ET2DS.

Plaque thickness, in contrast to cIMT, showed a significant increase between year 1 and year 4 of the ET2DS. As discussed previously, carotid plaque may represent a more advanced and active disease process than cIMT and therefore, active rapid change in these measures may not be surprising as continued exposure to vascular risk factors has a more acute effect on the nature of atherosclerotic plaques, with rupture and fibrosis of plaques being influenced by these factors. The constant active remodelling of plaques may explain why a change in plaque thickness was noted in contrast to cIMT. Plaque score and morphology were also significantly altered at year 4 follow up in comparison with year 1. Plaque was more extensive at year 4 than at year 1, as demonstrated by the higher plaque score at year 4, and individuals tended to have more high risk plaque at follow up. Only 9% of individuals had no plaque or echogenic plaque at year 4, compared with 44% at year 1. This means that 91% of the study population had at least 1 high risk plaque after 3 years of follow up, compared with 56.1% at year 1. This is a considerable increase in such a short period. It is possible that this may be attributable to variability in the way the sonographer classified plaque morphology between year 1 and year 4. However it is difficult to comment on this as no validation study was performed to assess this in the ET2DS. A study by Joakimsen in 1997 revealed that in general, reproducibility of plaque assessment was good. Some aspects of plaque assessment had better inter and intra-reader variability (plaque presence and plaque morphology) in comparison with others that displayed only moderate agreement (plaque thickness) (Joakimsen O, Bønaa KH et al. 1997). In view of this, in the ET2DS, it is perhaps most likely that the change in plaque morphology and distribution is simply related to the ongoing influence of vascular risk factors with time rather than any error in plaque assessment.

7.5 Association of cIMT with cardiovascular risk factors and prevalent CVD

This section discusses the reported associations of cIMT with risk factors and prevalent CVD.

7.5.1 Cardiovascular risk factors

In this study, all cIMT parameters were significantly higher in males than females, and increased with age for both sexes which is in keeping with current research (Joakimsen O, Bønaa KH et al. 1999). Mean cIMT was associated (after adjustment for age and sex) with increased duration of diabetes, raised systolic and reduced diastolic blood pressure, as well as cigarette smoking. Full multifactorial analysis then revealed that the independent predictors of cIMT in the ET2DS were increased age, male sex and raised systolic blood pressure. However, it should be noted that the effect sizes determined in this thesis are in some cases small. For example, the age and sex adjusted correlation coefficient between systolic BP and mean cIMT is only 0.075 but is statistically significant. While there is statistical significance, the clinical significance of such a small correlation must be questioned. In the case of the parameters where the correlation coefficient is <0.1, it is possible the association may only be significant because of the sample size. Similarly, the small ANOVA statistics also raise a similar question with regards to the categorical variables.

Associations between risk factors and cIMT have been reported in several general population studies. The most common risk factors associated with cIMT are increasing age, male sex, raised systolic and diastolic blood pressure, reduced HDL and elevated total cholesterol. An early study by Gariepy et al, of 788 men and women aged 17 to 65 years old, found associations between increasing cIMT and cardiovascular risk factors such as systolic and diastolic blood pressure, total and HDL cholesterol (women only) and blood glucose (men only) (Gariepy J, Salomon J et al. 1998). A study from 2000 using data from 963 Italian adults attending a metabolic study centre in Milan found that cIMT correlated with systolic blood pressure, total, LDL and HDL cholesterol and triglycerides (Baldassarre, Amato et al. 2000). In studies of cIMT and vascular risk factors in people with Type 2

diabetes (which are less widespread), several risk factors have been associated with cIMT, including serum triglycerides and HDL cholesterol (Temelkova-Kurktschiev, Koehler et al. 1999; Temelkova-Kurktschiev, Koehler et al. 2000), Butt 2009). Other studies identified associations with age, BMI (Güvener, Tütüncü et al. 2000) and microalbuminuria (Agewall S, Wikstrand J et al. 1995; Mykkänen L, Zaccaro DJ et al. 1997).

The most striking difference between these studies and the ET2DS is the lack of an association between cIMT and blood lipids in the ET2DS. The most likely explanation for this discrepancy lies in the high use of lipid lowering medication in the ET2DS, which may have masked the true relationship between cIMT and blood lipids. Another surprising finding is the lack of association of cIMT with smoking. The reasons for this are not clear but it may hint at the underlying pathology of cIMT as a precursor of atherosclerosis rather than atherosclerosis itself.

