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CAROTID THREE-DIMENSIONAL ULTRASOUND: LONGITUDINAL MEASUREMENT AND CARDIAC-GATED ACQUISITION

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CAROTID THREE-DIMENSIONAL ULTRASOUND: LONGITUDINAL MEASUREMENT AND CARDIAC-GATED ACQUISITION

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Abstract

Carotid atherosclerosis is the main cause of stroke – the fourth leading cause of death in Canada – and can be quantified by ultrasound measurements. Intima-media thickness (IMT), total plaque area (TPA) and 3-dimensional ultrasound vessel wall volume (3DUS VWV) were compared in a longitudinal study of 71 patients with diabetic nephropathy randomized to vitamin B or placebo. Only 3DUS VWV was sensitive to a difference in change between treatment groups. We developed and tested cardiac-gated 3DUS acquisition for use in younger subjects with compliant arteries; images were acquired from 400 ms after the start of the cardiac cycle to the beginning of the next cardiac cycle. In healthy volunteers and rheumatoid arthritis patients, change in area over the cardiac cycle was reduced to below that seen in moderate atherosclerosis patients. 3DUS VWV can measure change in atherosclerosis and can now be used in younger patients at risk of atherosclerosis in future studies.

Keywords: carotid, atherosclerosis, imaging, ultrasound, intima-media thickness, total plaque area, vessel wall volume, cardiac gating, ECG gating

Co-Authorship

The following thesis contains manuscripts prepared for submission for publication to scientific journals. Chapter 2 forms the basis of an original manuscript entitled "Longitudinal Measurement of Atherosclerosis in One, Two and Three Dimensions" submitted to the journal *Ultrasound in Medicine and Biology* on June 23, 2008 and accepted for publication September 10, 2008. This manuscript was coauthored by Christiane Mallett, Andrew House, J. David Spence, Aaron Fenster and Grace Parraga. Chapter 3 forms the basis of an original manuscript in preparation for submission to the journal *Medical Physics*, entitled "Prospective Cardiac Gating of Three-Dimensional Carotid Ultrasound" and coauthored by Christiane Mallett, Lori Gardi, Aaron Fenster and Grace Parraga. As principal author and Master's candidate, Christiane Mallett performed all vessel wall volume measurements, designed and carried out the cardiac gating experiments and analysis methods, processed and analysed all the data, performed all statistical analyses and wrote the original drafts of the papers contained in the manuscript. Lori Gardi designed and wrote the software to implement cardiac gated acquisition in consultation with Christiane Mallett. Dr. Parraga, as the principal author's supervisor, helped to determine project objectives, reviewed the results, provided mentorship, editorial assistance, and overall guidance. .Dr. Fenster provided ongoing guidance and feedback and reviewed the results. Dr. Andrew House and Dr. J. David Spence provided clinical expertise and helped in the interpretation of the results. Clinical and ultrasound data analysed for Chapter 2 of this thesis were acquired as part of the CIHR- and Kidney Foundation-sponsored study, Diabetic Intervention with Vitamins to Improve Nephropathy (DIVINe). Ultrasound image acquisition was performed by Maria DiCicco and Janine Desroches at the Stroke Prevention and Atherosclerosis Research Centre as well as Christiane Mallett and Ashley Opperman at Robarts Research Institute.

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List of Abbreviations

ACE	Angiotensin-converting enzyme
AGE	Advanced glycosylation end product
ApoB	Apolipoprotein B
AHA	American Heart Association
CAC	Coronary artery calcification
CAS	Carotid artery stenting
CCA	Common carotid artery
CAE	Carotid arterial endarterectomy
CHD	Coronary heart disease
CRP	C-reactive protein
COV	Coefficient of variation
CT	Computed tomography
DMARD	Disease-modifying anti-rheumatic drug
DUS	Doppler ultrasound
ECA	External carotid artery
ENHANCE	Ezetimibe and Simvastatin in Hypercholesterolemia Enhances Atherosclerosis Regression
HDL	High density lipoprotein
HMG-Co-A	3-hydroxy-3-methylglutaryl-coenzyme A
HRT	Hormone replacement therapy
ICA	Internal carotid artery
ICC	Intraclass correlation coefficient
IMT	Intima-media thickness
IVUS	Intravascular ultrasound
LDL	Low density lipoprotein
MA	Moderate atherosclerosis
MRI	Magnetic resonance imaging
MWT	Mean wall thickness
NA	Not applicable
NASCET	North American Symptomatic Carotid Endarterectomy Trial
PPAR γ	Peroxisome proliferator-activated receptor γ
RA	Rheumatoid arthritis
SD	Standard deviation
SPARC	Stroke Prevention and Atherosclerosis Research Centre
tHcy	Total homocysteine, also denoted homocyst(e)ine
TIA	Transient ischemic attack
TPA	Total plaque area
TPV	Total plaque volume
US	Ultrasound
VLDL	Very low density lipoprotein
VWV	Vessel wall volume
2D	Two-dimensional
3D	Three-dimensional

Chapter 1: Introduction

1.1 Overview

Cardiovascular disease is the leading cause of death worldwide, resulting in myocardial infarction, stroke and congestive heart failure; it is estimated that in 2005, of the 17.5 million people who died from cardiovascular disease, 7.6 million had myocardial infarction and 5.7 million had strokes (1). The picture is similar in Canada, where in 1998 heart disease and stroke combined were estimated to have large direct (6.8 billion dollars) and indirect (11.6 billion dollars) costs (2). Strokes are debilitating not only because of mortality risks, but also due to morbidity; accordingly, stroke is the leading cause of disability in the United States (3).

The main cause of cardiovascular disease is atherosclerosis, the accumulation of lipids and other particles in the arterial walls. A common location for plaque formation is the carotid artery; this is a concern because plaque rupture and thrombus in the carotid can lead to ischemic stroke. Atherosclerosis develops in stages from early, non-symptomatic lesions to advanced event-causing lesions by an inflammatory process that is also dependent on plasma cholesterol carried by low- and high-density lipoproteins.

Some risk factors for atherosclerosis such as smoking, diabetes and cholesterol were identified through a large ongoing cohort study, the Framingham Heart Study (4), while others have more recently been postulated and tested. Several diseases can also accelerate the atherosclerosis process. Treatments related to risk reduction are focused on reducing hypertension, decreasing plasma LDL cholesterol and increasing HDL cholesterol, as well as prevention of events through surgery.

Increasingly, medical imaging is playing an important role in the characterization of disease and in the evaluation of new treatments (5-7). Imaging techniques allow for direct quantification of atherosclerosis extent and composition and can be used in serial studies to monitor response to treatment. One such technique developed in our lab is three-dimensional ultrasound vessel wall volume (3DUS VWV), which quantifies wall

and plaque changes (8). The first part of this thesis is focused on the use of 3DUS VWV to quantify atherosclerosis change over time in patients with diabetic nephropathy, in comparison to 1- and 2-dimensional carotid ultrasound phenotypes. VWV has been validated in subjects with moderate atherosclerosis in their 60s, but we wish to extend this imaging technique to younger subjects in vulnerable populations, such as those with rheumatoid arthritis or diabetes. Carotid ultrasound imaging in younger subjects is complicated by arterial compliance that is not an issue for older subjects. The second part of this thesis discusses the development of a new technique for ultrasound image acquisition, cardiac-gated 3DUS and its implementation in healthy volunteers and rheumatoid arthritis patients.

1.2 Carotid Atherosclerosis

1.2.1 Anatomy

The carotid arteries (Figure 1-1) originate from the aorta distal to the aortic arch, and are located on either side of the neck. At approximately the level of the jaw, the common carotid artery (CCA) branches to form the external carotid artery (ECA), which supplies blood to the face and neck, and the larger internal carotid artery (ICA), which leads to the brain.

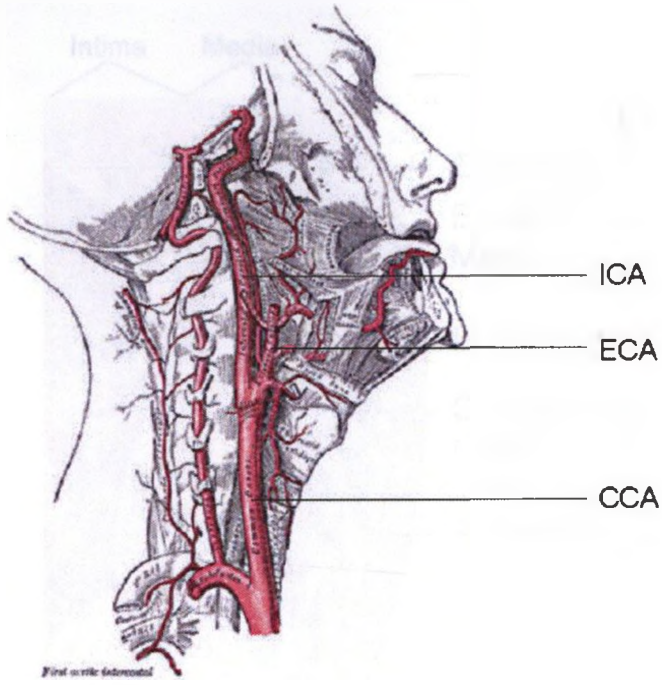

Figure 1-1: Carotid Artery Anatomy.

The common (CCA), internal (ICA) and external (ECA) carotid arteries in their anatomical positions in the head and neck. Adapted from (9).

The carotid artery is classified as an elastic artery, with a complex wall structure, seen in simplified form in Figure 1-2. The outermost layer is the adventitia, composed of connective tissue, which anchors the artery to the surrounding tissue. The media is composed of 40-70 alternating layers of elastic lamellae and smooth muscle cells in a matrix of chondroitin sulphate, collagen, reticular and elastin fibres. The innermost layer is the intima, composed of fibroblasts, smooth muscle cells and collagen. Adjacent to the lumen is a thin layer of endothelial cells. The intima and media are separated by the internal elastic lamina, and the media and adventitia by the external elastic lamina (10).

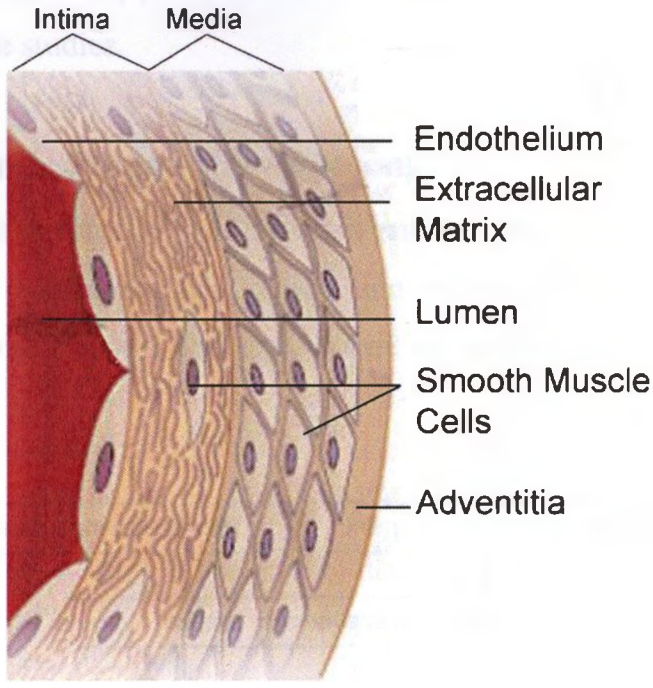

Figure 1-2: Artery Wall Anatomy.

Simplified scheme of the layers of the arterial wall. Adapted from (11).

The anatomy of the artery wall is critical to the normal function of the cardiovascular system. As the heart pumps blood, some of the energy is stored in the elastic arteries (including the carotid artery) so that there is constant pressure and flow to the smaller arteries and capillaries (12). It is the elastic nature of the media, primarily due to the elastin content, which allows this expansion to occur (10). Arterial expansion can be quantified by compliance, the change in volume per change in pressure, or by distensibility, the percentage change in volume per change in pressure (13).

Compliance decreases with age (14) as the elastin is replaced by fibrous tissue (13,15). Compliance is also affected by a decrease in estrogen; post-menopausal women who are not on hormone replacement therapy (HRT) have been found to have stiffer arteries than pre-menopausal women (16). The link between estrogen and compliance has been postulated to involve increased bioavailability of nitrous oxide (NO), which relaxes smooth muscle cells (17), as well as with an increase in media thickness, with an attendant increase in elastin (18). There are mixed reports about an improvement in

compliance with HRT (19-21), possibly due to varying delays between menopause and start of therapy in these studies.

1.2.2 Atherosclerotic Plaque Development

Atherosclerotic plaque development is complex and involves many stages. Figure 1-3 indicates the types of plaque and their progression as described by the American Heart Association, in a scheme that was developed based on histological examinations of post-mortem samples.

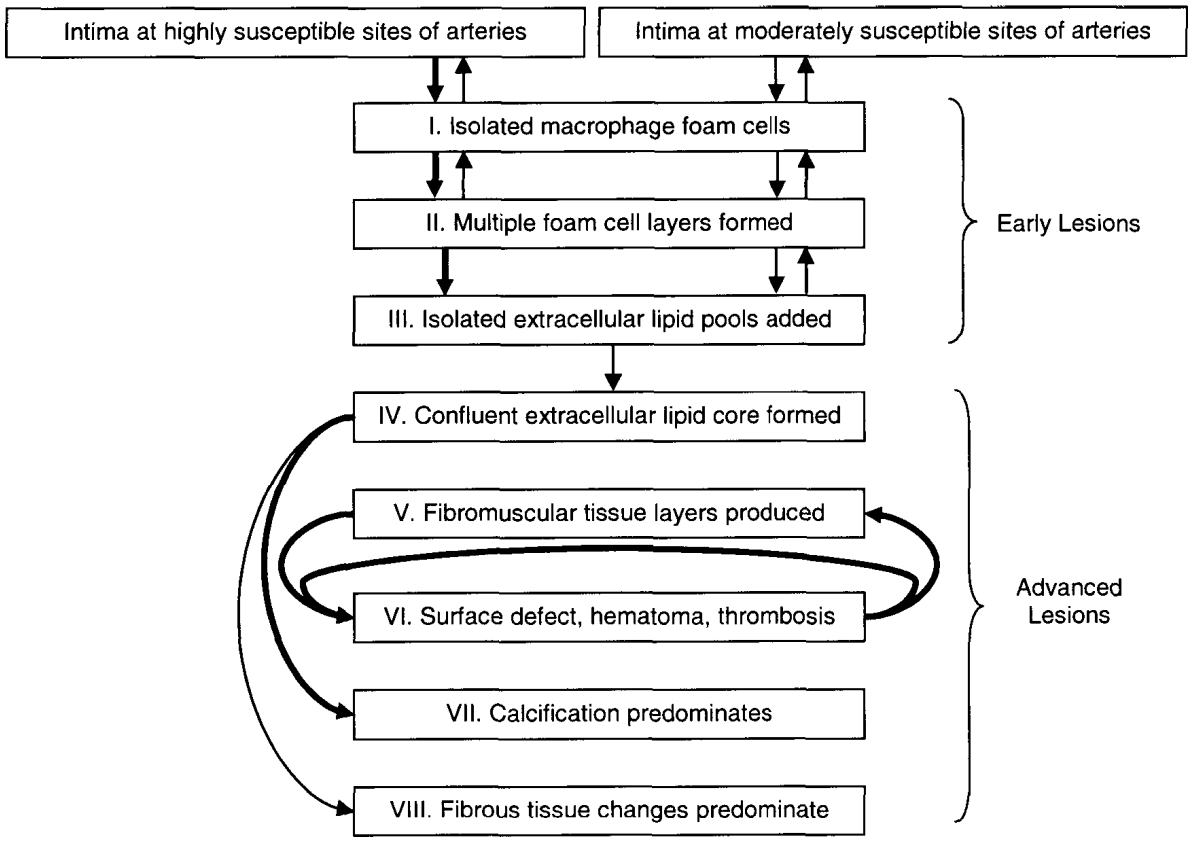

Figure 1-3: AHA Plaque Classification Scheme.

Plaque is most likely to develop as indicated by the bold arrows, but additional pathways are possible as indicated by the fainter arrows. Adapted from (22).

There are several critical molecules involved in atherogenesis whose role will be discussed below. Cholesterol is synthesized in the liver from acetyl coenzyme A by the HMG-reductase pathway and transported in the blood by lipoproteins. Low-density lipoprotein (LDL) is involved in transportation from the liver to tissues, and high-density

lipoprotein (HDL) is responsible for clearance of cholesterol from peripheral tissue to the liver, termed reverse transport. Apolipoprotein B (ApoB) on the surface of the LDL particle mediates binding to hepatic and arterial wall receptors (23,24).

The initial stage of atherosclerosis is the development of foam cells of macrophage origin. In the presence of hypercholesterolemia, the endothelium undergoes focal activation as LDL particles infiltrate the intima and cause inflammation (25). This inflammation activates endothelial cells and causes the expression of leukocyte adhesion molecules, adhering monocytes to endothelial cells on the arterial wall instead of allowing them to flow past (11). These monocytes then migrate into the intima where they begin to bind lipoprotein particles and become known as 'foam cells'. A feedback loop is initiated in which the foam cell increases the inflammatory reaction and reactive oxygen species in the lesion by expression of pro-inflammatory cytokines (11,26). These scattered foam cells are present even in infants (26). Type II lesions are characterized by the layering of the foam cells, which can lead to fatty streaks in the artery, visible to the naked eye. In type II lesions, lipid droplets are present in smooth muscle cells, with little extra-cellular lipid content (26). The fatty streak then increases in size and complexity over time, a process currently hypothesized to be influenced by physical disruption of the plaque (11). Progress of these lesions occurs mostly at highly susceptible sites which are characterized by more lipid macrophages and a thicker intima (26). Type III lesions, when scattered extracellular lipid pools develop, represent the next stage of plaque formation and the last stage of the 'early lesions' (26).

In type IV lesions, the scattered lipid pools have merged to form a lipid core, which continues to accumulate lipid from the plasma (27). At this stage, the atheroma does not cause narrowing, or stenosis, of the vessel because of compensatory enlargement of the adventitia (28). The organelles may show signs of calcium accumulation (27). As the atheroma begins to encroach on the lumen, and the cap composition contains more collagen and other fibrous tissue, the lesion is classified as type V (Figure 1-4) (27). Collagen content of the cap is decreased when the intima is inflamed, making it more prone to rupture (11).

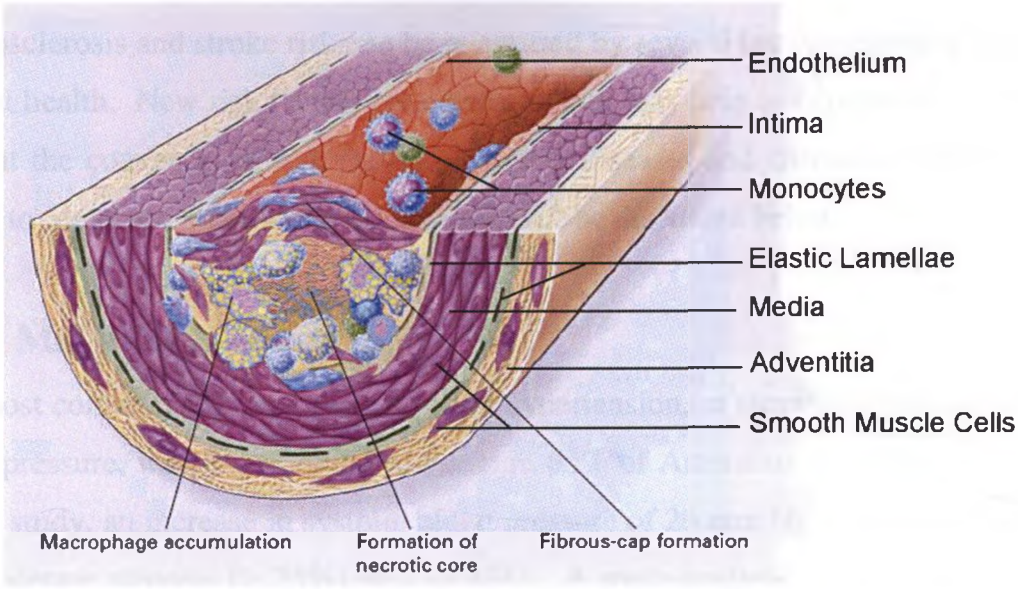

Figure 1-4: Complicated Lesion.