7.5.2 Prevalent vascular disease

Individuals in the ET2DS with a history of previous CVD displayed an altered cardiometabolic risk profile to those who with no history of CVD. They were older and more likely to be male, and had a longer duration of diabetes - all of which are well-established risk factors for CVD. There was no difference in HbA1c or systolic blood pressure, which probably reflects the increased use of insulin and antihypertensives in the disease group of high risk individuals. Medication use may also explain the lower total cholesterol in the group with disease. They also demonstrated a higher prevalence of smoking, which is in keeping with increased risk of vascular disease. They had significantly poorer renal function, increased inflammatory markers and a higher NTproBNP than individuals without disease, all suggestive of an increased vascular risk (Blankenberg, McQueen et al. 2006; Chronic Kidney Disease Consortium 2010). (Ridker PM, Rifai N et al. 2000)

All measures of cIMT were significantly higher in individuals who had ever been diagnosed with cardiovascular disease (MI, angina, TIA or stroke). This was also seen when individuals with and without CAD and with and without cerebrovascular disease were compared. However, logistic regression modelling revealed that

although cIMT was associated with prevalent CVD, this relationship lost significance once traditional risk factors were adjusted for and was the same for CAD and cerebrovascular disease. This contrasted markedly with measures of carotid plaque, all of which remained associated with prevalent CVD, CAD and cerebrovascular disease, even after adjustment for traditional risk factors and novel risk factors. These results suggest that it may in fact be carotid plaque that may be more useful in the prediction of cardiovascular disease, given the strong association between plaque and CVD.

Studies in both general populations and Type 2 diabetes have reported the association of cIMT with prevalent vascular disease. In 1999, Ebrahim et al reported an association between cIMT and prevalent vascular disease (Ebrahim, Papacosta et al. 1999). They identified that common carotid artery cIMT was associated with prevalent stroke whereas cIMT at the bifurcation of the carotids was more strongly associated with ischaemic heart disease. A 2002 study by Baldassarre et al also reported higher cIMT in individuals from the general population with CHD (Baldassarre, Amato et al. 2000). A more recent study by Polak et al, using data from the Framingham Offspring cohort found a stronger relationship between cIMT and prevalent vascular disease than was seen in the ET2DS (Polak, Pencina et al. 2010). Using multifactorial logistic regression models, they identified both CCA cIMT and ICA cIMT as independent predictors of prevalent cardiovascular disease. Critically, several studies in people with diabetes revealed that individuals with both stroke and CAD, identified using CT and MR, had increased cIMT (Lee, 2007, Djaberi 2009, Kasami 2011). The results of this thesis, in particular in relation to CAD and general CVD, further support the findings of these studies.

7.6 Association of Carotid Plaque with cardiovascular risk factors and prevalent CVD

7.6.1 Cardiovascular risk factors

Carotid plaque thickness in the ET2DS demonstrated a different cross sectional relationship with cardiovascular risk factors than cIMT. This difference in risk factor

associations has been reported by other studies including the British Regional Heart Study (Ebrahim, Papacosta et al. 1999). In the ET2DS, following multifactorial adjustment mean and maximum plaque thickness were associated with several risk factors – increasing age, male sex, longer duration of diabetes, increased systolic BP, reduced diastolic pressure and smoking history. However, like cIMT, the effect size of the correlations was in some cases small and may not represent a strong relationship between the risk factors and plaque thickness (the strongest age and sex adjusted correlation was with systolic blood pressure (r=0.119)) and care must be taken not to overstate their importance. The major difference between these associations and those seen for cIMT is the association with smoking. Cigarette smoking is known to be a direct cause of atherosclerosis so it is not surprising to find this relationship with carotid plaque thickness but not with cIMT. Carotid plaque burden as represented by individual plaque score in the ET2DS, appeared to be associated with a poor cardiometabolic profile however many of the differences were difficult to interpret as the relationships were not always completely linear. In order to address this, plaque score was considered as a bivariate factor (score ≤4 or >4) and a similar risk factor profile to that of plaque thickness was identified for those people with a high plaque score (>4), with the additional exception of BMI, suggesting that plaque score may not provide any additional information on cardiovascular risk than plaque thickness. The presence of high risk plaque was also associated with raised systolic and diastolic blood pressures, as well as also low BMI. Interestingly, there was no association with smoking history.

There are not many previous studies specifically of carotid plaque (in particular plaque thickness) and vascular risk factors in people with Type 2 diabetes. One recent study by Irie et al demonstrated that male sex, BMI and low-HDL-cholesterol were strongly associated with plaque morphology in people with Type 2 diabetes (Irie, Katakami et al. 2014) which is in support of the finding of this thesis. Cardoso et al also reported an association with smoking, which has been replicated in this thesis (Cardoso et al, 2012). In the general population, a review by Wyman et al highlighted that associations have been demonstrated between carotid plaque and age, systolic blood pressure, smoking, total/HDL cholesterol ratio and BMI (Wyman,

Fraizer et al. 2005). It is interesting to note that the relationship between carotid plaque and blood lipids demonstrated by other studies is absent in the ET2DS.

In terms of plaque morphology in the general population, Joakimsen et al noted that high risk plaques were more common in men from the general population (at all ages) although they do not describe the association of high risk plaques with risk factors (Joakimsen O, Bønaa KH et al. 1999). In the ET2DS, while men were more likely to have high risk plaque than women, this difference disappeared following multifactorial adjustment. Data from the Tromsø Study established that HDL cholesterol was associated with echolucent carotid plaques (adjusted OR 0.69 (95%CI 0.52-0.93) in 6727 participants in a population health survey (Mathiesen EB, Bønaa KH et al. 2001). Again, this relationship with HDL cholesterol was not demonstrated in the ET2DS.

7.6.2 Prevalent vascular disease

All parameters of carotid plaque considered in this thesis were more prevalent in individuals who had a history of any CVD. The absolute differences in mean and maximum plaque thickness were considerable (0.6mm for mean plaque thickness and 0.5mm for max plaque thickness).

In a study by Lee et al, which examined the relationship between cIMT, plaque and stroke in 133 people with Type 2 diabetes, the authors found that individuals who had experienced a stroke tended to have a higher plaque score than those who had not (Lee et al, 2007). This finding has been replicated in the ET2DS, where individuals with prevalent cerebrovascular disease (stroke or TIA) had a higher plaque score than those without. This finding was also replicated for CAD and any CVD. Also in accordance with the Lee study were the results of logistic regression models in which the association between plaque score and prevalent cerebrovascular disease was attenuated by adjustment for risk factors in logistic regression models. However, this thesis did find that the association between plaque score and CAD, as well as any CVD, survived full cardiovascular risk factor adjustment (including novel risk factors in the case of any CVD), suggesting a stronger relationship between plaque score and prevalent CAD and CVD rather than stroke. This is

supported by the findings of Akazawa et al who reported that plaque score was associated with the extent of CAD after risk factors adjustment (Akazawa et al, 2012). In addition, high risk plaques were more common in people with prevalent CVD than those without, suggesting that they may also be associated with an increased risk for CVD.

7.7 Usefulness of cIMT and Carotid Plaque in prediction of incident CV events over and above conventional risk factors in the ET2DS

In the ET2DS, cIMT was independently associated with established cardiovascular risk factors and demonstrated a modest association with prevalent cardiovascular disease while carotid plaque showed a more robust relationship with both cardiovascular risk factors and prevalent vascular disease. This suggested that carotid plaque may be more useful in the prediction of cardiovascular events than cIMT. However, the results of the analysis of these factors with incident cardiovascular disease demonstrate that cIMT had a stronger relationship with incident vascular disease than carotid plaque.

7.7.1 cIMT and incident vascular events

Cox proportional hazards' modelling was chosen to analyse the relationship between cIMT measurements made at year 1 in the ET2DS and incident cardiovascular events. Unadjusted models for all cIMT variables and incident events revealed that all measures of cIMT were associated with incident CVD and most with CAD. There was no such association for fatal events or cerebrovascular disease, which may reflect the small number of events seen in each of these categories. This finding supports much of the research from general population studies which demonstrates a stronger link between cIMT and both general CVD and CAD, rather than stroke.

Adjustment for age, sex, previous CVD and UKPDS cardiovascular risk factors attenuated the predictive ability for any CVD as well as CAD, for all measures of cIMT with the exception of mean cIMT, and mean maximum cIMT for CAD only.

Assessment of the usefulness of mean cIMT in prediction of cardiovascular disease over and above UKPDS risk factors was assessed using area under the curve and NRI. AUC improved marginally on addition of mean cIMT to the model containing risk factors (0.691-0.704) and NRI was found to be 0.25% suggesting that there is an overall minimal improvement in risk prediction for cardiovascular events when mean cIMT is added to traditional risk factors, the clinical significance of which is uncertain. These findings are similar to several studies that are discussed below, in addition to a large meta-analysis which will be discussed separately.

Five epidemiological cohort studies (3 prospective and 2 retrospective) and one meta-analysis assessing the usefulness of cIMT on top of traditional cardiovascular risk factors in people with Type 2 diabetes were identified in chapter 1 of this thesis. It is important to compare the demographic and cardiometabolic characteristics of these studies with the ET2DS in order to make meaningful comparisons with their results. The study population used in this thesis is a cohort of older adults with Type 2 diabetes living in Edinburgh and the Lothians. The average age of the participants at the time of carotid ultrasound assessment was 68.9 years, making the cohort slightly older than previous studies of cIMT and future cardiovascular risk in people with Type 2 diabetes (range 51.4-65.0 years) (Yamasaki Y, Kodama M et al. 2000; Bernard S, Sérusclat A et al. 2005; Ataoglu, Saler et al. 2009; Malik S, Budoff M et al. 2011). The ET2DS also differed from previous study populations in a number of other respects. For example, while the use of medication to manage diabetes was of a similar prevalence to that seen in the study by Bernard et al (Bernard S, Sérusclat A et al. 2005) the use of antihypertensive and lipid lowering medications was higher. Mean systolic and diastolic pressure was similar to that in the study by Yamasaki et al but lower than that in Ataolugo et al (Yamasaki Y, Kodama M et al. 2000; Ataoglu, Saler et al. 2009), which may be explained by the high prevalence of anti-hypertensive use in the ET2DS. Mean lipid levels in the ET2DS were poorer than that reported by Bernard et al but modestly better than that reported by Yamasaki et al (Yamasaki Y, Kodama M et al. 2000; Bernard S, Sérusclat A et al. 2005).