In this schematic, the atheroma encroaches into the lumen and has an accumulation of macrophages, a necrotic core and a fibrous cap, corresponding to a type V lesion in the AHA classification scheme. Adapted from (29).

Type VI lesions may arise from type IV or type V; in these lesions at least one of surface defect, hematoma or thrombosis are present (22,27). Surface defects, or fissures and ulcerations, can lead to plaque rupture, and the formation of a thrombus on the plaque surface when the thrombogenic contents of the plaque are released (11). If the thrombus remains on the surface of the plaque, the cap can be rebuilt with fibrous tissue; sequential events of this type will stabilize the plaque in type V lesions but also encroach on the lumen (11,22). Lesions may also become calcified as minerals replace the dead cells at the centre of the plaque (the necrotic core); these are termed type VII lesions in the AHA scheme (27). Type VIII lesions have a fibrous cap instead of one made of intimal tissue (27).

1.3 Risk Factors for Atherosclerosis and Stroke

Atherosclerosis and stroke risk can be quantified by several factors related to lifestyle and general health. New risk factors are emerging that may help to explain events in subjects without the conventional risk factors. Several diseases and chronic conditions are also linked to increased atherosclerosis risk and will be discussed below.

1.3.1 Major Risk Factors

The most common risk factor for stroke is hypertension, or elevated systolic and diastolic blood pressure, which was found to occur in 65% of Americans over the age of 65 (30). In one study, an increase in systolic blood pressure of 20 mm Hg increased the odds ratio for moderate stenosis ($\geq$ 25%) to 2.21 (31). A meta-analysis found that a decrease in diastolic blood pressure by 5-6 mm Hg was associated with a 35-40% decrease in stroke incidence and a 20-25% reduction in coronary heart disease (CHD) (32).

Smoking increases atherosclerosis risk as well, with an odds ratio for moderate stenosis of 1.08 (31). In animal models, exposure to cigarette smoke increases plaque volume and extent (33,34). The exact mechanism for the increased risk is not well understood, but there are several hypotheses related to endothelial effects, including inflammation and decreased vasodilation, as well as lipid modification (35). Smoking is correlated with increased arterial wall thickening and with decreased flow-mediated dilation (36). Tobacco smoking may also enhance LDL oxidation; smokers with hypercholesterolemia had significantly higher levels of LDL antibodies than controls (37).

Elevated levels of LDL cholesterol are associated with risk of cardiovascular disease, particularly of CHD (4,38,39). It appears that there is a 'U-shaped' relationship between LDL cholesterol and stroke; low levels of LDL are protective against ischemic stroke but may increase the risk of hemorrhagic stroke (40,41). Cardiovascular risk is inversely proportional to levels of HDL cholesterol (4,38,39,42). Even when LDL cholesterol has been reduced to below treatment targets, risk of CVD is related to low levels of HDL (43). For example, a 1 mg/dL increase in HDL resulted in a 2-4% reduction in coronary heart disease outcomes (42). Some studies have found no association between HDL and

ischemic stroke while others have demonstrated that increased HDL reduces ischemic stroke risk (44,45).

1.3.2 Genetic Factors

Genetic variation plays an important role in atherosclerosis risk as well; we shall outline a few of the many possible atherogenic mutations. In familial hypercholesterolemia, there is a mutation in the LDL receptor gene which impedes the clearance of LDL from the blood; the increase in serum cholesterol is much higher in homozygotes than heterozygotes (46). If left untreated, heterozygotes develop CVD by their 30s and 40s and homozygotes even earlier than that (47). This population is often used as a patient population for studies of new atherosclerosis treatments (6,48,49). Another genetic mutation causing increased serum cholesterol occurs in the gene for apolipoprotein B-100; when this protein is coded incorrectly, LDL cannot bind to hepatocytes and so serum cholesterol levels increase (47). Another harmful mutation occurs in the gene for peroxisome proliferator-activated receptor γ (PPAR γ), which in its usual form decreases the cholesterol content of macrophages (among other effects) (50). In one population, a mutation of PPAR γ was associated with increased atherosclerosis as assessed by ultrasound (51).

1.3.3 Framingham Risk Score

Cardiovascular risk related to these risk factors can be quantified by the Framingham Risk Score, derived from the Framingham Heart Study (38,52). Conducted beginning in 1948 and initially including over 5000 residents of Framingham, Massachusetts, the study has expanded to include offspring of the original study participants (53). Subjects underwent biennial cardiovascular exams and multiple logistic analysis was used to assess the effect of risk factors on cardiovascular outcomes (54). Significant risk factors extracted from the Framingham Heart Study have been incorporated into risk prediction tools that take into account age, sex, LDL cholesterol, HDL cholesterol, smoking, blood pressure, and diabetes and can predict 10-year CHD and overall CVD risk with a discriminatory power of approximately 75% (38,55). Some drawbacks of the study are that the subjects were primarily white, and aged 30-65 at inclusion; Framingham risk

scores may perform less well in non-white populations (56) as well as in diabetics (57) and other disease-specific populations that were not represented in the study.

1.3.4 Emerging Risk Factors

There is an emerging understanding that atherosclerosis development is also associated with risk factors not previously identified. Some data suggests that many patients with cardiovascular disease do not exhibit elevated total cholesterol (58,59), and that up to 50% of CHD patients have no risk factors (60). Others have refuted these claims; one meta-analysis estimated the proportion of CHD patients with no major risk factors as only 10-15% (61) and another found that 87%-94% of CHD patients had at least one major risk factor (62). Nonetheless, identification of additional risk factors beyond the traditional ones remains important, particularly in determining individual risk (63).

As an example, inflammatory markers such as C-reactive protein (CRP) are being considered as indicators of CVD risk. CRP is an acute-phase reactant that is released by the liver (63) and is one of several markers that are expressed as a response to inflammation (25). CRP may be involved in the recruitment of monocytes in the intima during atherogenesis (64) and in the regulation of LDL metabolism (65). Despite findings of increased relative risk of a first cardiovascular event, MI and stroke (59,66) with higher levels of CRP, its additional predictive value when added to standard risk assessment frameworks such as the Framingham Risk Score is modest (63).

Another emerging risk factor is the metabolic marker homocysteine, which is the breakdown product of the sulfur-containing amino acid methionine. For example, a genetic mutation that causes increased homocysteine production is related to increased risk of ischemic stroke (67,68). Plasma total homocysteine (tHcy) measurements include 3 components: homocysteine the disulfides homocystine and cysteine-homocysteine (69). High levels of tHcy are associated with increased atherosclerosis burden (70) and are predictive of MI and stroke (71,72). Possible mechanisms include an association with dyslipidemia (73), with increased production of adhesion molecules (74), with impaired vasodilation in response to stimulus (74,75), and with increased thrombosis and plaque

development (76). Since these effects are moderated by anti-oxidants such as vitamin E (74) and homocysteine concentration can be regulated by vitamin B therapy, clinical trials have been designed to test the causal relationship between homocyst(e)ine and cardiovascular events using various doses of B vitamins; the results of these trials have been inconclusive (77-80).

1.3.5 Other Diseases Affecting Atherosclerosis Risk

1.3.5.1 Rheumatoid Arthritis

Rheumatoid arthritis (RA) is an autoimmune disease that affects 300,000 Canadians (81). RA patients are at increased risk of atherosclerosis; it is estimated that RA patients experience cardiovascular events approximately 10 years before the general population (82). This risk is measured even when traditional risk factors are taken into account (83,84). Both traditional and disease-specific risk factors such as radiographic score, polymorphonuclear cell counts and joint space narrowing in the hands are associated with carotid plaque (85). One component of RA is systemic inflammation which is hypothesized as a contributor to the increased atherosclerosis risk seen in RA patients. Other possible influences are disease-modifying anti-rheumatic drugs (DMARDs), which also increase the risk of atherosclerosis (83).

1.3.5.2 Diabetes

Both type 1 and type 2 diabetes are also associated with an increased risk of atherosclerosis (4,86). As with rheumatoid arthritis, this increased CV risk is complex, arising from many factors, including hyperglycemia, inflammation and proteinuria. Hyperglycemia was found to increase risk of CHD, thromboembolitic stroke and overall mortality, when risk factors were taken into account (87,88). Glycosylated hemoglobin was associated with ischemic heart disease in type 1 diabetes and with ischemic heart disease and stroke in type 2 diabetes (89). One mechanism for this effect is an increase in oxidative stress from the production of reactive oxygen species in intracellular spaces (90). Additionally, glycosylation of proteins and lipids occurs; advanced glycosylation end products (AGEs) can accumulate on LDL particles, decreasing their clearance from the artery wall and increasing their susceptibility to oxidation (91). AGEs can interact

with endothelial cells to increase endothelial permeability and allow lipids to enter the artery wall (91). AGEs also contribute to the inflammatory process, which increases atherosclerosis as previously described.

1.3.5.3 Renal Insufficiency

Excretion of albumin in the urine is an independent risk factor for atherosclerosis. In one large study, microalbuminuria (classified as urinary albumin excretion of 20-200 μg/min) occurred in 30% of subjects with diabetes, but also in 5% of those without diabetes, hypertension or other potential confounders (92). In a cohort of subjects of African descent, microalbuminuria was associated with IMT in a model that was adjusted for traditional cardiovascular risk factors (93). Japanese type 2 diabetes patients with microalbuminuria had increased atherosclerosis as measured by aortic stiffness compared to age-matched controls (94). Increased albuminuria was associated with coronary artery stenosis and with increased coronary plaque thickness in type 1 diabetes (95). Diabetic nephropathy is characterised by increased proteinuria and retinopathy (95) and is associated with a sevenfold risk in cardiovascular mortality above that experienced by diabetic subjects without nephropathy (96).

1.4 Treatment of Atherosclerosis and Stroke Risk

The first line of treatment for atherosclerosis is to address risk factors, which can include weight loss and diet change to reduce cholesterol levels, smoking cessation, and control of blood sugar and hypertension, as recommended by the Canadian Association of Cardiologists (97). However, more aggressive treatment is often necessary as diet and exercise may not be sufficient to lower primary risk factors.

1.4.1 Hypertension Treatment

Control of hypertension is critical for reduction of stroke risk, given its prevalence in older adults (30). In one meta-analysis, a reduction in systolic blood pressure by 6 mm Hg was measured to decrease MI risk by 14% and stroke risk by 42% (32). Treatments for hypertension include diuretics and angiotensin-converting enzyme (ACE) inhibitors. Thiazide-type diuretics lower peripheral resistance, thus reducing hypertension (98) and

have been found to reduce stroke incidence by 36%. Because of their low cost and effectiveness, diuretics are the recommended first line of treatment for reducing hypertension (99). Angiotensin-converting enzyme converts angiotensin I to angiotensin II; the latter is responsible for vasoconstriction (100) as well as increased reactive oxidative species such as nitric oxide (100). Treatment with the ACE inhibitor rampiril resulted in a significant decrease in stroke risk as well as cardiovascular mortality (101).

1.4.2 Serum Cholesterol Alteration

As previously mentioned, high LDL cholesterol and low HDL cholesterol are risk factors for atherosclerosis. Both LDL and HDL cholesterol are targets of drug therapies to optimize their levels. Currently, a common treatment for elevated LDL cholesterol is statin therapy, but there are other possible treatments as well. HDL cholesterol is also being targeted to reduce atherosclerosis risk.

1.4.2.1 LDL Lowering Treatments

Statins, the class of HMG-CoA reductase inhibitors, target the rate-limiting step of the synthesis of cholesterol in the liver. This increases the production of LDL receptors on the hepatic surface, so that more cholesterol is cleared from the bloodstream. Statins are widely used to reduce levels of LDL cholesterol. Statin treatment significantly reduces CHD risk (102), and has been shown to reduce intima-media thickness (103) and coronary atherosclerosis burden (104,105). Statins reduce the risk of stroke or transient ischemic attack (TIA) in subjects with CHD (7) and are valuable in primary (102) and secondary stroke prevention (106).

The mechanism of action of statins in stroke prevention may be linked to factors other than a reduction of LDL cholesterol, such as an increase in plaque stability. A recent study found that although LDL levels decreased with statin treatment, carotid plaque volume did not change over time; however, the amount of lipid-rich necrotic core decreased (107). Other studies have found that statins reduce the macrophage count, concentration of oxidized LDL and smooth muscle cell proliferation in endarterectomy

specimens (108) as well as reducing the expression of adhesion molecules (109), suggesting that there is more to be understood about how statins reduce stroke risk.

Therapies that further lower LDL cholesterol beyond what is accomplished by high-dose statins are used to treat some patients. Ezetimibe lowers serum cholesterol by reducing absorption through the intestines, inhibiting Niemann-Pick C1-like protein (NPC1L1), a sterol transporter (110,111). Plasma LDL-C concentrations are decreased because of the resulting up-regulation of LDL receptors in the liver (112,113). In humans, ezetimibe can be added to standard statin therapy and is effective at reducing LDL-C levels in homozygous familial hypercholesterolemia patients with primary hypercholesterolemia (114). However, a recent study with an imaging endpoint found that a combined treatment of simvastatin and ezetimibe decreased LDL-C more than the statin alone, but with no corresponding decrease in intima-medial thickness (IMT) (115). In addition to its effects on LDL cholesterol, ezetimibe also modestly increases HDL cholesterol when combined with statins (116,117), as well as having beneficial effects on triglycerides (114,118) and C-reactive protein (118).

Several other drugs have been investigated for their LDL-cholesterol lowering effects. These include bile-acid binding resins such as cholestyramine, which inhibit the re-absorption of bile acids in the intestine. Cholesterol stores are used to produce new bile acids and thus serum cholesterol decreases (119). Significant reduction in LDL cholesterol and coronary events has been measured with cholestyramine (120). Fibrates such as gemfibrozil reduce LDL cholesterol levels by activating lipoprotein lipases that reduce triglyceride and LDL levels. In the Helsinki Heart Study, gemfibrozil decreased LDL cholesterol and increased HDL cholesterol (121). Niacin, also called nicotinic acid, inhibits the release of free fatty acids from adipose tissue, which decreases serum triglycerides and cholesterol (122,123). Its effectiveness is further discussed below.

1.4.2.2 HDL Raising Treatments

Niacin is one of the few therapies approved to raise HDL levels (124,125). It increases HDL cholesterol by increasing reverse transport of cholesterol from the vessel wall (126). Niacin monotherapy increases HDL by approximately 20%, with a small decrease in LDL (2%) (127). Combined niacin and statin therapy resulted in increased HDL compared to statin monotherapy after 1 year of treatment (125) with a regression in IMT after 24 months of treatment (124). Outcome trials have found a decrease in cardiovascular events with niacin treatment both alone (128) and in combination with statins (129). The common side effect of flushing is the dose-limiting factor and may contribute to low treatment compliance in patients (125,126).

HDL cholesterol can also be raised by inhibiting the action of cholesteryl ester transfer protein (CETP), which transfers cholesteryl from HDL cholesterol to lipoproteins containing apolipoprotein-B, thus increasing the availability of cholesterol-containing LDL and VLDL particles (130,131). A genetic deficiency of CETP is associated with high HDL (132) and levels of CETP are related to risk of coronary artery disease in healthy subjects (133).

Four randomized, placebo-controlled, double-blind trials were undertaken to test the effects of torcetrapib in combination with atorvastatin: RADIANCE-1, a carotid imaging trial in familial hypercholesterolemia subjects (48); RADIANCE-2, similarly conducted in mixed dyslipidemia patients (134); ILLUSTRATE, which examined coronary atherosclerosis (135); and the ILLUMINATE trial which tracked cardiovascular event rates (136). In all trials, HDL cholesterol was significantly higher in the torcetrapib/atorvastatin group than in the atorvastatin treatment arm, with increases of approximately 50-70% (48,134-136). In the imaging studies, there was no difference between treatments in the primary endpoints, but there was evidence of increasing atherosclerosis in some secondary endpoints (48,134,135). The trials were ended early because cardiovascular events were significantly higher in the torcetrapib/atorvastatin treatment group than in the placebo/atorvastatin group (136). This has been attributed in part to an increase in systolic blood pressure in the treatment group (48,134-136), but

other possibilities include effects on vasorelaxation and modification of HDL particles to change their functionality (48,134-136). Development of torcetrapib has ceased (136).

Anacetrabip is a new CETP inhibitor in the development phase. Early tests have shown a decrease in LDL and a larger increase in HDL than with torcetrapib, with no increase in blood pressure (137,138).

1.4.3 Surgical Treatment

In carotid arterial endarterectomy (CAE), a surgeon operates on the carotid artery and removes plaque from the inside of the artery (Figure 1-5). NASCET (North American Symptomatic Endarterectomy Trial) and ECST (European Carotid Endarterectomy Trial) studied endarterectomy in patients with symptomatic and asymptomatic carotid stenosis. They found a clear benefit for symptomatic high-grade stenosis (a reduction in luminal diameter of 70-99%) (139,140). After pooling the data from the two studies with re-analysis of ECST angiograms using the NASCET criteria (for consistent stenosis determination), there was a modest benefit in patients with 50-69% stenosis, but no benefit in patients with 30-49% stenosis and an increase in stroke with surgery in patients with less than 30% stenosis (139,141). Surgery is of the most benefit 2 weeks after the initial event (142). Risks from CAE include stroke, nerve injury, MI, coronary heart failure and infection (139,143), which must be considered before it is advisable in patients with asymptomatic stenosis, since the absolute risk reduction is 6% or less in these patients (144,145). Another possible complication is recurrent stenosis from recurrent atherosclerosis, which occurred in 5% of subjects after 60 months (146).

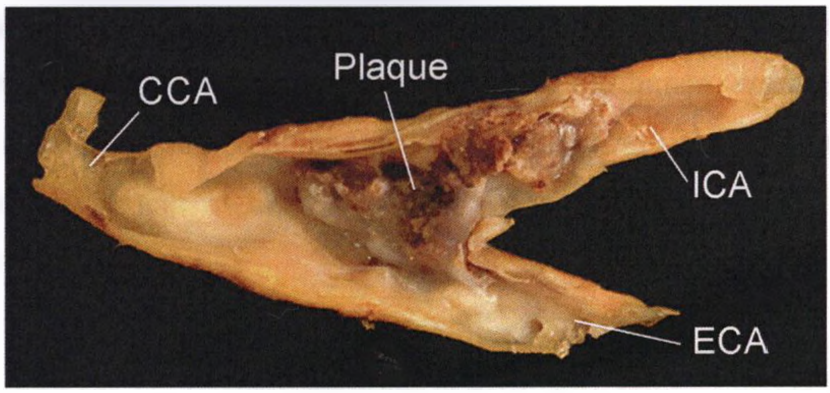

Figure 1-5: Endarterectomy Specimen.

Specimen from endarterectomy with arterial origin and plaque labelled. Approximate length, 10 cm. (Source: Ed Uthman, MD. Used with permission.)

In light of the complications inherent in an invasive surgical procedure such as CAE and the rate of restenosis, carotid angioplasty and stenting (CAS) has been investigated. In this catheter-based procedure, an angioplasty balloon is inflated inside the carotid artery and then a metal stent is inserted. However, the 30-day stroke risk is higher in CAS than in CAE (147). Randomized trials in symptomatic and asymptomatic (148,149) stenosis patients found that the relative risk of stroke was higher with stenting than with endarterectomy. It is possible that as surgeons gain more expertise with this technique it may be more appropriate (150); however, it is currently only recommended for patients with cerebral ischemia symptoms and greater than 70% stenosis who are at a high surgical risk from CAE (151).