The earliest of the prospective studies was that of Yamasaki et al who, in 2000, found that for a 1 unit increase in cIMT there was an almost 5 fold increase in risk of

non-fatal CAD after adjusting for cardiovascular risk factors in multifactorial logistic regression models (Yamasaki Y, Kodama M et al. 2000). This thesis has identified that a 1 SD increase in mean cIMT led to a 1.5 fold increase in risk for incident non-fatal CAD in the fully adjusted Cox regression models. Whilst this is lower than the risk identified by Yamasaki et al, it should be noted that their logistic regression model takes no account of time to event, as is seen in Cox regression; therefore the risks may not be directly comparable. However, the overall trend remains the same – that cIMT increased prediction of coronary artery disease over and above traditional risk factors. Unfortunately, they do not report any comparison of the models eg area under the curve or net reclassification, so it is impossible to comment on clinical impact of adding cIMT on risk classification in this study.

In 2005, a further study by Bernard et al prospectively assessed the predictive ability of cIMT for cardiovascular events. They found that for a 1 SD increase in mean cIMT there was an OR of 1.63 (95%CI 1.01-2.63) for cardiovascular events, after adjustment for age, sex, physical activity, microalbuminuria and HDL cholesterol. Their model for cardiovascular events containing only Framingham risk score had a similar AUC to the model containing only cIMT (0.720 vs 0.715 respectively) (Bernard S, Sérusclat A et al. 2005). The AUC for the model containing only Framingham risk score was slightly higher than the same model in the ET2DS (AUC 0.691). When they combined both Framingham risk factors and cIMT into their model using a combined index, there was a significant improvement in prediction as demonstrated by increased survival in Kaplan-Meir curves (16.1, P=0.0003) - AUC is not reported for that model. They also assessed Cox regression models of continuous cIMT in addition to FRS and found a global increase in chi square from 14.1 to 18.1 (p=0.0035) when cIMT was added, suggesting that cIMT may be predictive of cardiovascular events over and above FRS. The results of this study are similar to the results found in the ET2DS. They report an increase in global chi squared of 4.0 on addition of cIMT to the Framingham score, which is similar to the increase seen in the ET2DS on the addition of cIMT to UKPDS risk factors (3.87). Again, AUC and NRI are not reported for this study so no comparison is made with regards improvement in prediction.

Results from the Multi-Ethnic Study of Atherosclerosis (MESA), a large study of 6814 participants aged 45-84, were published in 2011 (Malik S, Budoff M et al. 2011). Using 881 MESA participants who met the criteria for diabetes, they analysed maximum cIMT in quartiles and compared its predictive ability for CVD and CAD in models containing Framingham risk factors with that of coronary artery calcium (CAC). In contrast to the results presented in this thesis, they found that while CAC adding to Cox models containing conventional risk factors significantly improved risk prediction for both CVD and CAD in people with diabetes, cIMT did not (4^{th} vs 1^{st} quartile cIMT HR 1.7 (0.7-4.3) and 1.0 (0.5-2.0) for CAD and CVD respectively). ROC curve analysis however did show a small increase in AUC for CHD when the risk factor model was compared to a model with risk factors and cIMT (0.72 -0.74 respectively). While the ET2DS cohort and the MESA participants included in this study were broadly similar in terms age and other risk factors, the MESA participants were drawn from a general population cohort, rather than being recruited into a diabetes specific study. This may have introduced an element of heterogeneity into the study population which may have impacted upon the results of the analysis.

The earliest retrospective cohort study that addressed the addition of cIMT to FRS for predicting vascular events in Type 2 diabetes was published in 2009 (Ataoglu, Saler et al. 2009). The authors reported that in 102 subjects with diabetes, cIMT could be useful in addition to Framingham risk scoring in predicting vascular risk. The OR for cIMT following adjustment for FRS factors was 7.92. However, caution must be applied to these results as they are the results of a retrospective cohort and the effect of bias and confounding may be greater in this cohort than in a prospective study. Additionally, they do not report any reclassification statistics so the improvement in risk prediction cannot be compared with the results of this thesis.