1.5 Measurement of Atherosclerosis

1.5.1 X-Ray Imaging

In traditional contrast angiography, an x-ray contrast agent is injected through a catheter into the coronary or carotid arteries to allow an x-ray image to be acquired in which the arterial lumens are visualized. This procedure allows for measurement of the percent stenosis, obtained by comparing narrow vessels to a vessel segment presumed to be normal. Because of the diffuse nature of atherosclerosis, there are likely no unaffected coronary arteries, so stenosis may be underestimated in this method (152). The use of

two orthogonal views partially overcomes limitations including irregular plaque shape and vessel tortuosity (152). Because it only images the lumen, contrast angiography cannot detect the earlier, remodelling stages of atherosclerosis, and so underestimates disease severity and extent of atheroma (152). This method can also be applied in imaging of the carotid arteries.

Some of the limitations of conventional x-ray angiography can be overcome by the use of multi-row detector CT angiography, which can locate plaques in the coronary arteries and can distinguish between calcified and non-calcified plaques. However, it performs poorer than intravascular ultrasound at distinguishing between fibrous and lipid-rich plaques (153,154).

X-ray CT and electron-beam CT can also be used to quantify coronary artery calcification (CAC) by the coronary calcification score. In this method, the coronary arteries are imaged without contrast agents. Calcified areas are identified based on pixel intensity above a given CT number threshold. An Agatson score is assigned based on peak pixel brightness and area of calcium deposition (155). CAC is associated with increased risk of coronary events (156). The Agatson score may also be a predictor of stroke, although not as strong a predictor as IMT (157). There is still a debate about the benefit of CAC in addition to other risk assessment measures (158).

1.5.2 Magnetic Resonance Imaging

MRI can be used in a variety of ways to provide information about carotid atherosclerosis. Mean wall thickness (MWT) (Figure 1-6) of the intima, media and adventitia is correlated with IMT with the added advantage of measuring around the entire circumference of the artery (159,160). MRI is also sensitive to plaque composition (161,162) and can distinguish between plaque types in a slightly modified version of the AHA classifications of plaque (163).

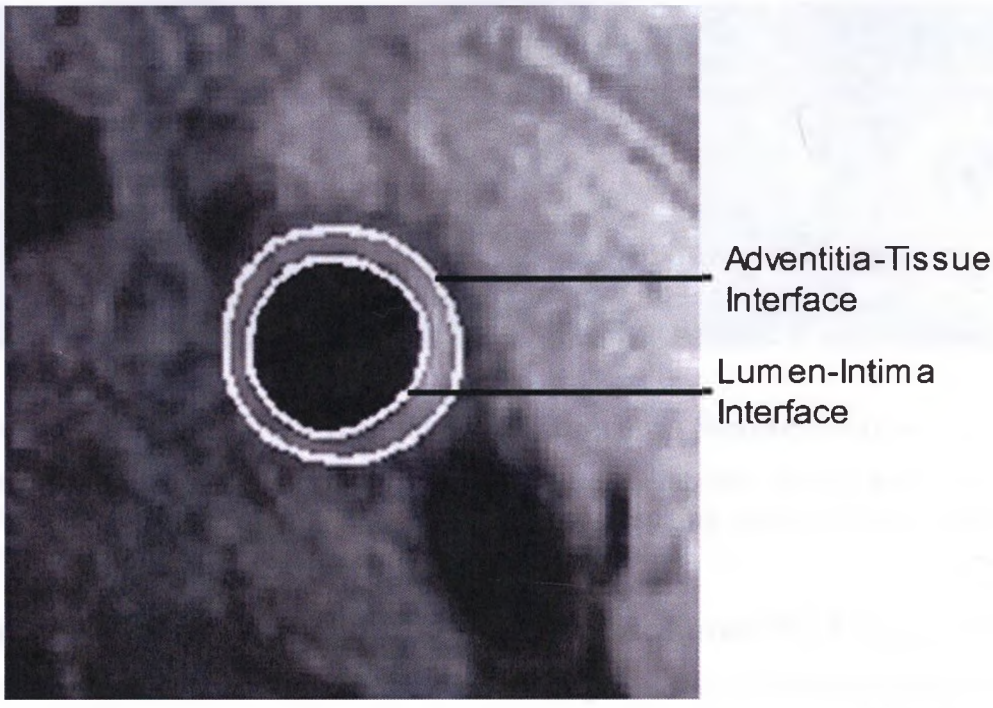

Figure 1-6: Sample Magnetic Resonance Imaging Mean Wall Thickness Image.

The lumen and adventitia of the carotid artery are segmented using an automated system. Adapted from (159).

An ongoing trial is testing the effect of lipid-modifying therapies on the lipid content of plaques using MRI as the imaging method (164). Previous studies using MRI phenotypes have examined the regression of plaque with statin treatment (165) and measured plaque volume and composition over time with statin treatment (107).

1.5.3 Intravascular Ultrasound

In intravascular ultrasound (IVUS), a catheter-mounted transducer is threaded through the arterial system through the femoral artery to the coronary arteries and pulled back at a constant speed during image acquisition, which can be gated to compensate for cardiac motion (166,167). Typical transducer sizes range from 0.9-1.2 mm, with frequencies ranging from 20-50 MHz, which corresponds to an axial resolution of 150 μm (168). Because of its invasive nature, diagnostic IVUS is performed only when angiography indicates a stenosis of at least 20% in one or more coronary arteries (169,170).

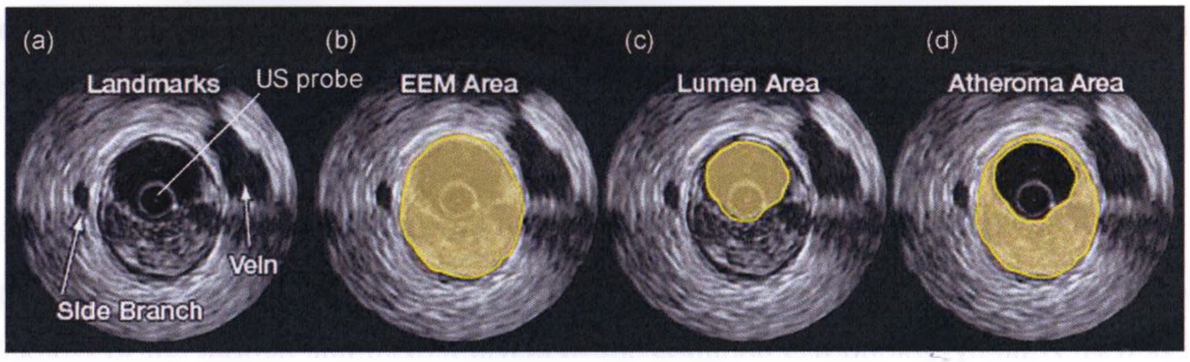

Figure 1-7: Sample IVUS Images with Atheroma Segmentation.

The coronary artery (a) with the external elastic membrane (media-adventitia boundary) segmented (b), the lumen segmented (c), and the resulting atheroma area highlighted (d). Adapted from (170).

Anatomical details of the artery wall can be visualised from IVUS images including the internal and external elastic laminae, as well as plaque accumulation (Figure 1-7). This structural information provides an advantage over angiography, which only visualises atheroma after it has begun to obstruct the lumen in the later stages of disease after compensatory enlargement has already occurred. Measurements derived from IVUS images include plaque area, and volumetric measures of total atheroma volume and percent atheroma volume (170-172) and have been used in clinical trials such as REVERSAL (Reversal of Atherosclerosis with Aggressive Lipid Lowering) (170) and ASTEROID (A Study to Evaluate the Effect of Rosuvastatin on Intravascular Ultrasound-Derived Coronary Atheroma Burden) (171).

There are some drawbacks to this imaging technique. Artefacts can result from slow-moving blood and an uncentred catheter (166). The invasive nature of IVUS means that there is a risk of side effects, the most common of which is vascular spasm (2.9% of cases), which is treatable with intracoronary nitroglycerin. Other complications, including thrombus, embolus, occlusion, myocardial infarction and emergency surgery occur but are much rarer (<0.8%). Overall, complications occur more frequently in patients with unstable angina or acute MI and are also more common in IVUS scans performed during interventions rather than for diagnosis (173). The potential for long-term effects from endothelial damage caused by the motion of the catheter in the

coronary arteries is a concern, but this has been examined in longitudinal studies and no adverse atherogenic effects due to imaging were seen (169,174).

1.5.4 Intima-Media Thickness

First developed in the mid 1980s (175), intima-media thickness (IMT) measures the thickness of the intimal and medial layers of the common carotid artery and is used as a surrogate biomarker for cardiovascular risk. IMT is measured from B-mode ultrasound images of the carotid artery and can be measured on the near or far wall (relative to the skin) of the CCA, ICA bulb and ICA, but in our lab it is measured on the far wall of the CCA (Figure 1-8). The choice of measurement location can have a large effect on the interpretation of the results (48).

IMT measurement has been extensively validated against histological samples (176,177). Increased IMT is associated with other biomarkers of atherosclerosis such as coronary stenosis (178) and with increased risk of stroke and MI (5,179,180). However, the primary cause of increased IMT is an increase in the thickness of the medial layer of the artery, while atherosclerosis occurs in the intima (181). Indeed, IMT is strongly associated with elevated blood pressure (182) and is more predictive of stroke than of coronary heart disease (180). IMT is widely used as a surrogate marker of atherosclerosis risk in trials of new treatments (6,48,134), as well as being used in cross-sectional studies as an indicator of future risk (5,179,183). However, it does not distinguish between age-related changes in the intima, such as intimal hyperplasia and intimal fibrocellular hypertrophy, and early atherosclerosis (184). Because of the slow rate of change of IMT, it is more suited to large population studies than to measuring individual risk (184,185).

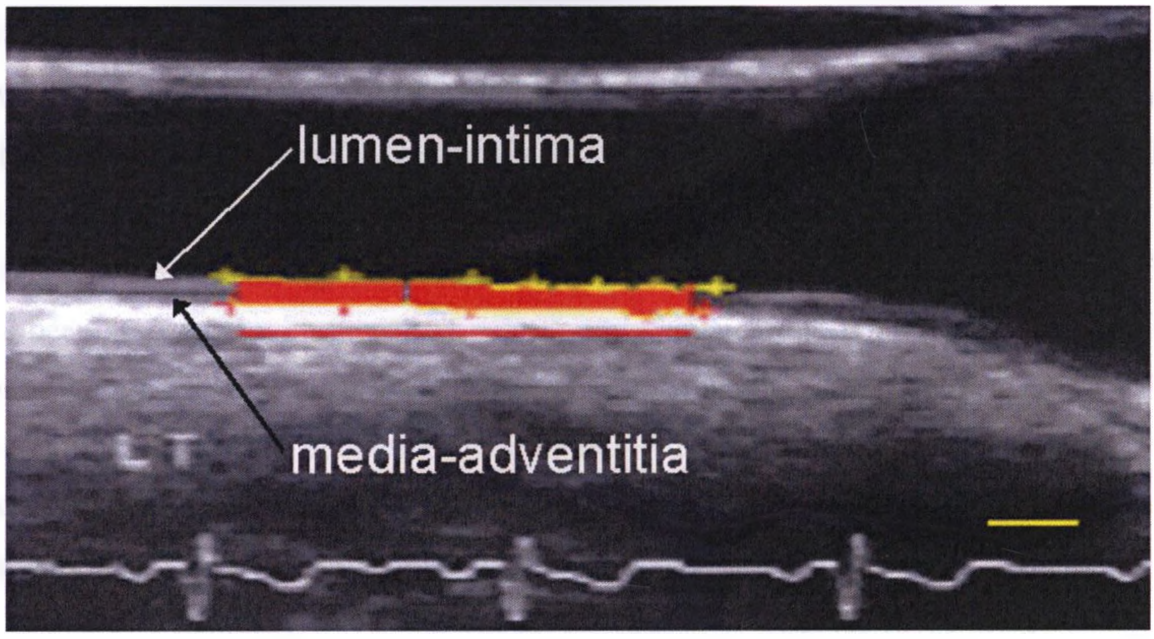

Figure 1-8: Sample Intima-Media Thickness Image.

Measurements are made on the far wall of the common carotid artery in a region 10 mm long (thin red line) which begins 5 mm proximal to the carotid bulb; the lumen-intima boundary is marked by yellow crosses and the media-adventitia boundary by red crosses with the measured thickness in red. Yellow scale bar indicates 2 mm.

1.5.5 Doppler Ultrasound

With Doppler ultrasound, degree of stenosis is assessed by measuring the speed of the blood in obstructed segments and comparing to disease-free segments. Carotid stenosis is an important indicator of stroke risk, as demonstrated by the reduction in stroke after endarterectomy (139,140). Doppler ultrasound can also be used in the cerebral arteries to detect microemboli by transcranial Doppler ultrasound (186). The value of Doppler-measured stenosis as compared to angiography is controversial. Eliasziw *et al* found only moderate accuracy of Doppler ultrasound compared to angiography, with no correlation between ultrasonographic findings and stroke (187). Others have disputed this result and found a consistent correlation between angiography and peak systolic velocity as measured by Doppler ultrasound (188,189).

1.5.6 Two-Dimensional Ultrasound Plaque Area

Atherosclerosis can also be quantified through measurement of plaque in the carotid arteries. In the phenotype of total plaque area (TPA), the maximum area of each plaque in a longitudinal view is calculated (Figure 1-9) and all plaques from the jaw to the clavicle in the left and right CCA, ICA and ECA are summed (190,191). Increased TPA has been associated with increased risk of stroke, myocardial infarction and death (192) and is a stronger predictor of myocardial infarction than IMT (193). However, TPA does not capture information about the circumferential distribution of plaque. As well, it is not well-suited for longitudinal studies because there are no anatomic markers of measurement location.

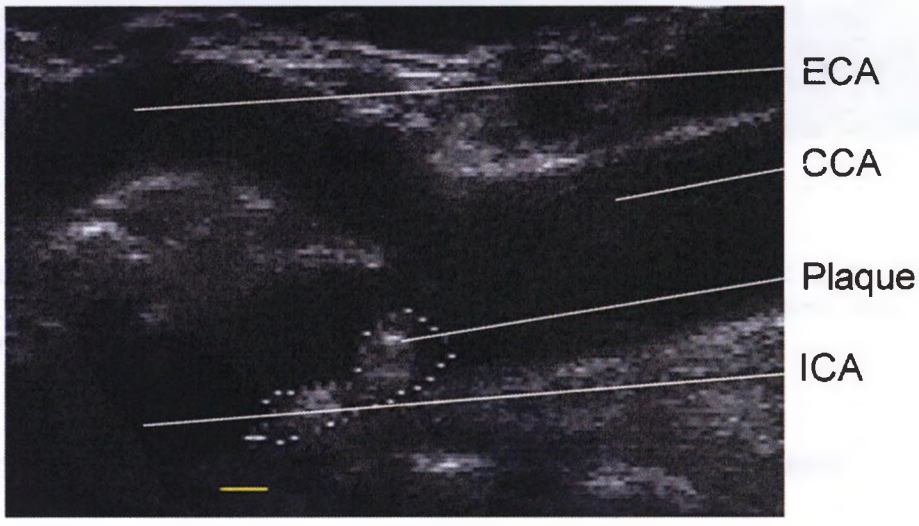

Figure 1-9: Sample Total Plaque Area Image.

B-mode ultrasound image of carotid artery bifurcation. The sagittal view in which the plaque has the largest area is isolated and frozen by the sonographer, the plaque is traced manually (in white) and its area measured. Yellow scale bar indicates 2 mm.

1.5.7 Three-Dimensional Ultrasound Total Plaque Volume

Total plaque volume (TPV) measurements are derived from 3-dimensional ultrasound (3DUS) images. These images are acquired by interpolating between sequential 2-dimensional images acquired at a known spatial location; this can be accomplished through either magnetic tracking of the ultrasound transducers or by the use of a mechanical acquisition system (194). Plaque area is manually segmented on sequential transverse slices through the common, internal and external carotid arteries in 1 mm

intervals, then the volume is calculated (Figure 1-10) (194-197). The apex of the carotid bifurcation is used as a reference point. TPV has been used to quantify change in plaque over time in a population treated with atorvastatin (198) and in cross-sectional studies of patients with diabetes (199,200) and with stenosis (200). Increased TPV has been associated with diabetes, plaque ulceration and age (199-201).

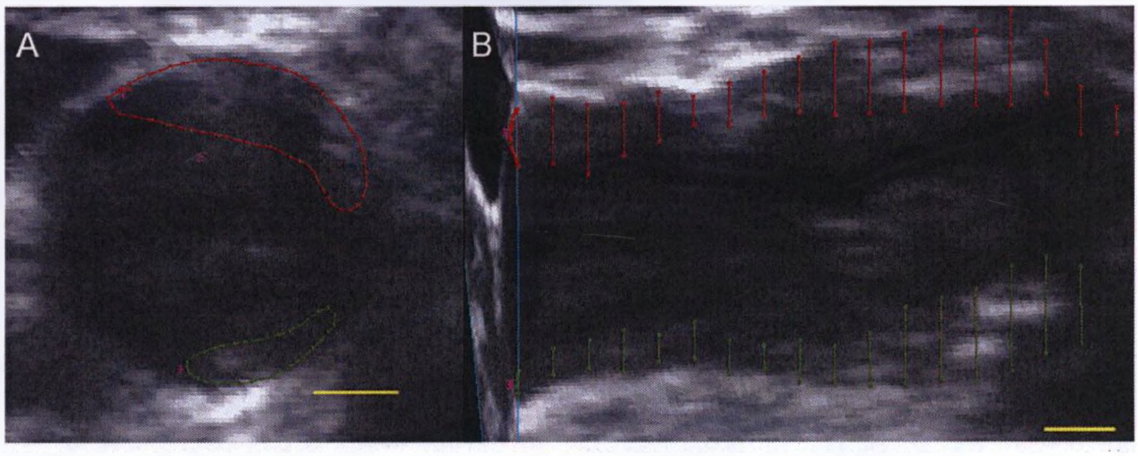

Figure 1-10: Sample Total Plaque Volume Images.

A: Cross-sectional view of carotid artery with 2 plaques segmented, one in red and the other in green. b) 3DUS image volume sliced to reveal plaque contours in sagittal view. Yellow scale bars indicated 2 mm. (Segmentations performed by Adam Krasinski.)

1.5.8 Three-Dimensional Ultrasound Vessel Wall Volume

Vessel wall volume (VWV) measurements are similarly derived from 3DUS images, but include wall thickening as well as plaque and have a lower inter- and intra-observer variability than TPV. The media-adventitia and lumen-intima boundaries are manually segmented in the common carotid artery and internal carotid artery (Figure 1-11). The volume of the inner surface is subtracted from that of the outer surface to yield a measurement of wall plus plaque thickness (8,202). The contours used to measure global VWV can be modified to provide information about local changes through the use of topology thickness maps (203). Surface contours are flattened from cylinders to sheets through an area-preserving algorithm and can then be displayed to show the distribution of wall thickness; maps from two examinations can be subtracted to show where change has occurred (Figure 1-12).