A further retrospective study from 2011 examined cIMT in relation to incident CVD events in a population of 783 type 2 diabetic adults (Yoshida, Mita et al. 2012). Using Cox regression models fitted for traditional cardiovascular risk factors, they identified that cIMT predicted risk for CVD events over and above the fitted risk factors (RR 2.39 (1.19-4.81, p=0.02). ROC curves of models containing cIMT and

as well as Framingham risk factors revealed improved risk prediction with the AUC increasing from 0.645 for risk factors alone, to 0.656 on the addition of cIMT. These results, in a study of comparable size, are very similar to the ET2DS.

Because of the mixed and modest results demonstrated by these studies, a meta-analysis was performed by den Ruijter et al using data from 4,220 individuals with diabetes (and mean follow up of 8.7 years) in a large ongoing individual participant collaboration (USE IMT) involving 56,194 subjects from 17 population-based cohorts worldwide (Den Ruijter, Peters et al. 2013). Similarly to the methods used in this thesis, the authors used Cox regression models to assess the predictive ability of cIMT over and above Framingham risk factors. They found a HR of 1.22 (1.14, 1.32) for CVD events (MI or Stroke) for a 1SD increase in mean cIMT. They then identified only a small improvement in NRI (1.7%). This is highly comparable with the HR identified in this thesis for a 1DS increase in mean cIMT for CVD (HR 1.26 (1.003-1.58). The results of this thesis also demonstrated only a small increase in NRI (0.25% for tertiles of risk).

Although the results of this thesis do not differ significantly from the findings of the somewhat larger meta-analysis performed by den Ruijter et al and some of the smaller individual studies, what this thesis adds to current research lies in addressing the methodological problems highlighted in the meta-analysis and the smaller studies. If we first address the smaller studies, the obvious advantage of the ET2DS over these is sample size. In contrast, while the numbers used in the meta-analysis are large, they were drawn from a large multi-centre collaboration. Individuals with diabetes were identified from with large general population studies and the data pooled. As den Ruijter et al highlight in their paper, there was considerable variation in the way that diabetes was defined and cIMT measured in each study. The ET2DS was developed specifically to recruit a representative sample of individuals with Type 2 diabetes and each participant was subjected to the same entry criteria and the same study protocols. In addition, important diabetes related factors such as duration of diabetes, which was controlled for in this thesis, were not available in the meta-analysis. The authors also identify that they were unable to address carotid plaque in any way in their study, which this thesis was able to do. In addition, to strengthen

the results of the models presented in this thesis, history of previous CVD was included in the models to account for the increased risk conferred by a previous cardiovascular event on future vascular risk. The high prevalence of CVD in the ET2DS excluded the possibility of selecting a study sample that was free of CVD as the reduction in sample size would have had a considerable impact on the power of the study. However, including previous CVD in the model goes some way to addressing this while maintaining a sufficient sample size.

Therefore, while the results of this thesis do not differ dramatically from published research, aspects of the study perhaps allow more weight to be added to their findings. In addition, the results from the ET2DS follow only 3.5 years of follow up, whereas the USE IMT meta-analysis was based on over 8 years of follow up. Given the strength of the relationship between cIMT and incident events in the ET2DS after a comparably short period of follow up, there may value in performing further data linkage to extend the follow up to explore whether a stronger relationship might be found.

7.7.2 Plaque and incident cardiovascular disease

One of the aims of this thesis was to explore the relationship between carotid plaque and incident cardiovascular disease. In this ET2DS, while individuals with incident vascular events had higher plaque thickness, higher plaque score and more higher risk plaques than those who did not have events, upon Cox regression modelling, no plaque parameters were associated with an increased risk for any type of incident event once cardiovascular risk factors were taken into account, and indeed most were not associated even in unadjusted models. This is particularly surprising for the outcome of cerebrovascular disease, given the well documented relationship between carotid plaque (particularly plaque morphology) and stroke in the general population (Pedro LM, Pedro MM et al. 2000; Grønholdt MLM, Nordestgaard BG et al. 2001), although a similar result was reported in a general population study by the MESA group in 2013 . There could be several potential explanations for this. Firstly, the size of the study, although considerable, may not have been large enough to capture the relationship between carotid plaque and vascular risk. The absolute differences

in carotid plaque between those with and without incident events were small and in addition, the small number of events in each outcome category may have weakened any associations between carotid plaque and incident events. Thirdly, the way in which plaque was characterized may not have been as optimal for identifying any relationships as some more extensive measures of plaque eg total plaque volume. More complex measures of plaque may have captured more fully the cardiovascular risk associated with carotid plaque. Although participants who experienced incident events had a higher plaque thickness than those who did not, the small absolute difference between the groups may not have clinical relevance in terms of risk prediction.