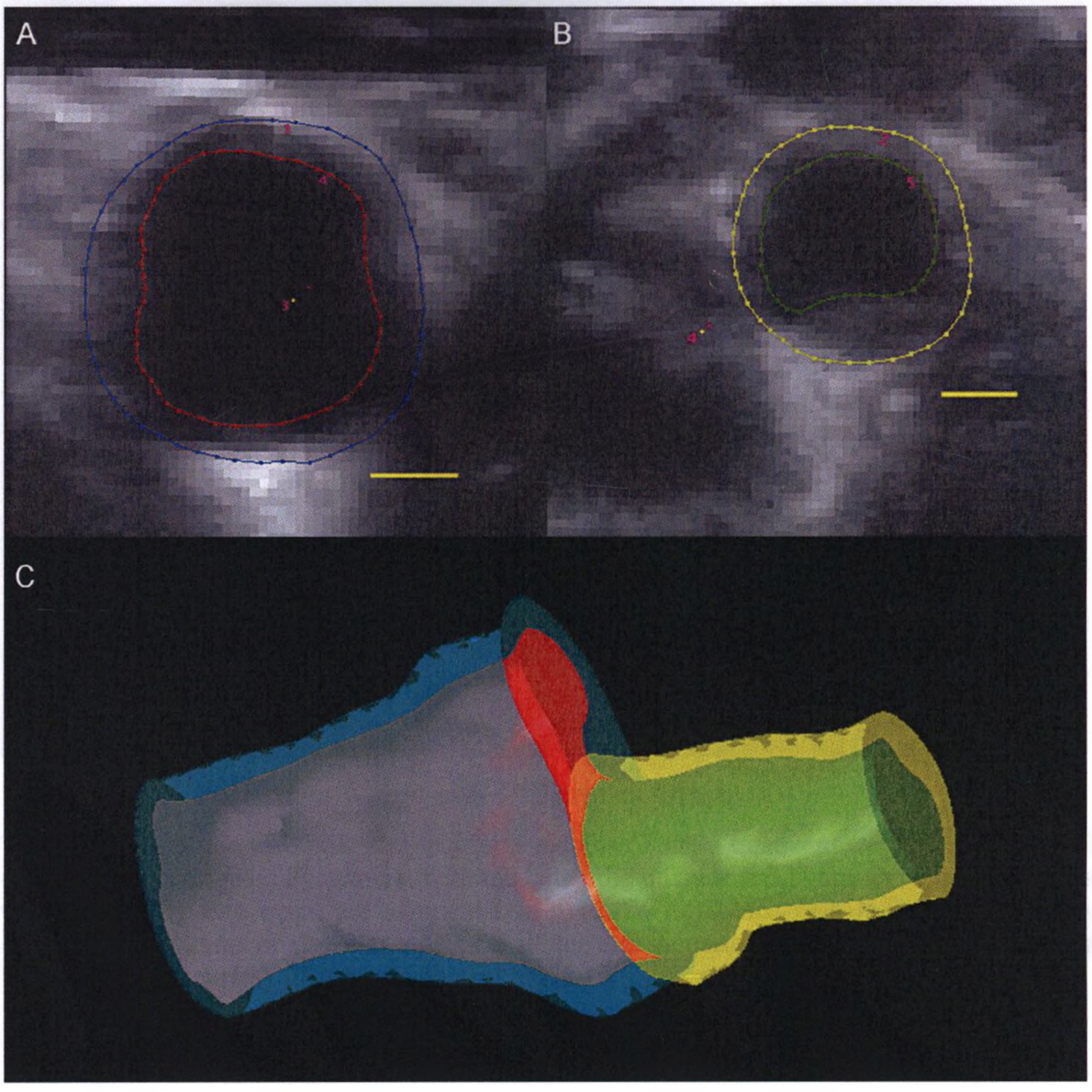

Figure 1-11: Sample 3DUS Vessel Wall Volume Images.

A: Axial view of common carotid artery with manual segmentation of lumen-intima boundary in red and media-adventitia boundary in blue. B: Axial view of internal carotid artery with manual segmentation of lumen-intima boundary in green and media-adventitia boundary in yellow. C: Sagittal view of three-dimensional surface rendering of volumes. Yellow scale bars indicate 2 mm.

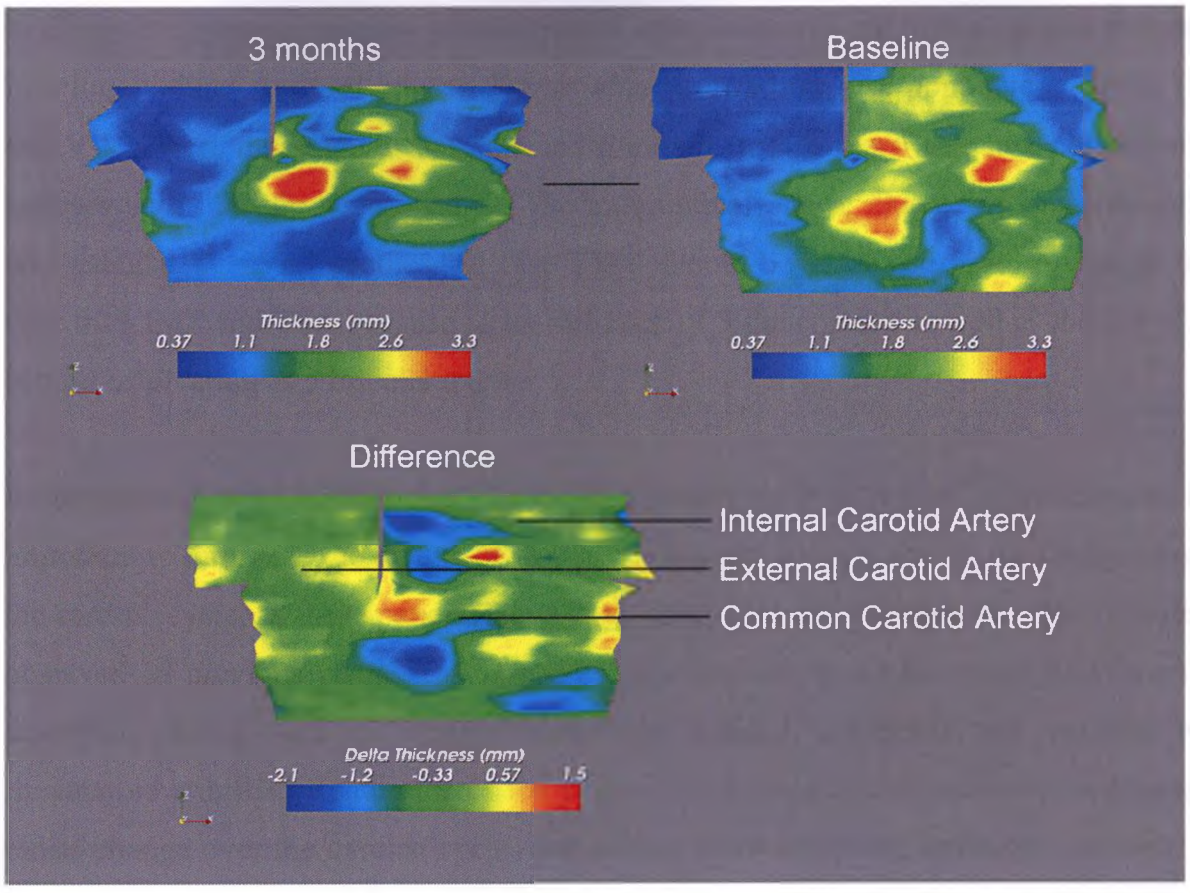

Figure 1-12: Thickness Maps and Difference Map.

The thickness of the wall plus plaque after 3 months of statin treatment (top left) and at baseline (top right) are indicated by colours, with red being the thickest at 3.3 mm and blue the thinnest at 0.37 mm. The two maps are subtracted to give the difference map (bottom) with areas of decrease in thickness of -2.1 mm indicated in blue and increase in thickness of 1.5 mm indicated in red. (Image courtesy of Adam Krasinski, 2008.)

1.6 Research Objectives

This project has two main research objectives: 1) to characterise the phenotype of 3-dimensional ultrasound vessel wall volume in a longitudinal study of patients at risk of atherosclerosis and 2) to design and implement cardiac gated acquisition fo 3-dimensional ultrasound images to increase precision of 3DUS VWV measurements. With the completion of these objectives, the utility of 3DUS VWV in longitudinal studies will be better understood, and image acquisition can be optimised for younger subjects in future studies.

In Chapter 2, three ultrasound phenotypes of atherosclerosis are evaluated and compared in a longitudinal study of atherosclerosis change in a high-risk population. IMT, TPA and VWV were measured at baseline and 6 months to 4.5 years later in a population of patients with diabetic nephropathy. The longitudinal rate of change of each phenotype was calculated. We hypothesised that VWV would be more sensitive to change over time than IMT and TPA because of its inclusion of 3 dimensions as well as the use of the carotid bifurcation as a fiducial marker.

In the acquisition of 3DUS images in younger patients at high risk of atherosclerosis, it is important to minimize the effects of changes in carotid artery diameter over the course of the cardiac cycle, as these patients have more compliant arteries than the elderly patients observed in previous studies. In Chapter 3, a method of cardiac-gated acquisition is described, along with its implementation in healthy volunteers and patients with rheumatoid arthritis. Requirements for acquisition include minimization of the effects of radial change over the cardiac cycle, and patient comfort during scanning. As well, the images had to be analysable and not distorted by the acquisition method. Our hypothesis was that a rapid method for ECG gating could quantitatively decrease arterial compliance effects in ultrasound images.

Chapter 4 describes the limitations of the research presented here, with proposed solutions. It also describes how 3DUS VWV could be used in the future for applications in clinical research and medical practice.

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Chapter 2: Longitudinal Ultrasound Evaluation of Carotid Atherosclerosis in One, Two and Three Dimensions

2.1 Introduction

Ultrasound methods play an important role in the evaluation and monitoring of carotid atherosclerosis, both in the clinical management of patients at risk of stroke and increasingly in clinical studies and trials of potential atherosclerosis treatments. For example, recently a number of key studies of established (1) and novel atherosclerosis treatments (2,3) have employed B-mode ultrasound measurements of intima-media thickness (IMT) as a surrogate or primary endpoint. In a comparison of the effects of atorvastatin alone and in combination with torcetrapib, IMT measurements at 12 locations in both carotid arteries were used as the primary endpoint (2,3). In this study, there was a significant difference in cardiovascular events between treatment arms (4), that was not reflected in the rate of change in IMT measurements (2,3). However, in the same study, the change in maximum and mean IMT at four carotid locations revealed a difference between treatment groups (2,3). Similarly, in the ENHANCE study of ezetimibe, a decrease in LDL cholesterol was observed in the group treated with both ezetimibe and atorvastatin compared to the group treated with atorvastatin only, but there was no difference in IMT between treatments (5).

Because of the apparent discordance in some studies between ultrasound measurements, clinical outcomes and plasma biomarkers, new multidimensional ultrasound imaging measurements of atherosclerosis are being developed that may provide more sensitivity or specificity to carotid artery changes that accompany treatment or other disease changes. As an example, B-mode ultrasound-derived 2-dimensional total plaque area (TPA) has been shown to be associated with increased risk of stroke, myocardial infarction and death (6). In addition, Johnsen et al. (7) also showed that TPA was a stronger predictor of myocardial infarction than IMT. We have also measured three-

dimensional ultrasound (3DUS) total plaque volume (TPV) (8,9) and vessel wall volume (VWV) (10,11), which quantifies plaque and wall thickness, length and circumferential distribution – in essence a 3D IMT measurement along the entire carotid artery. The measurement of 3DUS VWV, which includes both plaque and wall thickness, was previously validated in a study of 10 patients with moderate atherosclerosis (10) and then applied to the measurement analysis of patients with carotid stenosis (11).

Here we directly compare changes in IMT, TPA and 3DUS VWV in a subset of patients from the Diabetic Intervention with Vitamins to Improve Nephropathy (DIVINe) trial. All subjects were at increased risk of cardiovascular mortality because of diabetic nephropathy and diabetic microvascular disease (12,13). The trial was a placebo-controlled randomized trial designed to measure renal and cardiovascular outcomes after vitamin B therapy, which was hypothesized to decrease total plasma homocysteine, an emerging risk factor for atherosclerosis (14-16). Here we focus directly on the ultrasound imaging results, comparing the sensitivity of the three ultrasound phenotypes. To our knowledge, this is the largest number of study subjects within a randomized, controlled study that incorporates 1D, 2D and 3DUS measurement evaluations as well as the first report of the longitudinal measurement of carotid atherosclerosis progression using all three ultrasound measurements.

2.2 Methods

2.2.1 Subjects

Subjects with diabetic nephropathy were recruited from the Nephrology Clinics and Diabetes Clinics at the London Health Sciences Centre (London, Canada) and enrolled after providing written informed consent to the study protocol approved by a local research ethics board. In order to be eligible for the study, subjects required a diagnosis of type 1 or type 2 diabetes and a clinical or histological diagnosis of diabetic nephropathy, with at least 300 mg per day of urinary albumin excretion or 500 mg per day of protein excretion. Subjects were excluded if they were currently undergoing

dialysis, or had creatinine clearance below 30 mL/min or an expectation of mortality or complications during the course of the study. There was no screening based on carotid atherosclerosis measurements as the analysis presented here was a site-specific addition to the protocol. Based on the primary outcome measure for the DIVINe trial of decline in renal function, a total of 300 patients were projected to be enrolled at 5 clinical sites. This sample size was based on expected change in glomerular filtration rate (GFR), a measure of kidney function, and was based on results from Parving et al. (17). At baseline, all patients were randomized to either treatment group A or treatment group B; one group received a daily self-administered tablet of placebo and the other a combination tablet of 2.5 mg folic acid (vitamin B9), 25 mg pyridoxine (vitamin B6) and 1 mg cobalamin. Treatment group assignments have not yet been unblinded due to ongoing analysis of the renal function clinical data.

2.2.2 Image Acquisition Parameters

High-resolution B-mode ultrasound images were acquired with an optimized US system for carotid imaging (ATL HDI 5000, Philips, Bothel, Washington) with a 50 mm L12-5 MHz transducer with a central frequency of 7 MHz (Philips, Bothel, Washington). Baseline images were acquired on the same day as the screening visit. Ultrasound imaging parameters such as gain, time gain compensation and focal points were optimized for each patient by the sonographer taking into consideration the neck size, carotid anatomy and tissue depth penetration required. Patients were scanned at approximately 6 month intervals for the duration of their follow-up time, which was a maximum of 4.5 years. For this analysis baseline scans and the scans furthest from baseline were measured for each subject.

2.2.2.1 Intima-Media Thickness Acquisition and Measurement

Images for IMT measurement were acquired in an antero-lateral longitudinal view of the left and right carotid arteries and recorded on videotape for analysis with concurrent 12 lead electrocardiogram outputs. IMT was measured by one observer, blinded to treatment, who measured mean combined thickness of intima and media as previously

described (18) using computerized edge-detection software (ProWin™ Medical Technologies International (19)). All image analyses were performed at end diastole in the cardiac cycle. Briefly, measurements were made over a 10 mm segment of the common carotid artery 5 mm from the bulb on the far wall; if that region was unclear or if there was a plaque (defined as focal thickening > 1 mm) the operator could choose to measure on the near wall, a segment further from the bulb or at a different phase of the cardiac cycle. The median measurement from 3 cardiac cycles was recorded; we report the mean of the left and right sides.

2.2.2.2 Total Plaque Area Acquisition and Measurement

TPA measurements were performed as previously described (20,21) by one of two ultrasound technicians who were blinded to treatment. Briefly, each plaque was scanned in a longitudinal view until the maximum area of the plaque was in the plane of view, the image was frozen and magnified, and the ultrasound technician measured the area by tracing the perimeter with a cursor. TPA was recorded as the sum of the areas of all plaques from the clavicle to the jaw in the right and left carotid arteries.

2.2.2.3 Three-Dimensional Ultrasound Vessel Wall Volume Acquisition and Measurement

Three-dimensional ultrasound scans were acquired by freehand scanning along the neck for a distance of approximately 5 cm. Probe orientation and position were tracked with a magnetic tracking system and 2-dimensional images were reconstructed into a 3-dimensional volume; the EchoScan system (TomTec, Boulder, Colorado) was used from 2001-2003, and the 3D Echotech system (GE Medical Systems, Milwaukee, Wisconsin) was used from 2003-2007.

3DUS VWV was measured from anonymized and randomized images by one observer who was blinded to timepoint and treatment; both timepoints were analysed as a pair for consistency of interpretation. A variability trial was included in which 6 pairs of images (baseline and follow-up) were embedded four additional times into the series of scans to

be measured, such that each image in the variability trial was measured five times. The lumen-intima and media-adventitia boundaries of the carotid artery were manually segmented in the transverse plane, although the image volume could be manipulated in any plane to verify the segmentation, as previously described (10). Using a WACOM Intuos pen and tablet (Wacom Technology Corporation, Vancouver, Washington), the common carotid artery was segmented 15 mm proximally from the bifurcation in 1 mm increments. Similarly, measurement of the internal carotid artery was performed distally into the ICA to a maximum of 10 mm; for just under half of the subjects, artifacts or incomplete images precluded a full measurement of the ICA. The area enclosed by each segmented boundary was multiplied by the interslice distance and all areas were summed using Simpson's rule to obtain the final volume. VWV of the CCA (VWV_{CCA}) and VWV of the CCA and the maximum measurable length of ICA ($VWV_{CCA+ICA}$) were calculated as the subtraction of segmented lumen and wall volumes. It is important to note that the lumen was delineated on the basis of echolucency using all viewing planes in the 3D volume to help delineate the blood-intima boundary. Although it is usually straightforward when using all viewing planes to delineate hypoechoic plaque from the echolucent lumen, there is the potential to have included some hypoechoic and echolucent plaque within the manually segmented lumen volume and not in the wall volume.

2.2.2.4 Statistical Tests

Statistical analyses were performed using SPSS 16.0 (SPSS Inc., Chicago, Illinois). Intra-observer measurement variability of VWV measurements was calculated using the coefficient of variation (COV), which is the standard deviation divided by the mean of the measurements. Treatment groups were compared using 2-sided independent sample t-tests and each group was compared to zero using one-sample t-tests. Significance was assessed at the $p<0.05$ level.

2.3 Results

2.3.1 Study Population

Subject demographics are provided in Table 2-1. Of the 106 randomized subjects, 77 were scanned at baseline and at least once in follow-up; 29 were withdrawn from the study for clinical reasons such as dialysis, dementia or other intercurrent illnesses or were lost to follow-up. We present ultrasound analysis for 71 subjects, 34 in group A and 37 in group B because six subject images were of insufficient quality for analysis and these subjects were not included in the analysis of all imaging measurements. There were no differences in baseline clinical and ultrasound measurements between the subjects who were randomized in the two treatment groups except for baseline VWV_{CCA} which was significantly larger in treatment group A ($p=0.04$). As shown in Table 2-1, baseline VWV_{CCA} was higher in group A (1200 ± 300 cm^3) as compared to group B (1060 ± 300 cm^3) ($p=0.03$). A similar trend was observed for $VWV_{CCA+ICA}$ and IMT although the differences were not statistically significant ($p=0.054$, and 0.28, respectively). As well, there was a difference in VWV between males and females; for VWV_{CCA}, 1200 ± 300 mm^3 vs 940 ± 160 mm^3 ($p<0.001$) and for $VWV_{CCA+ICA}$, 1700 ± 400 mm^3 vs 1300 ± 300 mm^3 ($p<0.001$).

2.3.2 Carotid Atherosclerosis Measurements

Figure 2-1 provides images for the evaluation of 1D, 2D and 3DUS measurements at baseline and follow-up for two representative subjects in each of group A and B. The mean time between the first study visit and the baseline imaging visit was 3 ± 7 months (range: 0-36 months) with no difference between treatment groups ($p>0.05$). The maximum measurable length of ICA varied from 0 mm to 10 mm, with a mean of 9 ± 3 mm. The mean COV for the VWV_{CCA} measurement of six image pairs that were measured each five times in randomized and blinded order within the analysis images was 10%.

Table 2-1: Subject Demographics at Baseline.

Clinical values and ultrasound measurements at baseline are provided for all subjects who were randomized, scanned and evaluated (by treatment subgroup). For all measures, values were available for each subject in the category; in exceptions, the number of subjects with available measurements is given in square brackets. Errors given are standard deviations. ND=not done.

	Randomized n=106	Scanned n=77	Evaluated n=71	Group A n=34	Group B n=37
Clinical Values					
Male n (%)	73 (69)	56 (73)	51 (72)	26 (77)	25 (68)
Age	60±11	60±11	60±11	59±11	59±11
Pack-years of smoking	19±24	18±24	17±23	16±23	18±23
Treatment for hypertension n (%)	99 (93)	73 (95)	67 (94)	32 (94)	35 (95)
Hypertensive n (%)	94 (89)	68 (88)	63 (89)	30 (88)	33 (89)
Treatment for hyperlipidemia n (%)	85 (80)	62 (81)	57 (80)	29 (85)	28 (76)
High cholesterol n (%)	90 (85)	65 (84)	59 (83)	29 (85)	30 (81)
HDL cholesterol (mmol/L)	1.1±0.4 [93]	1.1±0.4 [69]	1.1±0.4 [63]	1.1±0.4 [28]	1.2±0.3 [35]
LDL cholesterol (mmol/L)	2.8±1.3 [83]	2.8±1.4 [62]	2.8±1.0 [56]	2.8±1.7 [27]	2.7±1.1 [29]
Homocyst(e)ine (µmol/L)	14.1±5.4 [64]	13.4±1.3 [48]	13.4±4.2 [44]	13.5±3.6 [21]	13.3±4.8 [23]
Ultrasound Measurements					
Mean IMT (mm)	ND	ND	0.9±0.2 [70]	0.9±0.3 [30]	0.8±0.2
Total Plaque Area (cm^2)	1.4±1.3 [103]	1.3±1.1	1.3±1.1	1.4±1.3	1.1±1.0
Total VWV_{CCA} (mm^3)*	ND	ND	1140±300	1200±300	1060±300
Total $VWV_{CCA+ICA}$ (mm^3)	ND	ND	1580±400	1680±470	1480±400

* Significant difference between subgroup A and subgroup B, p=0.03

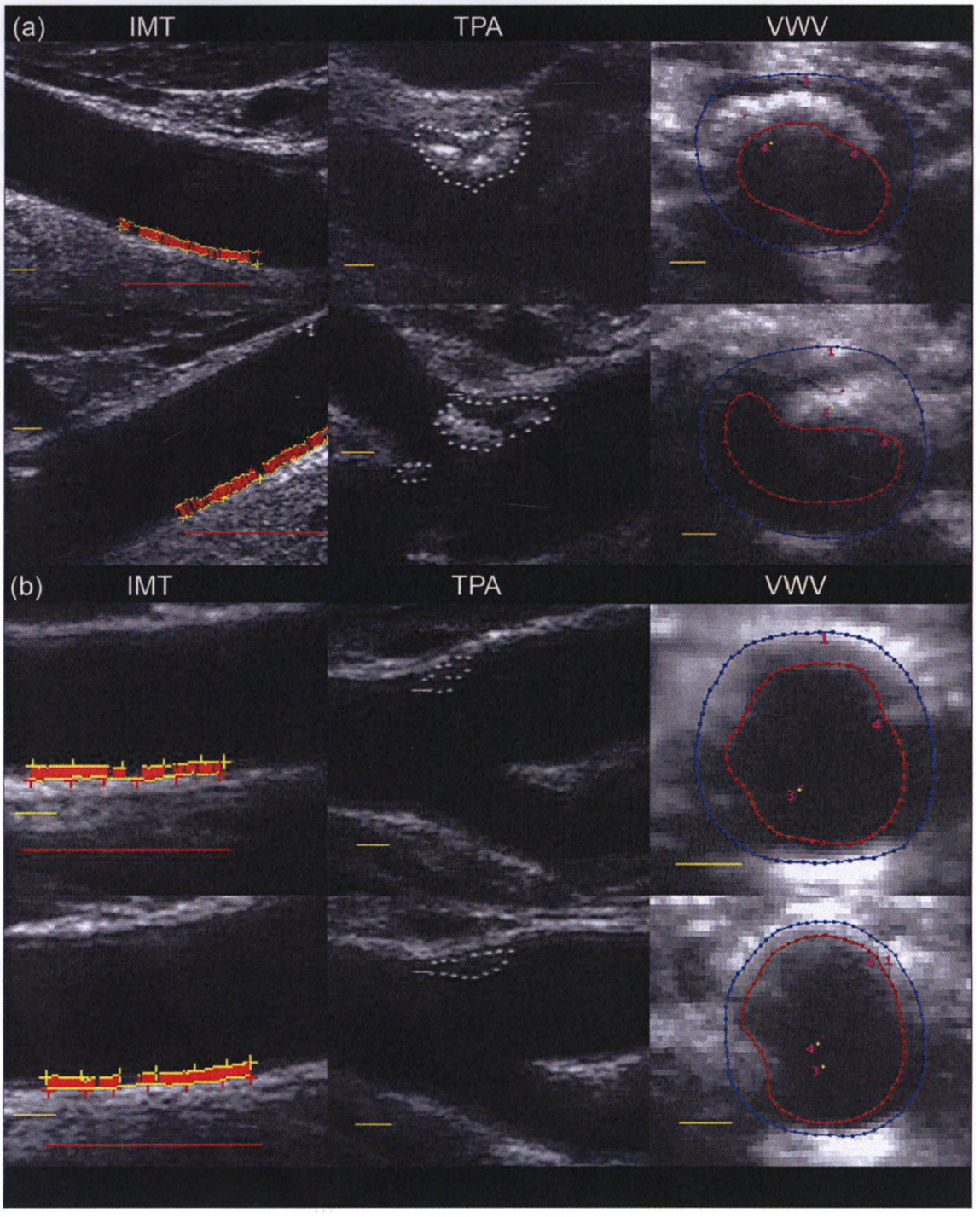

Figure 2-1: Ultrasound Measurements in Representative Subjects.

From left to right: Sagittal view of common carotid artery with IMT measurement, sagittal view of carotid artery with TPA outline for one plaque (in white), cross-sectional view of common carotid artery with VWV segmentation (lumen-intima boundary in red and media-adventitia boundary in blue). Yellow scale bars indicate 2 mm. (a) Images of the right carotid artery for a subject in group A. First row: images from baseline scans; second row: images from follow-up (1 year later) at approximately the same location. (b) Images of the left carotid artery for a subject in group B. First row: images from baseline scans; second row: images from follow-up (2 years, 7 months later) at approximately the same location.

2.3.3 Longitudinal Assessments

As shown in Figure 2-2, follow-up time varied among subjects, with a mean of 2.3 ± 1 years (range: 0.5 – 4.5 years). There was no difference in follow-up time between groups (p=0.08), but there were more subjects with a follow-up time less than 2 years in group B than group A (17 vs 8, p=0.049). Table 2-2 shows the measurements at baseline and follow-up and the rate of change (slope) for each of the measurements.

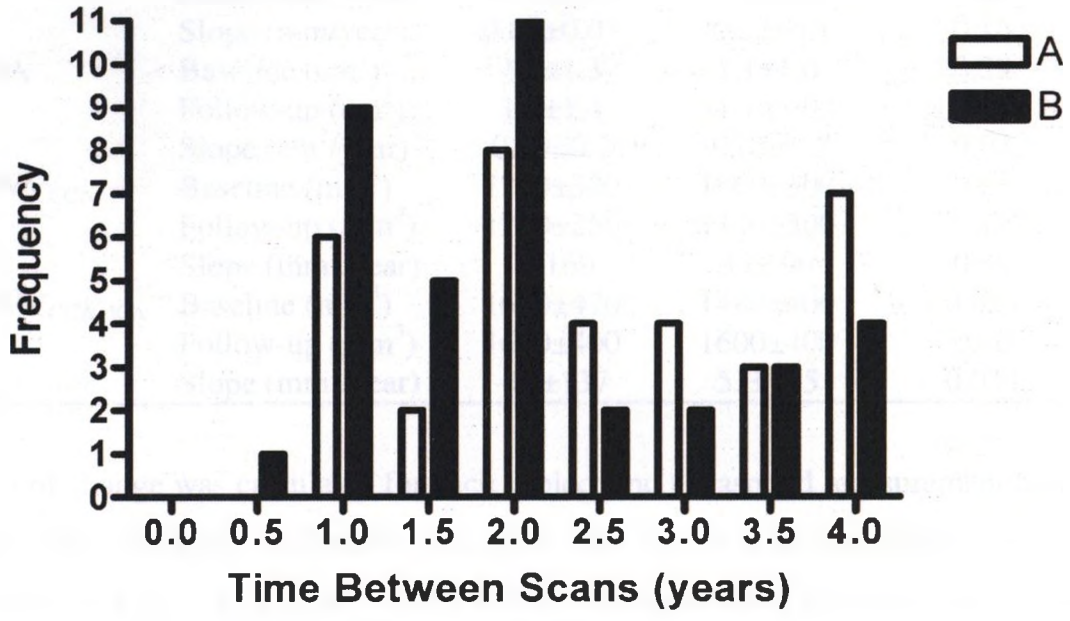

Figure 2-2: Ultrasound Scan Follow-Up Distribution.

The distribution of follow-up times for the 71 evaluated subjects, by treatment group.

Table 2-2: Ultrasound Measurements at Baseline and Follow-Up for each Treatment Group.

Follow-up time was considered when calculating the slope, which is the change measured over time. Significance of between-group differences is given. Errors given are standard deviations. Mean follow-up time is not significantly different between treatment groups.

Ultrasound Measurement		Group A n=34	Group B n=37	Significance
IMT	Baseline (mm)	0.90±0.3	0.84±0.2	0.28
	Follow-up (mm)	0.90±0.2	0.82±0.2	0.10
	Slope (mm/year)	0.02±0.07	-0.02±0.1	0.16
TPA	Baseline (cm^2)	1.4±1.3	1.1±1.0	0.29
	Follow-up (cm^2)	1.6±1.4	1.1±1.0	0.08
	Slope (cm^2/year)	0.09±0.2	-0.03±0.3	0.08
VWV_{CCA}	Baseline (mm^3)	1200±300	1060±300	0.03
	Follow-up (mm^3)	1220±250	1140±300	0.19
	Slope (mm^3/year)	7±160	33± 90	0.39
$VWV_{CCA+ICA}$	Baseline (mm^3)	1680±470	1480±400	0.054
	Follow-up (mm^3)	1670±400	1600±400	0.40
	Slope (mm^3/year)	-11±137	53±115	0.034

The rate of change was calculated for each subject and ultrasound measurement based on the time between scans, and mean rate of change for each measurement provided as shown in Figure 2-3. As provided in Table 2-2, the mean rate of change of IMT was 0.02 ± 0.07 mm/year in group A and -0.02 ± 0.1 mm/year in group B and neither slope was significantly different from 0 (p=0.15 and 0.43, respectively) with no difference between groups (p=0.15). The mean TPA rate of change was 0.09 ± 0.2 cm^2/year in group A, which was significantly different from 0 (p=0.013) and -0.02 ± 0.3 cm^2/year in group B which was not significantly different from 0 (p=0.6). In addition, for TPA there was no difference in rate of change detected between treatment groups (p=0.08). The mean rate of change of VWV_{CCA} was significantly greater than 0 (p=0.03) for group B (30 ± 90 mm^3/year) and not for group A (7 ± 150 mm^3/year) (p=0.8), but there was no difference detected between groups (p=0.4). The mean rate of change of $VWV_{CCA+ICA}$ was significantly greater than 0 for group B (53 ± 110 mm^3/year) (p=0.008) which was significantly (p=0.034) higher than the rate of change of $VWV_{CCA+ICA}$ for group A (-12 ± 137 mm^3/year). The group A $VWV_{CCA+ICA}$ rate was not significantly different than 0 (p=0.6). The rates were also calculated separately for short follow-up time and long

follow-up time. As provided in Table 2-3, there were no significant changes in subjects who were followed for less than 2 years. For subjects who were followed more than 2 years, there was significant progression of TPA in group A (0.08 ± 0.2 cm^2/year, p=0.041). In group B, there was significant progression of VWV_{CCA} (30 ± 50 mm^3/year, p=0.017) and $VWV_{CCA+ICA}$ (45 ± 70 mm^3/year, p=0.013), although only the change in $VWV_{CCA+ICA}$ was significantly different between groups (p=0.039).

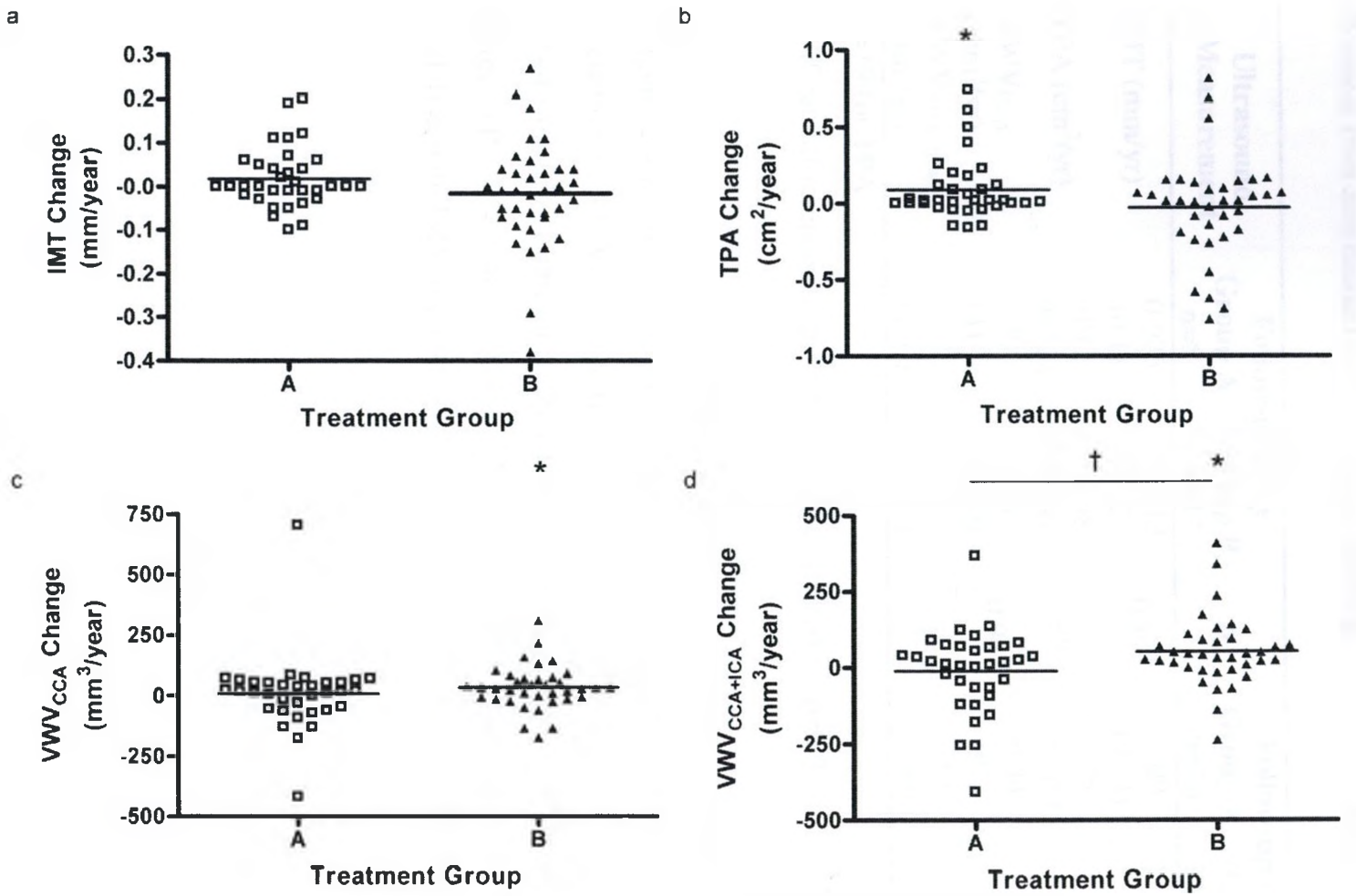

Figure 2-3: Ultrasound-Measured Change in Carotid Atherosclerosis.

a: IMT; b: TPA; c: VWV_{CCA}; d: $VWV_{CCA+ICA}$. Significant differences from 0 are marked with *, between groups are marked with † ($p<0.05$).

Table 2-3: Change in Ultrasound Measurements by Follow-Up Time.

Changes are presented for follow-up time of less than two years and of two years and over. Significance of between-group differences are provided, with any individual significant differences from zero denoted in the footnotes. Errors given are standard deviations.

Ultrasound Measurement	Follow-up < 2 y			Follow-up ≥ 2 y		
	Group A n=8	Group B n=17	p	Group A n=26	Group B n=20*	p
IMT (mm/yr)	0.075 (0.1)	-0.013 (0.2)	0.192	0.00 (0.04)	-0.18 (0.6)	0.244
TPA (cm^2/yr)	0.12 (0.13)	-0.08 (0.4)	0.248	0.08 (0.2)**	0.018 (0.2)	0.265
VWV_{CCA} (mm^3/yr)	30 (310)	35 (120)	0.947	0.44 (67)	30 (50)†	0.097
$VWV_{CCA+ICA}$ (mm^3/yr)	-8 (230)	62 (150)	0.364	-12 (100)	45 (70)‡	0.039

* n=19 for TPA

Significant differences from 0: ** p=0.041, † p=0.017, ‡ p=0.013.

Correlations were calculated change in all measurements by treatment group. As shown in Figure 2-4 the relationship between ΔVWV and ΔIMT was significant. In group A, the change in VWV_{CCA} over time was positively correlated with the change in IMT ($r=0.44$, $p<0.05$). In group B, the change in VWV_{CCA} was negatively correlated with the change in IMT ($r=-0.44$, $p<0.01$). If $VWV_{CCA+ICA}$ was considered, there was a significant correlation with IMT in group A ($r=0.395$, $p<0.05$) but not in group B ($p=0.08$).

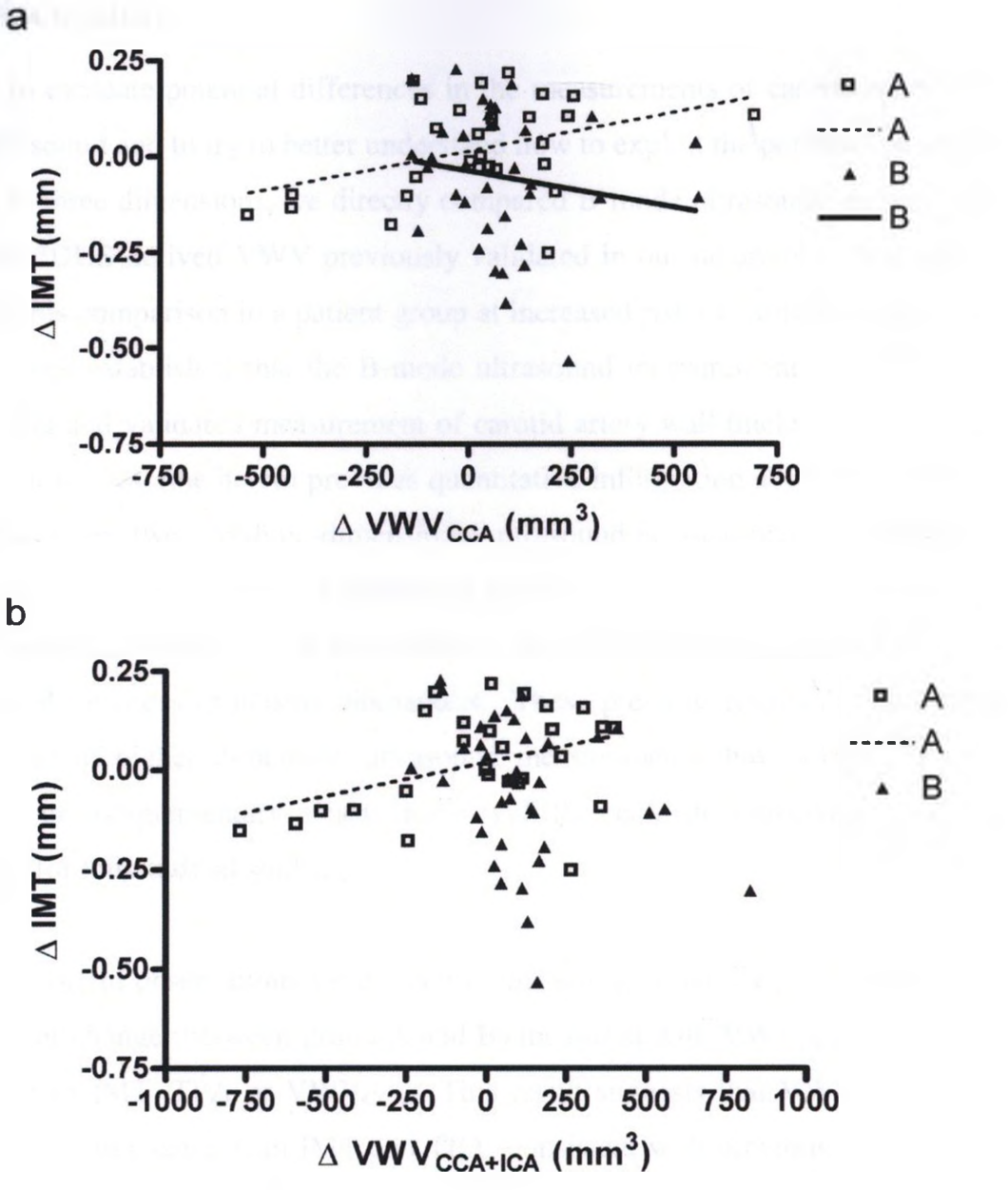

Figure 2-4: Relationship between 1-Dimensional and 3-Dimensional Longitudinal Ultrasound Measurements of Carotid Atherosclerosis

a: ΔIMT : VWV_{CCA}. There were significant Pearson correlations ($p<0.05$) in group A (dashed line) and group B (solid line); b: ΔIMT : $VWV_{CCA+ICA}$. Only the correlation in group A was significant (dashed line).