A further possible explanation for the lack of association between carotid plaque and incident vascular disease may lie in the underlying pathology. Carotid plaque is an active endothelial pathology. It is possible that the very nature of carotid plaque means it is more closely associated to the current intravascular milieu, which is supported by the strong association of carotid plaque with prevalent vascular disease, rather than to any future vascular risk.

There is evidence for a relationship between different measures of carotid plaque and incident cardiovascular disease in individuals from the general population (Wyman, Mays et al. 2006; Naqvi T and Lee M-S 2014), and a large meta-analysis has been conducted that concluded that in the general population, carotid plaque more accurately predicted CAD events than cIMT (Inaba, Chen et al. 2012). However, data concerning the use of carotid plaque for cardiovascular risk prediction in Type 2 diabetes are sparse (Den Ruijter HM, Peters SA et al. 2012) and indeed, literature searching revealed only a handful of papers that addressed mostly cross sectional relationships between carotid plaque and prevalent vascular disease in Type 2 diabetes, rather than incident disease (Charvat, Michalova et al. 2006; Lee EJ, Kim HJ et al. 2007; Ostling G, Hedblad B et al. 2007; He C, Yang Z et al. 2010; Akazawa, Tojikubo et al. 2012). Only two prospective studies in people with Type 2 diabetes were identified (Katakami, Takahara et al. 2012; Irie, Katakami et al. 2013), both reporting results from the same study population. A comparison of the ET2DS with these studies revealed that they were broadly similar in terms of demographics

and risk factors (Katakami, Takahara et al. 2012; Irie, Katakami et al. 2013). Katakami et al reported that low calibrated-IBS values (a measure of plaque echolucency) in a pilot study of 85 Type 2 diabetic persons could improve the risk prediction of cardiovascular events in asymptomatic type 2 diabetic patients with carotid plaque (Katakami, Takahara et al. 2012). To expand this research, Irie et al demonstrated that in 287 individuals (from the same study population) with Type 2 diabetes, echolucent plaques improved risk prediction after accounting for traditional risk factors (HR 4.55) and indeed, adding plaque echolucency to a model contained traditional risk factors and plaque thickness significantly increased the area under the curve for the models (Irie, Katakami et al. 2013). In the ET2DS, while echolucent, high risk plaque was associated with cardiovascular risk factors and prevalent vascular disease, and indeed, was more prevalent in people with incident vascular disease, it did not demonstrate significant predictive value for incident events after traditional cardiovascular risk factors were accounted for.

Although the results presented in this thesis do not support the current (limited) literature, the size of the ET2DS study in comparison with the two smaller studies discussed may point to the ET2DS being a closer reflection of the real relationship between carotid plaque and incident vascular disease in people with Type 2 diabetes. However, caution must be taken when interpreting this as the measures of carotid plaque in the two studies are not the same as those in the ET2DS so it is difficult to make a direct comparison. Larger studies, with more extensive plaque assessment will be required to confirm the true relationship between carotid plaque and cardiovascular risk, including an analysis of which aspects of carotid plaque might be most useful in risk prediction in people with Type 2 diabetes.

7.8 Novel biomarkers and cardiovascular disease in the ET2DS

As this thesis progressed, it was noted that current literature was increasing regarding other potential markers of vascular risk (Ridker PM, Rifai N et al. 2000; Blankenberg, McQueen et al. 2006; Fowkes FG, Murray GD et al. 2008;

Donfrancesco C, Palleschi S et al. 2013). While time limitations excluded the possibility of a full exploration of the potential for such markers to predict cardiovascular events in this thesis, exploratory analyses of the association between cIMT, carotid plaque, vascular disease and other novel markers of cardiovascular risk were performed. Ankle brachial index (ABI), renal function (eGFR and microalbuminuria), markers of inflammation (CRP and IL-6) and NTproBNP (a marker of heart failure) were included. cIMT and plaque were predominantly associated with ABI and NTproBNP following multifactorial regression.

All measures of cIMT and plaque in the ET2DS were negatively associated with ABI. A negative association between these two markers is expected as a decreasing ABI represents lower arterial distensability and subsequently, increased vascular risk whereas increasing cIMT and plaque represents increasing risk. The relationship of cIMT and plaque with a proven marker of vascular risk could add weight to the evidence for the use of cIMT as a marker of risk, and conversely, may also support the use of ABI in people with diabetes. Fowkes et al demonstrated that ABI is a reliable marker of cardiovascular risk in the general population and have validated its use as a risk model in a large collaboration of 18 cohorts (Fowkes FG, Murray GD et al. 2008; Fowkes, Murray et al. 2014). In another Edinburgh based cohort, Price et al demonstrated that the addition of cIMT to ABI increased prediction of cardiovascular events (Price JF, Tzoulaki I et al. 2007) and the relationship between cIMT and ABI demonstrated in the ET2DS could suggest that combining cIMT and ABI may improve risk prediction in the cohort.