2.4 Discussion

In order to evaluate potential differences in the measurements of carotid atherosclerosis using ultrasound and to try to better understand how to exploit the potential advantages of imaging in three dimensions, we directly compared B-mode ultrasound derived IMT and TPA with 3DUS-derived VWV previously validated in our laboratory. We provide the results of this comparison in a patient group at increased risk of cardiovascular events. It has been well-established that the B-mode ultrasound measurement of IMT provides a reproducible and validated measurement of carotid artery wall thickness (22-24) and the comparison we describe herein provides quantitative information about the differences in sensitivity of one, two and three-dimensional ultrasound measurements. This comparison is especially relevant because of a number of recent longitudinal studies (2,3) that showed no significant differences in 1-dimensional carotid ultrasound measurements despite significant differences in plasma biomarkers. These previous results are motivating the development of higher dimension ultrasound measurements that include plaque as an alternative or complementary measurement with the requisite sensitivity, precision and specificity for longitudinal studies.

Several important observations were made in this study. First, we observed a difference in the rate of change (between group A and B) measured with $VWV_{CCA+ICA}$ that was not observed with IMT, TPA or VWV_{CCA}. This result suggests that $VWV_{CCA+ICA}$ may be more sensitive to change than IMT and TPA, consistent with previous observations that lesions may change more quickly along the length of the artery than in thickness (20) and of the added value of information regarding circumferential distribution of plaque (10). However, it is important to acknowledge that the difference measured in $VWV_{CCA+ICA}$ must also be considered in the context of measurement variability provided by the coefficient of variability (COV). While the mean COV for one carotid side was 10%, this mathematically corresponds to a COV for two sides of 14% once the errors are added in quadrature. In other words, it is critical to consider the balance of both precision and sensitivity of the measurements given that mean $VWV_{CCA+ICA}$ of 1580 ± 400 mm^3, and 14% COV would reflect a difference of 221 mm^3, which is approximately equal to measurement differences at four years (at a measured rate of 53 mm^3/year).

We observed an increased slope or rate of change in VWV only in Group B and there was an expectation of such changes in carotid disease in at least one treatment arm (either placebo or vitamin B-treated), particularly given that this is a high-risk population. As a comparison, IMT progression of 0.021 ± 0.007 mm/year was measured in a type 2 diabetes population (25) and 0.02 ± 0.11 mm/year was measured in a decreased kidney function population (26). In a previous pilot study of 101 patients from a stroke prevention clinic, TPA showed a progression of 0.21 ± 0.41 cm^2/year in patients with plasma H(e) >14 µmol/L and 0.13 ± 0.24 cm^2/year in patients with plasma H(e) <14 µmol/L (27). As the current study is the first to measure change in 3DUS VWV over longer periods of time, no direct comparison is available in the literature. However as a first approximation, if only vessel wall thickness changes are considered, a 2% change in IMT (a 1-dimensional measurement) would directly mathematically correspond to an approximate 6% change in VWV (a 3-dimensional measurement) (28,29).

It is also important to consider the disparate measurement sites of the three ultrasound phenotypes in the interpretation of the results. IMT is measured in the common carotid artery, proximal to the bulb. TPA includes measurements from the entire common, internal and external arteries from the clavicle to the jawbone. The CCA component of VWV includes the carotid bulb and some of the common carotid artery. The increased sensitivity of VWV_{CCA} and $VWV_{CCA+ICA}$ compared to IMT and TPA suggests that change occurs in the bulb and internal carotid artery, where it would not be measured by IMT. Various strategies have been implemented to circumvent the localized nature of IMT, for example by measuring wall thickness in up to 12 segments in the common, bulb and internal carotid artery on the near and far wall (1-3). However, 3DUS VWV includes both the near and far walls, as well as the side walls, and as such it can be used as an indicator of global change (10) with the additional advantage that the segmented contours can be used to generate thickness maps to visualize the location of such changes (11).

There were a number of limitations of this study, the first of which is the limitation of the analysis to a subset of subjects with image quality necessary and sufficient for segmentation; this reduced the study population to approximately 70% of the entire group

of subjects enrolled. It is possible that with more subjects, differences in IMT and TPA progression between groups would have been observed on the order of those reported in previous studies of similar populations. However, with our measured standard deviations, at a power of 80% and a significance level of 0.05, we calculate this would require over 1500 subjects to observe a 0.02 mm/year change in IMT and over 500 subjects required to observe a 0.2 cm^2/year change in TPA, which could not have been achieved with this study, even if all subjects enrolled had images of adequate quality for analysis. Poor 3DUS image quality made VWV measurement an occasional challenge and necessitated the removal of the ICA from consideration for much of the population; in future 3DUS VWV studies more rigorous quality control procedures must be used to address this important issue. Subject selection in the enrollment process may have also played a role in our results since subjects were included into the study based only on renal function tests, with no requirement for a diagnosis of atherosclerosis. However, this is a patient population with a known increased risk for atherosclerosis so they are an important group to characterize.

A final interesting observation we point out was the difference in correlation between IMT and VWV changes in group A and group B, with a positive IMT/VWV correlation for group A and a negative IMT/VWV correlation for group B. This difference may be explained by a few factors. At baseline, there was a trend to lower IMT and TPA in group B as compared to group A, with a significant difference for VWV_{CCA} and a nearly significant difference for $VWV_{CCA+ICA}$. This difference in VWV was no longer present at follow-up. One difference between group A and group B is that there were more females (32%) in group B than in group A (23%), which may explain the difference in VWV at baseline, given that males had larger VWV than females in this study. The difference in correlation of ΔIMT and ΔVWV between groups may also be explained by the location and nature of the measurements: IMT is essentially a measurement at a single location of the common carotid artery whereas VWV provides a more global measurement from the entire carotid volume. The inverse correlation between ΔIMT and ΔVWV in group B might also indicate that two processes were occurring in that group, an increase in plaque and wall thickness in the bulb and ICA, with a decrease in wall thickness in the CCA.

While the positive correlation between ΔIMT and ΔVWV in group A is more intuitive to understand, the reverse correlation in group B may also reflect other, as yet unknown, differences in blood pressure, renal function or a treatment effect, which will be explored in future once all the clinical data are analysed and the treatment blind is broken.

2.5 Conclusion

In a relatively small group of subjects at risk of atherosclerosis from diabetic nephropathy, 3DUS VWV is more sensitive to temporal changes in carotid atherosclerosis as compared to IMT or TPA. A comparison two treatment groups, one treated with vitamin B and the other with placebo suggested that 3DUS VWV provided adequate sensitivity to observe post-baseline changes. There was a difference in atherosclerosis progression between treatment groups measured with VWV but not with the other phenotypes. Although this result suggests that 3DUS VWV is an adequate measurement tool for longitudinal studies of carotid atherosclerosis, there is, nonetheless, a trade-off between sensitivity and precision that must be considered when comparing 1D measurements like carotid IMT with 3D measurements such as 3DUS VWV.

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Chapter 3: Prospective Cardiac Gating of Carotid Three-Dimensional Ultrasound

3.1 Introduction

Carotid atherosclerosis results from a gradual process involving inflammation and accumulation of lipid-laden macrophage lesions in the sub-endothelial layer of the carotid arterial wall. Plaque vulnerability and rupture can lead to stenosis, thrombosis or embolism, resulting in cerebral infarcts or stroke. While stroke risk is associated with carotid stenosis (1-3) and plaque composition (4-7), clinically, the risk of major atherosclerosis events is typically evaluated using indirect measurements associated with risk of stroke and coronary events (8-10), such as serum low density lipoprotein (LDL) cholesterol, diastolic and systolic blood pressure and plasma triglycerides. In addition to indirect plasma biomarkers, diagnostic and quantitative imaging methods have also played important roles in the clinical measurement and management of atherosclerosis. Moreover, in longitudinal studies and clinical trials of new treatments, the focus has moved to include direct imaging measurements of atherosclerotic lesions and arterial wall changes. The growing trend of using imaging measurements in clinical studies of new atherosclerosis treatments (11-16) stems from the notion that direct measurements of atherosclerosis such as arterial wall thickness, luminal stenosis, atherosclerotic plaque volume, plaque area and plaque composition provide a direct assay for measuring the effects of intervention. Because the size, composition, morphology and disposition of atherosclerotic lesions themselves are the determinants of major cardiovascular events, these direct imaging measurements have potential clinical relevance.

A large body of evidence from observational (17,18) and interventional studies (11,19) supports the use of ultrasound imaging for quantifying carotid atherosclerosis using B-mode ultrasound measurements of carotid intima media thickness (IMT). While the measurement of IMT is well-validated (20,21), widely accepted and cost-effective to implement, other imaging methods may also provide additional, unique and complementary information. Three-dimensional ultrasound imaging methods are being

investigated that may be inherently more sensitive to changes associated with interventions (22,23). Towards the goal of developing new 3D carotid atherosclerosis measurements, 3DUS measurements have been validated and assessed such as 3DUS carotid total plaque volume (TPV) (24,25), which quantifies plaque volume, and vessel wall volume (VWV) (26-28), which quantifies plaque and wall thickness. The measurement of 3DUS VWV was previously validated (26), and applied to the analysis of a handful of elderly patients with carotid stenosis (27).

One of our research goals is to expand the utility of 3DUS VWV to younger subjects with more compliant vessels. Atherosclerosis and risk of events is now increasingly a disease of younger and middle-aged adults because of the increased incidence of obesity, type-2 diabetes and lifestyle factors that put younger subjects at risk. As well, diseases such as rheumatoid arthritis (RA) and type 1 diabetes are associated with increased risk of atherosclerosis at younger ages (29-34). It is well-established that as part of the aging and atherosclerosis processes, arterial wall changes occur that result in decreased compliance (35,36). As carotid volumes are acquired using 3DUS, aortic pulsations that are in synchrony with the cardiac cycle can be readily observed as radial changes in younger subjects, whereas for older subjects with significant atherosclerosis, vessel walls are less compliant and show little or no radial changes during the cardiac cycle. Accordingly, and towards the goal of expanding the utility of 3DUS VWV measurements in younger subjects at risk of atherosclerosis, here we describe the development of a 3DUS cardiac gating methodology and its application in a pilot test in healthy volunteers and patients with rheumatoid arthritis. Our goal was to develop a method which reduced effects of carotid pulsatility with a rapid scan time to minimize patient discomfort.

3.2 Methods

3.2.1 Cardiac Gating Design and Implementation

We have previously described the design and implementation (24,26-28,37-39) of a mechanical ultrasound scanning system that acquires evenly-spaced two-dimensional (2D) images and reconstructs them into a three-dimensional (3D) volume. Briefly, a

conventional ultrasound probe mounted on a linear motor-assembly is moved with a constant speed and two-dimensional B-mode images from the US machine are digitally captured by a video frame-grabber (Matrox Meteor II MC, Matrox Electronic Systems Ltd, Dorval, Quebec) and stored to create a 3D volumetric image. In this scheme, there is an electronic delay, or "image lag" of approximately 66 ms between the generation of the ultrasound image in the ultrasound machine and its capture and addition to the image volume, which is an important feature to consider in the acquisition of cardiac gated 3D ultrasound images. It is within this general image acquisition approach that a prospective gating program was designed and implemented.

To enable prospective cardiac gating of 3DUS volumes, image acquisition must be timed precisely with, and triggered by, the cardiac cycle. Towards this goal, we aimed to design a method in which the electrocardiographic (ECG) signal was monitored continuously and 2DUS images captured by the frame grabber were saved during specific times of the cardiac cycle, as shown in schematic in Figure 3-1. The sharp peak in the ECG trace is called the R wave, and corresponds to ventricular systole; it was used to denote the start of each cardiac cycle. The T wave corresponds to ventricular diastole. For image acquisition with no gating (Ai), the carotid artery near and far wall respond in synchrony with the cardiac cycle (Aii) and there is a large difference between the maximum and minimum cross-sectional area of each cardiac cycle. In our approach, the ECG signal (Bi) is used to time acquisition to occur during diastole of the cardiac cycle (ΔI) so that effects of pulsatility (Bii) and variation in cross-sectional area are minimized. There is no image acquisition during systole (Δt).

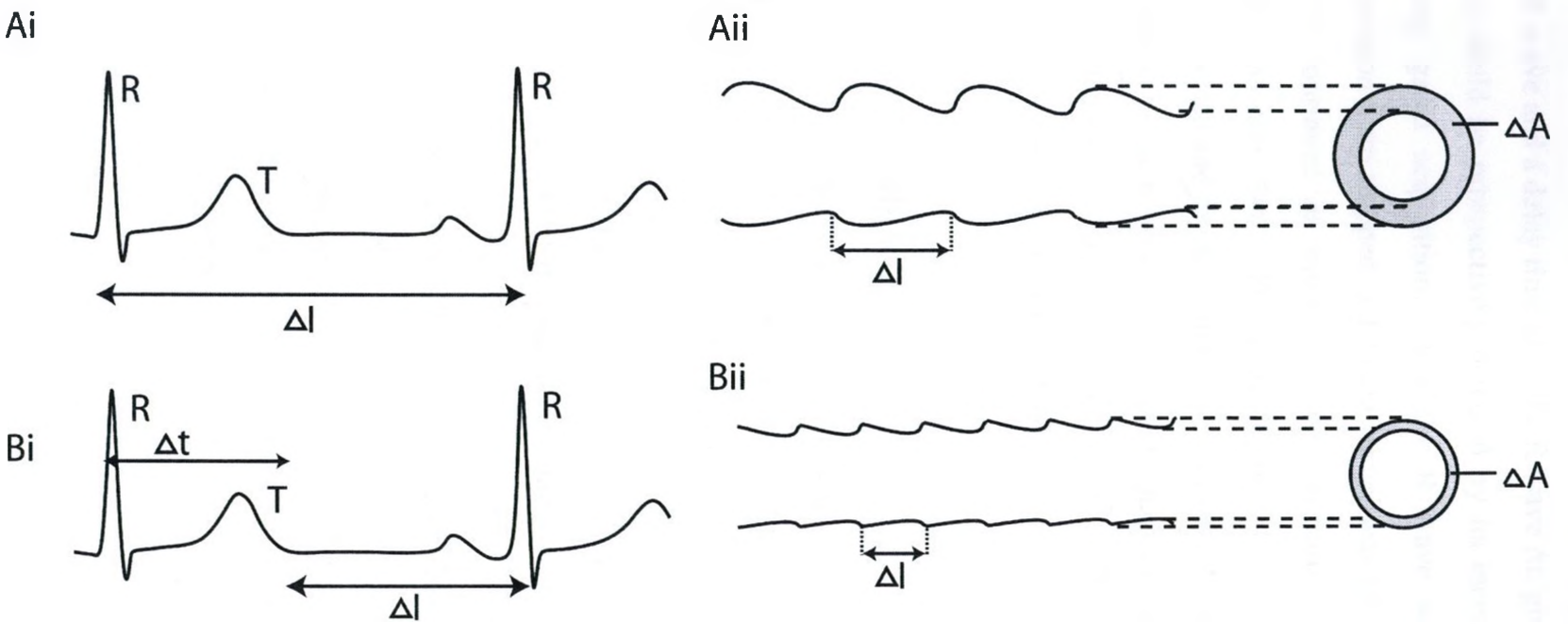

Figure 3-1: Schematic of Logic for Cardiac Gating of Ultrasound Images.

Ai: ECG for ungated imaging. The imaging interval ΔI covers the entire cardiac cycle. Aii: Sagittal view of carotid artery showing variation in diameter over cardiac cycle, with transverse view showing change in area (ΔA) over the cardiac cycle (grey shading). Bi: ECG for gated imaging. Δt after the R wave, imaging begins and occurs for a time ΔI until the next R wave. Bii: Sagittal view of carotid artery showing reduced variation in diameter, with transverse view showing reduced change in area (ΔA) over the cardiac cycle (grey shading).

In order to implement this method, after a calibration period to centre and display the ECG trace on the screen, the operator selected a threshold amplitude exceeded only by the R wave and a delay time after the R wave Δt, given in milliseconds. Thereafter, the R wave could be prospectively detected by its increased amplitude above the threshold. During gated acquisition, when the R wave was detected, the mover and image acquisition were stopped and a delay timer was set. After the delay time had elapsed, the mover continued its motion in the direction of the scan with synchronous image acquisition now "on". This process continued until all the images for the 3D volume were captured and digitally stored. This method used the two-frame processing lag to our advantage; as the R wave was detected, images from 66 ms before the R wave were being processed, and therefore image acquisition was halted before the peak systole increase in pressure affected the artery wall diameter or thickness.

3.2.2 Study Subjects

All subjects enrolled for imaging in this study provided written informed consent to the study protocol that was approved by our local research ethics board. Volunteers were classified as "healthy" (HV) if they had no history of cardiovascular disease and no other chronic conditions or acute intercurrent illness. Subjects with moderate atherosclerosis (MA) were enrolled from a local stroke prevention clinic where they were being treated and had a documented B-mode ultrasound measurement of total plaque area (TPA) >0.5 cm^2 (40). These patients are representative of typical patients used in the previous validation of the 3DUS VWV measurement (26). Patients with a clinical diagnosis of rheumatoid arthritis (RA) were recruited and enrolled from a local rheumatoid arthritis clinic and had an ongoing history and treatment of rheumatoid disease for at least 2 years. Additionally, all subjects were aged between 18-80 years and were capable of providing written informed consent.

3.2.3 Three-Dimensional Ultrasound Imaging

3.2.3.1 Ungated Three-Dimensional US Imaging

Five healthy volunteers and three moderate atherosclerosis patients were scanned to compare vessel compliance as quantified by change in area over the cardiac cycle. Images were acquired with a 35 mm L7-4 transducer (Philips, Bothel Washington) and an ATL HDI5000 ultrasound machine (Philips, Bothel, Washington). For each subject, a scan was acquired with the transducer held stationary over the common carotid artery with simultaneous recording of the ECG signal. Sequential 2D Images were imported into the image volume to create a 3D image volume with time as the third dimension instead of axial position.

3.2.3.2 Cardiac-Gated Three-Dimensional US Imaging

Three healthy volunteers and three patients with rheumatoid arthritis were evaluated first without cardiac gating, and then with the delay time Δt= 250 ms after the R wave and again with Δt =400 ms after the R wave. The 50 mm L12-5 transducer (Philips, Bothel, Washington) was mounted on a computer-driven motor assembly and the ultrasound machine was on the SonoCT (compound imaging) setting. The motor assembly moved at a speed of 0.15 mm per frame, with simultaneous recording of the ECG signal. Images were centred on the apex of the carotid bifurcation with a total scan length of 5.1 cm.

3.2.4 Analysis

3.2.4.1 Image Analysis

Images were analysed by manual segmentation of the media-adventitia boundary and the cross-sectional area of each slice was calculated. Segmentations were performed using a WACOM Intuos pen and tablet (Wacom Technology Corporation Vancouver Washington USA). As demonstrated in Figure 3-2, segmentations were performed in the transverse plane, but the volume could be manipulated in any direction to verify the segmentations.

Images acquired at with the transducer held stationary over the common carotid artery in HV and MA patients were segmented in 66 ms intervals for at least 5 cardiac cycles; each slice was segmented three times by one observer and the average for each slice was calculated. The R wave of the ECG signal was used to determine the beginning of each cardiac cycle and the percent change in area (%ΔA) was calculated for each cycle.

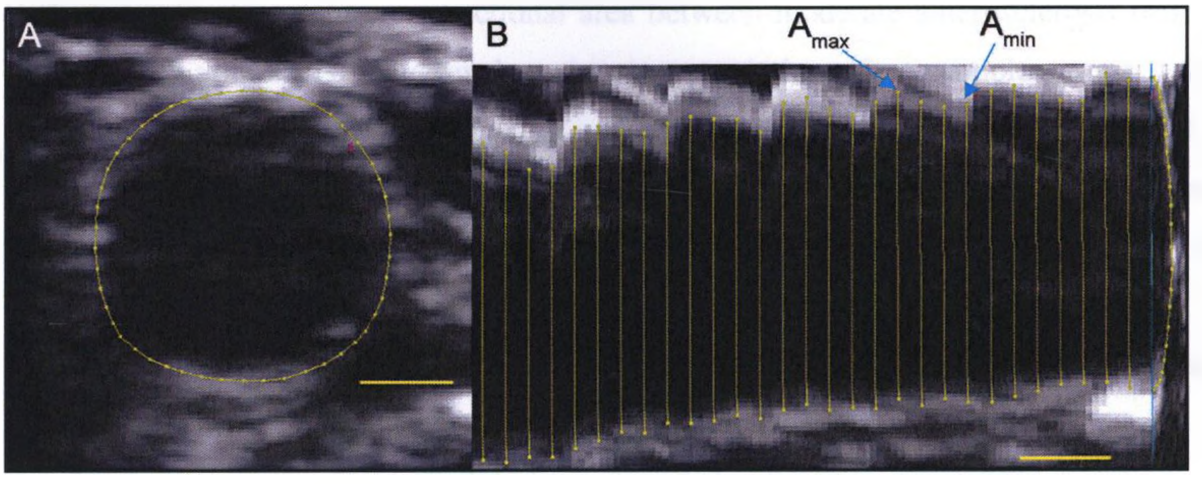

Figure 3-2: Ultrasound Image Analysis.

Right carotid artery of 38 year old female with rheumatoid arthritis. A: Transverse view of common carotid artery with media-adventitia boundary segmented in yellow. B: Sagittal view of common carotid artery with segmentations. For each cardiac cycle, the maximum (A_{max}) and minimum (A_{min}) areas were identified and used to calculate %ΔA. Images acquired with 50 mm L12-5 transducer on a mechanical acquisition system without cardiac gating. Yellow scale bars indicate 2 mm.

$$\%\Delta A = \frac{A_{max} - A_{min}}{A_{min}} \times 100\%$$

Equation 3-1: Percent Change in Area.

Calculation of percent change in area over one cardiac cycle. A_{max}: maximum cross sectional area; A_{min}: minimum cross-sectional area.

Images from HV and RA patients were segmented in 0.5 mm intervals in the common carotid artery distal to the bulb over a length of 2 cm or to the end of the image volume, whichever was greatest; segmentations were performed once by one observer. The percent change in area was calculated for each cardiac cycle and the mean calculated.

For ungated images, the R wave of the ECG signal was used to determine the beginning of each cardiac cycle. For gated images, the cardiac cycles were determined from the sagittal plane of the image volume, where images from each new cardiac cycle are slightly offset from the previous cardiac cycle, creating a vertical stripe.

3.2.4.2 Statistical Analysis

SPSS version 16.0 (SPSS Inc., Chicago, IL, USA) was used for all data analysis. Differences in change in cross-sectional area between moderate atherosclerosis patients and healthy volunteers were evaluated using an independent samples t-test. The relationship between %ΔA and age was evaluated using linear regression. The effect of cardiac gating on change in cross-sectional area was evaluated using analysis of variance to determine any significant differences between the gated and ungated images, with the Tukey's honest significant difference post-hoc test applied for multiple comparisons. In all statistical analyses, results were considered significant when the probability of making a Type I error was less than 5% ($p < 0.05$).

3.3 Results

3.3.1 Study Population

Table 3-1 provides the characteristics of all subjects imaged in these experiments. The mean age of the healthy volunteers (HV) was 34 ± 8 years, with 6 females and 2 males. The mean age of the moderate atherosclerosis patients (MA) was 67 ± 12 years, with 2 female patients and one male. Additionally, for MA patients, mean LDL cholesterol was 1.51 ± 0.3 mmol/L and mean TPA was 1.56 ± 0.3 cm^2. For the 3 female rheumatoid arthritis patients (RA), the average age was 51 ± 11 years.

Table 3-1: Pilot Study Subjects.

Description of subjects. Errors given are standard deviations, with ranges indicated in square brackets.

	HV n=8	MA n=3	RA n=3
Age (SD)	34 (8)	67 (12)	51 (10)
[range]	[26-46]	[57-80]	[38-60]
Male sex n (%)	2 (25%)	1 (33%)	0 (0%)

3.3.2 Pulsatility Measurement

In Table 3-2 we compare mean percent change in cross-sectional area (%ΔA) of a segment of the common carotid artery for 5 HV and 3 MA subjects for images acquired without cardiac gating. Mean %ΔA for HV subjects (16 ± 4 %) was significantly higher than for MA subjects (12 ± 3 %) ($p<0.01$). Mean %ΔA with no gating for all subjects is provided in Figure 3-3 as a function of age; the linear regression was statistically significant ($r^2=0.27$, $p=0.01$).

Table 3-2: Mean Change in Area over Cardiac Cycle with and without Cardiac Gating.

Measurements are provided for healthy volunteers (HV), moderate atherosclerosis patients (MA) and rheumatoid arthritis patients (RA). The mean %ΔA was calculated for each artery; n indicates the number of arteries included in each calculation. Standard deviation is provided in brackets (). Significance of differences between groups is given: $p^{\dagger}$ denotes significance of independent-samples t-test; $p^{\ddagger}$ denotes significance of analysis of variance.

	HV No gating n=8	MA No gating n=5	$p^{\dagger}$	No gating n=10	HV+RA Δt=250 ms n=11	Δt=400 ms n=9	$p^{\ddagger}$
%ΔA (SD)	16 (4)	12 (3)	<0.01	14 (5)	8 (5)	7 (1)	0.002

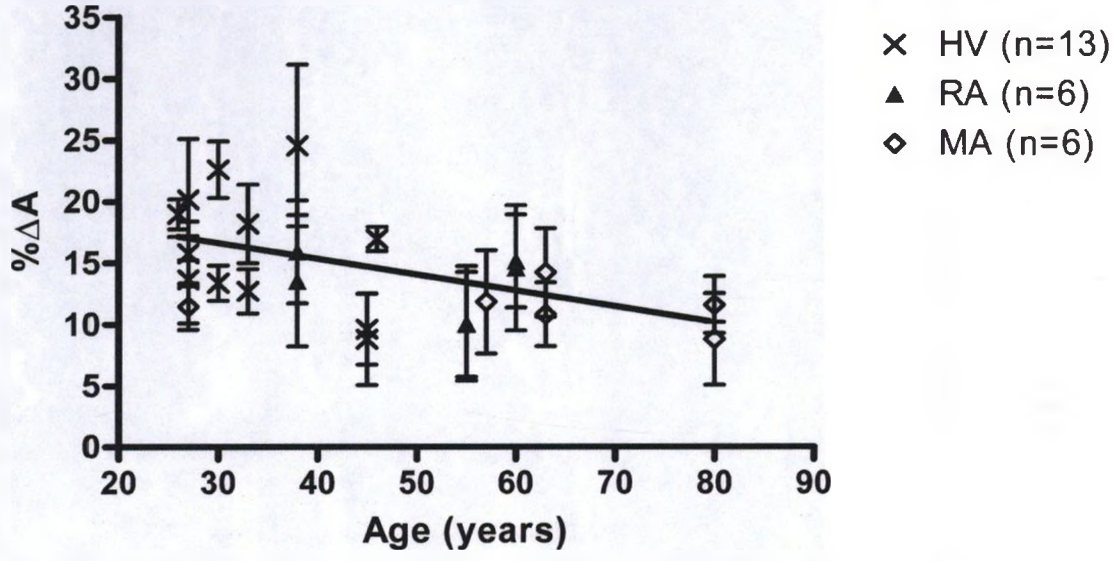

Figure 3-3: Change in Cross-Sectional Area with Age.

%ΔA for healthy volunteers, rheumatoid arthritis patients and moderate atherosclerosis patients, with left and right arteries treated independently. Error bars are standard deviation of all measured cardiac cycles for each artery. The linear regression for all subject groups is provided (r^2=0.27, p=0.01).

3.3.3 Cardiac-Gated Acquisition

Implementation of cardiac gating increased the time required to acquire 3DUS volumes. For example, in a 27 year old female HV, the time to acquire ungated images was 35 s, for Δt=250 ms it was 51 seconds and for Δt=400 ms it was 58 s. Figure 3-4 qualitatively compares images acquired with and without cardiac gating for two subjects showing differences in the radial change over the cardiac cycle.

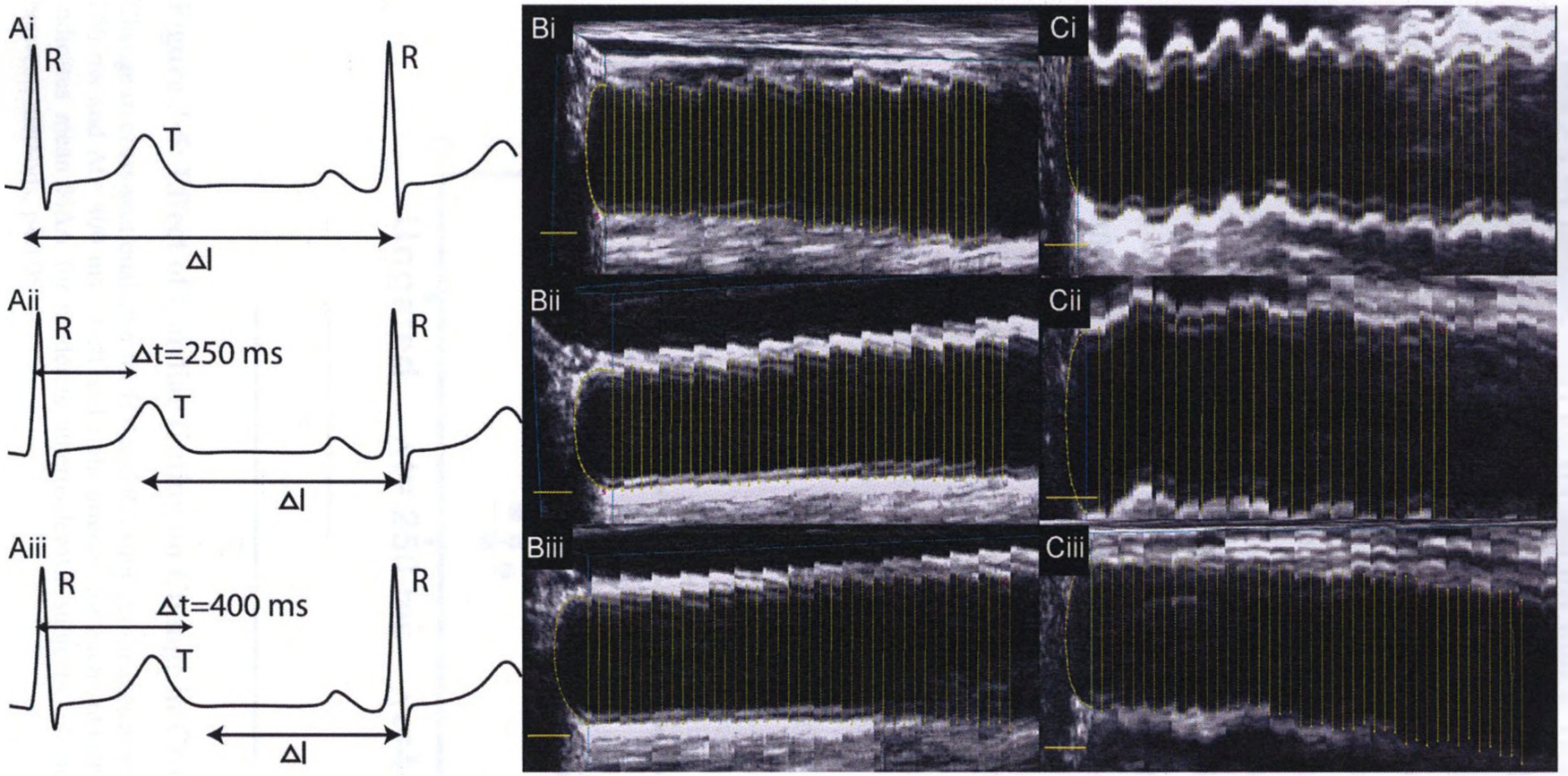

Figure 3-4: Effect of Cardiac Gating on Carotid Artery Image.

Three-dimensional ultrasound images of sagittal views of common carotid artery with segmentation of media-adventitia boundary in yellow. Yellow scale bar indicates 2 mm. Images acquired with 50 mm L12-5 transducer.
Row legend: i: no gating; ii: Δt=250 ms; iii: Δt=400 ms.
Column legend: A: schematic of gating method with delay time after R wave given by Δt and time when imaging occurs by ΔI; B: 38 year old female healthy volunteer; C: 65 year old female rheumatoid arthritis patient.

In Table 3-2 we compare %ΔA for HV and RA without cardiac gating, and for cardiac gating with Δt=250 ms and Δt=400 ms. Some subjects only provided data from one carotid artery because of ECG signal noise for that acquisition which did not allow for determination of the cardiac cycle. There was no main effect of health on %ΔA (p=0.25) in a two-way analysis of variance, so HV and RA were analysed as one group. Analysis of variance for differences in %ΔA between gating methods was significant (p=0.002), so the Tukey honest significant difference post-hoc test was used to determine between-group differences. As shown in Figure 3-5, there was a statistically significant difference between %ΔA for ungated images and both gating methods (p=0.013 for Δt=250ms and p=0.003 for Δt=400 ms) but there was no difference in mean %ΔA between gating methods (p=0.7). However, the range of values of %ΔA is noticeably smaller for the images acquired with Δt=400 ms than for images acquired with Δt=250 ms.

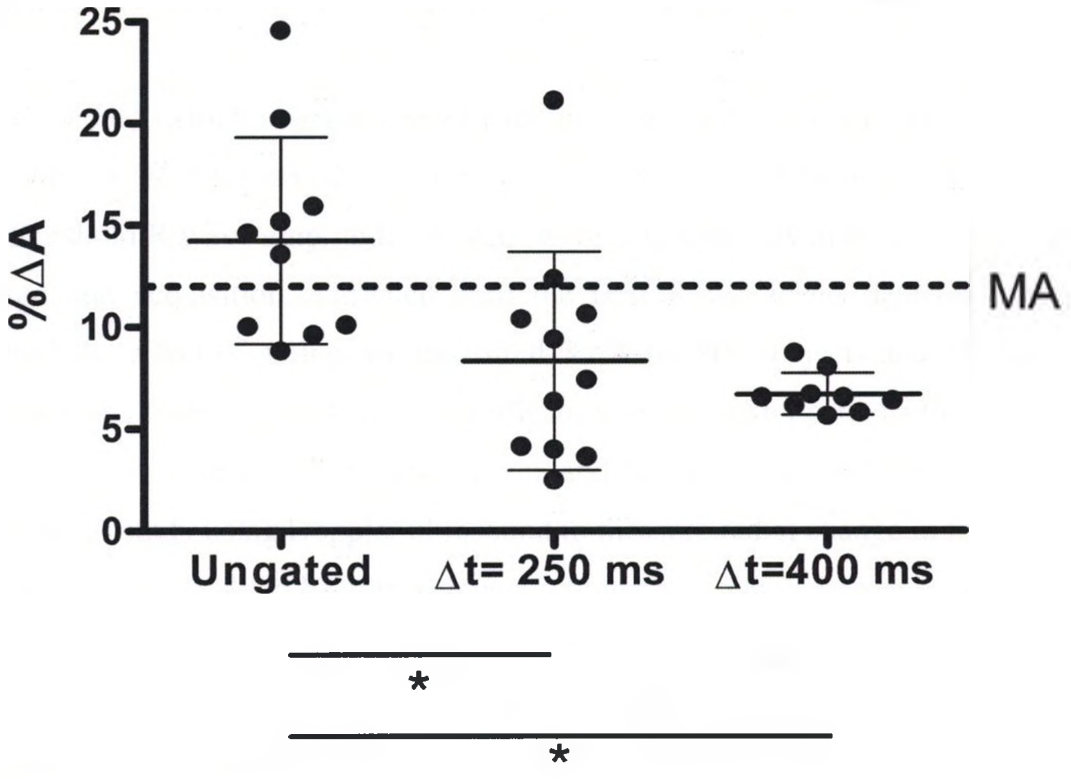

Figure 3-5: Effect of Cardiac Gating on Change in Cross-Sectional Area.

Change in cross-sectional area for HV and RA subjects; images acquired with no gating, with Δt= 250 ms and Δt= 400 ms. Left and right arteries for each subject are plotted separately. MA indicates mean %ΔA for moderate atherosclerosis subjects. * indicates significant difference between methods, p<0.05.

3.4 Discussion

The growing inclusion of imaging endpoints in clinical studies of new atherosclerosis treatments (11-16) and the fact that atherosclerosis and vascular risk is increasing in younger subjects has driven our development of cardiac gating for 3DUS image acquisition for use in longitudinal studies and clinical trials of new treatments. Here we provide the details of a method developed for the acquisition of 3DUS images with prospective cardiac gating in order to extend our imaging capability to younger subjects with more compliant arteries. We have previously described the development of a 3DUS system (37,38) and using this system we have measured 3DUS total plaque volume and vessel wall volume in a variety of older subjects at risk of atherosclerosis (23,26-28,41). We provide: 1) details of the cardiac gating method, 2) application of our method to young healthy volunteers and patients with RA as well as older patients with MA, and 3) evaluation of the effect of cardiac gating on arterial pulsatility effects.

In our method, which is implemented with an ultrasound transducer mounted on a motor assembly, the R wave was detected by its amplitude and a delay timer was set according to a threshold R wave amplitude. Images were acquired only after the delay time Δt had elapsed and acquisition continued until the next R wave was detected. In order to evaluate the effect of gating, we measured $\%\Delta A$ for HV subjects and MA patients as a reference and found that $\%\Delta A$ was significantly lower in MA patients than in HV. There was a significant relationship between age and $\%\Delta A$. This is consistent with previous research. A study using Doppler ultrasound to measure radial change in the carotid artery over the cardiac cycle in healthy volunteers found that female subjects aged 10-19 had a significantly lower percent change in diameter than those aged 50-59 (13 ± 2 % vs. 6 ± 2 %) Similarly, male subjects aged 10-19 had a percent change in diameter of 14 ± 3 % and those aged 50-59 had a percent change in diameter of 5 ± 2 % (42,43).

Our goal was to reduce $\%\Delta A$ in younger HV and RA subjects to values comparable to those measured in older MA patients. When our method of prospective cardiac gating was applied with delay times of Δt=250 ms and Δt=400 ms, $\%\Delta A$ was significantly lower than without cardiac gating for both delay times, and both delay times reduced $\%\Delta A$ to

well below that of MA patients. However, there was a smaller range of %ΔA with the delay time Δt=400 ms. Importantly, scan time was maintained at approximately one minute.