Interestingly, no measures of cIMT or plaque were independently associated with renal function in the ET2DS. Several recent studies, including a large meta-analysis, have identified eGFR and microalbuminuria (as measured by ACR) as potential markers of cardiovascular risk in the general population (Chronic Kidney Disease Consortium 2010; Donfrancesco C, Palleschi S et al. 2013), so the absence of any relationship between cIMT and eGFR in this study is perhaps surprising given the increased prevalence of chronic kidney disease and reduced renal function in people with Type 2 diabetes. However, eGFR was significantly lower in participants who

had an incident event, as well as in those who had prevalent vascular disease, which may hint at the potential for eGFR to predict risk in this population.

When inflammatory markers were assessed, cIMT was positively associated with IL-6 but not with CRP. Following adjustment for age and sex, correlation coefficients for IL-6 were attenuated somewhat but significance was retained; however they did not survive multivariate adjustment. Type 2 diabetes is known to be associated with low grade inflammation (Pickup 2004) and there is also considerable literature describing the relationship between inflammatory markers and cardiovascular disease (Epstein and Ross 1999), an indeed, the relationship between inflammation and CVD and cIMT in Type 2 diabetes (de Jager J, Dekker JM et al. 2006; Ray A, Huisman MV et al. 2009), Kang, 2004). Therefore, it is surprising to find such a limited relationship between markers of inflammation and cIMT in the ET2DS. However, again, there was an association between higher inflammatory markers and the presence of prevalent and incident vascular disease, which may suggest the potential for markers of inflammation to be used in risk prediction in people with diabetes.

cIMT and plaque were also associated with NTproBNP, even after multifactorial adjustment. NTproBNP is a novel marker of heart failure that has received increased interest recently as a potential marker of cardiovascular risk (Blankenberg, McQueen et al. 2006). As well as being associated with cIMT, a higher NTproBNP was seen in individuals with prevalent and incident vascular disease in the ET2DS, suggesting NTproBNP could have the potential for use in risk prediction in this cohort.

7.9 Conclusions

The main aim of this thesis was to describe the frequency and distribution of cIMT and carotid plaque in people with Type 2 diabetes, and to explore the association of cIMT and carotid with cardiovascular risk factors and prevalent cardiovascular disease. This allowed for a subsequent well informed analysis of the predictive abilities of IMT and carotid plaque for incident cardiovascular disease, over and

above cardiovascular risk factors that are included in common risk prediction scores, which was achieved in two stages. Firstly, cIMT and carotid plaque were described in the study population, followed by a cross sectional analysis of cIMT and carotid plaque with cardiovascular risk factors and prevalent cardiovascular disease. Finally, a longitudinal survival analysis of cIMT and carotid plaque with incident events was performed, adjusting for traditional cardiovascular risk factors and previous vascular disease. Additional aims of this thesis were also to analyse the measurement of cIMT in the ET2DS and to address the relationship of cIMT, carotid plaque and both prevalent and incident vascular disease with more novel markers of cardiovascular risk, including ABI, renal function, inflammatory markers and NTproBNP.

Mean values of cIMT in the ET2DS were comparable with other studies of cIMT in people with Type 2 diabetes and may indeed be higher than cIMT in the general population. Increasing cIMT was found to be associated with several cardiovascular risk factors including increased age, male sex and raised systolic blood pressure, as well as adverse levels of the more novel markers of vascular risk such ABI and NTproBNP. Mean cIMT was associated with prevalent vascular disease and was predictive of incident cardiovascular events and coronary artery events (but not stroke) over and above UKPDS risk factors, although the clinical impact of this on reclassification of vascular risk (as demonstrated by NRI) may be limited.

There was a high prevalence of carotid plaque in the ET2DS, and in particular, so called "high risk" plaques. The different measures of carotid plaque were associated with several cardiovascular risk factors including increasing age, male sex, longer duration of diabetes, hypertension, smoking and low BMI. In addition, all measures of carotid plaque were associated with the novel biomarkers ABI and NTproBNP. Carotid plaque was associated with a slightly more extensive range of cardiovascular risk factors than cIMT and was strongly associated with prevalent vascular disease. However, despite this strong association with risk factors and prevalent vascular disease, carotid plaque did not have any additional predictive value for incident cardiovascular events over and above UKPDS risk factors.