Cardiac gating has been previously described for 3DUS image acquisition. Prospective cardiac gating systems were previously described (44,45) in the measurement of plaque volume in elderly subjects with the US transducer mounted both on a motor assembly and tracked by a magnetic sensor. In these studies, the R wave was used to trigger acquisition of one image per cardiac cycle, which extended the scan time to 5-10 minutes. In a comparison of the cardiac-gated and ungated scans acquired with freehand acquisition, there was no increase in inter- or intra-observer variability with cardiac gating (45). However, the subjects in that study were of mean age 63, and based on the fact that older subjects have more rigid vessels, they would not be expected to have significant changes in cross-sectional area over the cardiac cycle.

Another study used retrospective cardiac gating of 3DUS for the reconstruction and modeling of the carotid artery bifurcation (46). 3DUS images were acquired with a freehand, magnetically-tracked system from a variety of angles and positions with simultaneous recording of an ECG in subjects aged 43-56 years. Then images from 50-95% of the cardiac cycle were used to reconstruct the vessel walls for 3 cm around the bifurcation. With this method, the coefficient of variation for repeated analysis of the same image was 5% and the time to acquire images was 3 minutes.

One limitation of our study was the small number of study subjects. However, although the total number of subjects was small, a statistically significant change in %ΔA was detected, likely because the subjects were very young where considerable carotid pulsatility without cardiac gating can be measured. Another limitation we encountered stemmed from the detection of the R wave as a trigger for the delay timer. In some subjects, the R wave and T wave were of similar size and thus the T wave sometimes re-set the delay timer. This would only result in increased scan time, however, as the image acquisition window was artificially and unnecessarily shortened. Alternately, sometimes

the R wave did not have enough amplitude to reach the threshold and signal the end of the cardiac cycle, resulting in image acquisition during the entire cardiac cycle. Both of these issues should be addressed in the future with on-the-fly calibration of the ECG signal for more amplitude consistency, and optimization of electrode placement to magnify the R wave. Another future modification will be to set the length of the delay timer for each patient based on the length of the cardiac cycle, instead of setting a constant delay in milliseconds.

3.5 Conclusion

We measured variation in cross-sectional area of the common carotid artery in young healthy volunteers (representative of the target patient population for future applications of 3DUS VWV) and in moderate atherosclerosis patients (typical of the previous patient populations in which this phenotype was applied) and found that healthy volunteers had more variation in cross-sectional area (%ΔA) than moderate atherosclerosis patients and that %ΔA was age-dependent. We designed a cardiac gating method based on the use of the R wave to set a delay timer. Because the end of the imaging interval was set by the new R wave, this technique could potentially be used in subjects with variable heart rates without acquisition of images from the systolic phase of the cardiac cycle. We attempted to minimize scan time to prevent patient discomfort, which is particularly important in specific subgroups at risk of atherosclerosis such as those with rheumatoid arthritis where long scanning times might result in pain; our scan time of approximately one minute compares favourably to other methods with previously reported scan times of 3-10 minutes. The effect of cardiac gating was measured using %ΔA of the common carotid artery in the region distal to the carotid bulb as an assay. In healthy volunteers and rheumatoid arthritis patients, %ΔA was significantly reduced with cardiac gated acquisition; there was no significant difference in %ΔA between delay times of 250 ms and 400 ms; however, the range of values was appreciably lower for the delay time of 400 ms. Importantly, cardiac gating reduced %ΔA to below the measured %ΔA in moderate atherosclerosis, suggesting that cardiac gating may be of use in older populations as well.

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Chapter 4: Conclusions and Future Directions

4.1 Rationale and Summary

The development of new, sensitive and precise measurements of carotid atherosclerosis will be critical in the future for estimating stroke risk as well as for determining the efficacy and safety of interventions and therapies. Over several decades, traditional plasma biomarkers such as low-density lipoprotein and high-density lipoprotein cholesterol have been widely used both in carotid atherosclerosis research and clinical care, whereas other emerging plasma biomarkers such as C-reactive protein and homocysteine have been validated and utilized in specific research studies. Although plasma biomarkers have provided a foundation for our understanding of atherosclerosis, these are indirect measurements of an anatomically heterogeneous disease. Imaging can be used to directly and non-invasively measure the structural, compositional, functional and anatomical manifestations of atherosclerosis such as arterial wall thickening and the size, shape, topology and composition of atherosclerotic plaque lesions. Such measurements include those made using magnetic resonance imaging, x-ray computed tomography and the use of Doppler ultrasound to measure blood velocity in arterial stenosis (1-3). Carotid atherosclerosis can also be directly visualized using B-mode ultrasound measurement of arterial intima-media thickness (IMT) (4) which serves as the gold standard measurement of carotid disease, and total plaque area (TPA) (5). In this thesis we focus on the development and application of another ultrasound imaging measurement of carotid disease, three-dimensional ultrasound vessel wall volume (3DUS VWV) which provides a quantitative assessment of both arterial wall thickening and plaque using a relatively rapid (5 minutes) and non-invasive imaging procedure.

Accordingly, the overall objective of this thesis was to develop a better understanding of how 3DUS VWV measurements could be used in longitudinal studies and in younger subjects. The primary objective of this thesis research was to compare the sensitivity of three different ultrasound measurements of atherosclerosis in a middle aged diabetic nephropathy patient group. A secondary objective was to develop cardiac gated 3DUS

and incorporate it within our system in order to extend our imaging capabilities to younger subjects with more compliant arteries.

In Chapter 2, 3DUS VWV was compared IMT and TPA in a longitudinal study in which images were acquired for subjects over 4 years. Patients were randomized to two treatment groups, denoted group A and group B, one treated with vitamin B therapy and one with a placebo. Patients were followed for a mean of 2.3 ± 1 years and ultrasound measurements of IMT, TPA and 3DUS VWV were made at baseline and follow-up. There were no statistically significant differences in IMT, TPA or VWV_{CCA} change between groups. For $VWV_{CCA+ICA}$ there was a mean increase in group B of 53 ± 115 mm^3/year that was statistically significantly different from the change in group A of -11 ± 137 mm^3/year. There was a positive correlation between change in IMT and change in VWV_{CCA} in group A ($r=0.44$, $p=0.03$), but a negative correlation in group B ($r=-0.44$, $p=0.008$). To our knowledge, this is the first reported study to use 3DUS VWV to measure changes in atherosclerosis over a two year period of time and furthermore it is the largest study to date using 3DUS VWV as an outcome measure.

In Chapter 3, a method for cardiac-gated acquisition of three-dimensional carotid ultrasound was presented. Measurements were made in 14 subjects – six subjects with pre-existing vulnerabilities to cardiovascular disease and eight healthy young volunteers – with and without cardiac gating. We established and tested a method that incorporates the timing of the cardiac cycle to decrease effect of arterial pulsatility on image acquisition; this was quantified as the percent change in cross-sectional area over the cardiac cycle (%ΔA). First, we measured the expected %ΔA with no cardiac gating in healthy volunteers (HV), patients with rheumatoid arthritis (RA) and patients with moderate atherosclerosis (MA). We found that the arteries of the younger subjects changed in cross-sectional area over the cardiac cycle significantly more than the moderate atherosclerosis patients (16 ± 4 % compared to 12 ± 3 %, $p<0.01$), and also that %ΔA decreased with age (r^2 for regression line 0.27, $p=0.01$). We then applied a cardiac gating method to acquire images a set delay time (Δt) after the R wave in HV and RA patients; delay times of Δt=250 ms and Δt=400 ms were tested. With this method, we

were able to statistically significantly reduce %ΔA in HV and RA patients from 14 ± 5 % with no gating to 8 ± 5 % with Δt=250 ms and 7 ± 1 % with Δt= 400 ms with a scan time of approximately 1 minute.

4.2 Conclusions

We had two original hypotheses that we tested within the research outlined in this thesis. First, we hypothesized that 3DUS VWV could be used to detect longitudinal changes in carotid atherosclerosis and that in a specific vulnerable patient group 3DUS VWV was more sensitive to changes over two years than 1-dimensional and 2-dimensional US measurements. We further hypothesized that prospective cardiac gating of carotid 3DUS would reduce the arterial compliance effects seen in the images of young subjects to be comparable to the compliance effects in older subjects.

In a longitudinal study of patients with diabetic nephropathy, 3DUS VWV was more sensitive to change in atherosclerosis than IMT and TPA. Only 3DUS VWV measurements yielded a statistically significant difference between treatment groups. The inverse correlation between IMT and VWV_{CCA} change in group B suggests a heterogeneous response to treatment in group B. We acquired cardiac-gated 3DUS images in approximately one minute, which is a short enough scan time to avoid discomfort for patients. Our target was to reduce the %ΔA to below 12 ± 3 % as measured in the older MA patients. Our measured %ΔA for HV and RA patients was 8 ± 5 with Δt=250 ms and 7 ± 1 % with Δt=400 ms, both significantly lower than the %ΔA for ungated measurements in these subjects of 14 ± 5 %. Although there was no significant difference in %ΔA between the two gating methods, the lower standard deviation of the measurements made with the longer Δt indicates that Δt=400 ms should be applied in future studies for a more consistent results. As well, %ΔA with gating was lower than the goal derived from the MA patients, which suggests that cardiac gating should be applied even when older subjects are being imaged.

In summary, we provided: 1) evidence that VWV may be more sensitive to longitudinal changes (over two years) in carotid atherosclerosis than IMT and TPA, 2) evidence of the different relationships between longitudinal changes in 3DUS VWV and IMT in a vulnerable patient group, and, 3) a new image acquisition method that utilizes prospective cardiac gating without significantly increasing acquisition time to expand the use of 3DUS VWV measurement in younger patients and subject populations.

4.3 Limitations of Current Tools and Solutions

As a result of the research performed in this thesis and previously in our lab, we established that 3DUS VWV provides a sensitive tool for the measurement of carotid atherosclerosis. However, several limitations of the 3DUS acquisition methods as well as the measurement and analysis of 3DUS VWV were encountered which will be discussed here with suggestions for improvements.

To increase the utility of 3DUS VWV and before it is translated to other clinical and research sites, limitations from measurement variability and time to make manual measurements must be addressed. As described in Chapter 2, the intra-observer coefficient of variability (COV) for VWV measurements of the common carotid artery was 14%; the intraclass correlation coefficient (ICC) was 0.89. Previous studies in our group have measured an intra-observer COV of 6.4% for 3DUS VWV and 22.7% for total plaque volume, with corresponding intraclass correlation coefficients of 0.95 and 0.85, respectively (6). In contrast, reliability of IMT measurement has variously been reported as an ICC of 0.98 (7), a Pearson correlation coefficient of 0.847 (8), and a coefficient of variability 1.3% (9) to 10.2% (10). For TPA, intra-observer ICC has been reported as 0.94 (11). As well, measurement of 3DUS VWV is time-consuming, taking about 45 minutes to 1 hour per image volume which for the 71 subjects analysed in the DIVINe study corresponds to 284 hours of analysis. This is in contrast to the 5-10 minutes per subject required to measure IMT and TPA.

One solution to the problems of VWV measurement variability and time for analysis could be addressed by the introduction of segmentation automation. With automated or semi-automated measurement tools, we expect to observe both reduced inter-observer and intra-observer variability. In the absence of automation, the addition of power Doppler image acquisition should be considered, as the indication of regions of blood flow could be helpful in defining the lumen boundaries and differentiation of luminal noise from plaque in manual segmentation. The addition of cardiac-gated imaging may also improve the ability of semi-automated algorithms to work by reducing inter-slice differences in wall and lumen size. The effect of cardiac gating on measurement variability in a variety of patient populations must also be further examined to determine if and when it is necessary. A previous study by another group found a non-significant decrease in measurement variability when cardiac-gated acquisition was used; however, the population in that study was relatively old (63 years) and only plaque was measured, not wall thickness (12). We have calculated that in order to measure a 25% improvement in variance, 40 arteries from 20 subjects must be measured as described below:

$$F = \frac{\sigma_u^2}{\sigma_g^2}$$

$$F = \frac{4^2}{3^2}$$

$$F = 1.78$$

$$df \approx 40$$

Equation 4-1: F-test for sample size calculation.

The number of subjects required for the gating variability experiment corresponds to the degrees of freedom of the F test. σ_u^2: variance of 3DUS VWV measurements from images acquired with ungated acquisition; σ_g^2: variance of 3DUS VWV measurements from images acquired with gated acquisition. The degrees of freedom for the F value are taken from a table of critical values of the F distribution, with $\alpha = 0.05$ (13).

A further limitation of the work presented in this thesis was the image quality of the 3DUS images used to measure VWV in Chapter 2; only 77 of the original subjects had sufficient image quality and extent for analysis and 7 subjects were eliminated from the analysis altogether because of poor image quality. This could be ameliorated by

improved quality assurance procedures as imaging is done or by changing the transducer used to acquire images to a lower frequency; however, the resulting increase in depth (and corresponding increase in measurable length of ICA) comes at the cost of a loss of image resolution.

A general limitation of 3DUS VWV is that it is purely a volumetric measure that does not incorporate change in plaque composition. One study conducted with MRI found that even if carotid plaque extent does not change with treatment, it can stabilize due to composition changes (14). If that study had been conducted with 3DUS VWV as the outcome measure, the information about composition change would not have been recognized. Carotid ultrasound has some ability to differentiate between plaque types based on echolucency (15,16) that could be exploited to detect compositional changes over time; this question is being examined in several research groups (17-19). Finally, the combination of plaque composition data with the volumetric and spatial information obtained from 3DUS VWV and associated 2-dimensional topology maps could increase the utility of 3DUS in clinical and research applications.

4.4 Roadmap for Future Studies

In the future, we think that 3DUS VWV will provide a sensitive, specific and precise measurement of carotid atherosclerosis in research and in clinical care. Before this goal can be reached, a complete characterization of 3DUS VWV and improvements in its specificity, sensitivity and precision are required.

First, in order to better understand 3DUS specificity, its relation to other stroke risk factors and outcomes must be further characterized, as well as the effect of changes in these risk factors on change in VWV. This information is partially available from an unpublished analysis of data in the DIVINe study, where baseline VWV_{CCA} was significantly ($p<0.001$ for linear regression) related to sex, hypertension and pack-years of smoking, and $VWV_{CCA+ICA}$ was related to HDL cholesterol, sex and age (Table 4-1); in contrast, IMT was related to age, high cholesterol and hypertension, and TPA was related

to age and pack-years of smoking. However, these preliminary findings must be verified in larger populations in the future.

Table 4-1: Relationship of Ultrasound and Clinical Measurements.

Forward stepwise regression of baseline values with clinical variables. Transformations were applied to IMT and TPA for normality. B indicates the standardized regression coefficient, R is the multiple correlation coefficient and p indicates the significance of the model. Significant predictors (p=0.05 for entry, 0.1 for removal) were selected from baseline values of: age, sex, pack-years of smoking, systolic blood pressure, diastolic blood pressure, pulse pressure, medication for hypertension (y/n), hypertensive (y/n), total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, medication for lipids(y/n), high cholesterol (y/n), homocysteine. Yes/no questions are scored as 0=no, 1=yes. Sex is scored as 0=male, 1=female.

Dependent Variables	Independent Variables	B	R^2	p
IMT^{-1}	Age	-0.412	0.507	<0.001
	High cholesterol	0.053		
	Hypertensive	-0.432		
$TPA^{1/2}$	Age	0.337	0.295	0.0004
	Pack-years of smoking	0.332		
VWV_{CCA}	Sex	-0.555	0.515	<0.001
	Hypertensive	0.398		
	Pack-years of smoking	0.295		
$VWV_{CCA+ICA}$	HDL cholesterol	-0.386	0.445	<0.001
	Sex	-0.360		
	Age	0.299		

The sensitivity of 3DUS VWV will be evaluated in younger subjects in ongoing natural history studies in two patient groups; one, in patients with rheumatoid arthritis, will include the effect of inflammatory factors and evaluated the effect of cardiac gating on 3DUS VWV measurement precision; another, in patients with a kidney transplant after diabetes-induced renal failure, will examine the effect of immuno-suppressant treatment on atherosclerosis; and a third will study the effect of dietary interventions.

3DUS VWV will be further developed as a measurement tool through these studies and others, paving the way for future applications in both clinical practice and research. These applications could include use of 3DUS VWV as a screening and monitoring tool for physicians to use to track atherosclerosis progression and response to treatment in

their patients. Clinical researchers could use 3DUS VWV as a primary outcome measure and surrogate for stroke risk in cross-sectional, natural history and treatment studies as a sensitive, specific and precise measure of carotid atherosclerosis.

4.5 References

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Appendix

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The University of Western Ontario
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Telephone: (519) 661-3036 Fax: (519) 850-2466 Email: ethics@uwo.ca
Website: www.uwo.ca/research/ethics

Use of Human Subjects - Ethics Approval Notice

Principal Investigator: Dr. J.D. Spence
Review Number: 07830 **Revision Number:** 8
Protocol Title: Diabetic intervention with vitamins to improve nephropathy - DIVINe Trial
Department and Institution: Medicine, Robarts Research Institute
Sponsor:
Ethics Approval Date: January 31, 2006 **Expiry Date:** December 31, 2007
Documents Reviewed and Approved: Revised study end date and increase in number of local participants to 150
Documents Received for Information:

This is to notify you that The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects (HSREB) which is organized and operates according to the Tri-Council Policy Statement and the Health Canada/ICH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted full board approval to the above named research study on the approval date noted above. The membership of this REB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

This approval shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the UWO Updated Approval Request Form.

During the course of the research, no deviations from, or changes to, the protocol or consent form may be initiated without prior written approval from the HSREB except when necessary to eliminate immediate hazards to the subject or when the change(s) involve only logistical or administrative aspects of the study (e.g. change of monitor, telephone number). Expedited review of minor change(s) in ongoing studies will be considered. Subjects must receive a copy of the signed information/consent documentation.

Investigators must promptly also report to the HSREB:
a) changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
b) all adverse and unexpected experiences or events that are both serious and unexpected;
c) new information that may adversely affect the safety of the subjects or the conduct of the study.

If these changes/adverse events require a change to the information/consent documentation, and/or recruitment advertisement, the newly revised information/consent documentation, and/or advertisement, must be submitted to this office for approval.

Members of the HSREB who are named as investigators in research studies, or declare a conflict of interest, do not participate in discussion related to, nor vote on, such studies when they are presented to the HSREB.

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Use of Human Subjects - Ethics Approval Notice

Principal Investigator: Dr. G. Parraga
Review Number: 12433E **Revision Number:** 2
Review Date: May 04, 2007 **Review Level:** Expedited
Protocol Title: 3-dimensional Ultrasound Software and Hardware Development
Department and Institution: Diagnostic Radiology & Nuclear Medicine, Roberts Research Institute
Sponsor: CIHR-CANADIAN INSTITUTE OF HEALTH RESEARCH
Ethics Approval Date: May 10, 2007 **Expiry Date:** December 31, 2010
Documents Reviewed and Approved: Letter of Information (dated May 2007).
Documents Received for Information:

This is to notify you that The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/ICH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced revision(s) or amendment(s) on the approval date noted above. The membership of this REB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

The ethics approval for this study shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the UWO Updated Approval Request Form.

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Use of Human Subjects - Ethics Approval Notice

Principal Investigator: Dr. J.E. Pope
Review Number: 15024E **Review Level:** Expedited
Review Date: March 26, 2008

Protocol Title: This is an observational study to determine if 3D ultrasound provides quantifiable and reproducible measurement of plaque progression in patients with mild to moderate rheumatoid arthritis and to evaluate the contribution of traditional and non-traditional risk factors including biomarkers for cardiovascular disease regarding the potential contribution to the occurrence of plaque and rate of plaque progression in these patients

Department and Institution: Rheumatology, St. Joseph's Health Care London
Sponsor: HOFFMANN LA ROCHE
Ethics Approval Date: June 03, 2008 **Expiry Date:** March 31, 2010
Documents Reviewed and Approved: UWO Protocol, Letter of Information and Consent (May 6, 2008), Authorization for Release of Medical Information (May 13, 2008).
Documents Received for Information: Roche Protocol WA21114A (RO4877533)

This is to notify you that The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/ICH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced study on the approval date noted above. The membership of this REB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

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