This thesis also aimed to address several cIMT measurement issues. A validation study of cIMT measurement in the ET2DS found that cIMT measurement was highly repeatable, giving weight to the conclusions based on the results in this thesis. As consensus statements recommend that cIMT is measured using automated edge detection methods, which were not possible in the ET2DS, measurement of cIMT using user defined, computer aided measurement algorithms were examined to compare multiple cIMT measurements with the more limited sonographer measurements. Measurements made using the computer algorithm were found to be lower than those made by the sonographer although the absolute difference was small and the two measures were highly correlated. Because this approach was tested on a small sub-sample of the ET2DS, further work is needed before firm conclusions can be drawn regarding the best method to use when measuring cIMT in the ET2DS.

Finally, measures of cIMT and carotid plaque in the ET2DS were associated with the novel markers ABI and NTproBNP. In addition these markers were significantly higher in those individuals with prevalent vascular disease, suggesting a more extensive exploration of the association of these markers in relation to cardiovascular disease in the ET2DS may be warranted.

Final Summary

In conclusion, this thesis reports that both cIMT and carotid plaque are modestly associated with traditional cardiovascular risk factors in people with Type 2 diabetes. Carotid plaque appeared to be associated with prevalent vascular disease but prospective analysis revealed that cIMT was predictive for incident vascular events in older adults with Type 2 diabetes, while carotid plaque was not. However, the exact clinical benefit of measuring cIMT over and above traditional cardiovascular risk factors is not clear. Additionally, cIMT and carotid plaque, as well as prevalent vascular disease, were associated with the more novel biomarkers ABI and NTproBNP. There may be potential to use these novel markers to improve

cardiovascular risk prediction in people with Type 2 diabetes but exploration of these factors is out with the remit of this thesis. Measurement of cIMT in this thesis appears to be accurate and broadly comparable with computer aided measurements in a subsample of participants, however further analysis of these measurements in the whole cohort are needed before a firm conclusion can be drawn as to the added value of multiple cIMT measurements.

7.10 Recommendations for Further Research

7.10.1 What this study contributes to current research

This thesis has confirmed current research suggesting that while cIMT seems to be predictive of incident cardiovascular disease in people with Type 2 diabetes, the clinical utility of the improvement over traditional cardiovascular risk factors remains to be proved. Although the findings are not dramatically different, they strengthen the findings of the meta-analysis by den Ruijter et al because the study sample was a specifically chosen, robustly phenotyped cohort that had cIMT measured in a clear and consistent way.

The results of the analysis of carotid plaque with vascular risk in people with diabetes are relatively unique in the literature and demonstrate that carotid plaque does not appear to be of use over and above traditional cardiovascular risk factors in the prediction of disease in people with diabetes, despite the strong association with risk factors and prevalent disease, and the potential evidence in the general population that carotid plaque may be more predictive for cardiovascular events than cIMT. However, more, large prospective epidemiological evidence is required before a conclusion regarding the use of carotid plaque in the prediction of cardiovascular risk in people with Type 2 diabetes can be made. Additionally, this thesis suggests that other more novel markers of vascular risk such as ABI and NTproBNP seem to be associated with vascular risk factors and prevalent disease, and may be of use in predicting cardiovascular risk in people with Type 2 diabetes.

7.10.2 Recommendations for future research

1. Longer follow up for the ET2DS may provide additional information on the relationship between cIMT and incident cardiovascular disease in the study. Follow up presented in this thesis was only for 3.5 years and follow up extended beyond this will increase the number of events available for analysis

2. Despite the negative results presented in this thesis for the use of carotid plaque, larger studies may be needed to investigate this relationship further. Indeed, over the course of my PhD, I have worked with a large collaborative study (USE-IMT) which has published data regarding cIMT and future vascular risk, both in general populations and in people with diabetes (Den Ruijter HM, Peters SA et al. 2012; Den Ruijter, Peters et al. 2013). Such a collaboration would be ideally suited to provide a robust analysis of carotid plaque. In addition, longer follow up of the ET2DS may allow for a fuller assessment of the relationship of carotid plaque with incident vascular disease within the study.

3. The association of cIMT, carotid plaque and prevalent vascular disease with ABI and NTproBNP in the ET2DS opens up the possibility of their use in CVD risk prediction in diabetes, particularly given the evidence for their use in the general population. A more in depth analysis of the relationship between incident CVD and these markers in the ET2DS, either compared with or in combination with cIMT or plaque, would be a sound primary step

4. The findings presented in this thesis that describe the change in cIMT in the ET2DS highlight the need for further research into the progression or change in cIMT and carotid plaque over time, especially in relation to the use of cIMT as a surrogate end point in clinical trials in people with diabetes. Another large collaboration that I have been involved with in the course of my PhD is the PROG IMT collaboration which has specifically examined cIMT progression and future vascular risk (REF) and a meta-analysis of cIMT and progression and incident vascular disease is currently in final draft pre-submission.

5. Given the modest effect size of cIMT for predicting cardiovascular events and the negative findings for carotid plaque, a potential further avenue of exploration might be the combined predictive power of these markers.

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