THREE-DIMENSIONAL ULTRASOUND QUANTIFICATION OF INTENSIVE STATIN TREATMENT OF CAROTID ATHEROSCLEROSIS

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(Received 17 September 2008; revised 13 April 2009; in final form 25 May 2009)

Abstract—This study was designed to evaluate 3-D ultrasound (3DUS)–derived vessel wall volume (VWV), a 3-D measurement of the carotid artery intima and media, including atherosclerotic plaque, in patients enrolled in a randomized placebo-controlled three-month study of intensive atorvastatin treatment. Thirty-five subjects with carotid stenosis >60% who provided written informed consent and completed a randomized, double-blind, placebo-controlled study were evaluated at baseline and at three months after receiving either 80 mg atorvastatin (16 subjects, nine male, mean age 68 ± 8.6 y) or placebo (19 subjects, 15 male, mean age 70 ± 9.4 y) daily. 3DUS images were acquired and 3DUS VWV was manually segmented by a single observer. Individual lumen and wall segmentation contours were also used to generate carotid atherosclerosis thickness difference maps by establishing correspondence between points along the vessel wall and lumen segmentation contour surfaces, and digitally subtracting registered baseline and follow-up thickness maps. 3DUS VWV increased by 70 ± 140 mm³ (1.49 ± 10.3%) in the placebo group and decreased by 30 ± 110 mm³ (-1.4 ± 7.7%) in the atorvastatin group (p, 0.05). Two-dimensional maps generated from the VWV measurements show localized heterogeneity and vessel wall thickness changes for all subjects, mainly in the common carotid artery. Carotid 3DUS VWV is a quantitative measure of atherosclerosis burden including the intima, media and plaque, with sensitivity to detect changes over short periods of time. Quantitative VWV thickness difference maps provide visual evidence of the spatial and temporal dynamics of carotid artery changes. (E-mail: gep@imaging.robarts.ca)

Key Words: Atherosclerosis, Statins, Ultrasonography.

INTRODUCTION

Despite the development of preventive strategies and efficacious therapies, atherosclerosis, the main underlying cause of cardiovascular disease (Libby 2002), still contributes 30% of worldwide mortality and is predicted to become the leading cause of global mortality by 2050 (SoRelle 1999). In the current therapeutic context, most patients at risk receive therapies with well-established efficacy and tolerability profiles, yet there is still a residual untreated risk (Hackam and Anand 2003). Accordingly, to achieve a greater reduction in risk for individuals and patient populations, there is a need for accelerated research and development of treatments that complement current therapeutic strategies. In concert with the requirement for additional and novel therapies is the fact that presently, all new interventions must demonstrate therapeutic efficacy that is additional to the measurable effects of statin therapy.

Surrogate endpoints and biomarkers such as blood pressure, serum cholesterol and triglyceride levels provide indirect measurements of therapeutic safety and efficacy and have been critical in the development of a wide range of currently approved therapies. Recently, in clinical studies of new atherosclerosis treatments (Kastelein and de Groot 2008; Yanai et al. 2007), the focus has moved to include not only these indirect plasma measurements, but quantitative imaging measurements of atherosclerosis. The inclusion of such imaging surrogate endpoints in clinical trials stems from the notion that direct measurement of atherosclerosis such as arterial wall thickness, luminal...
stenosis, atherosclerotic plaque volume, plaque area and plaque composition provide direct assays for measuring the effects of intervention. It is the fact that the size, composition and morphology of atherosclerotic lesions themselves are the physiological determinants of major cardiovascular events and mortality (Golledge et al. 2000; Takaya et al. 2005; Virmani et al. 2006) that confer significant clinical relevance of these direct imaging measurements.

A large body of evidence from observational (O’Leary et al. 1999; Chambless et al. 2000) and interventional studies (Yanai et al. 2007; Kastelein and de Groot 2008) supports the use of B-mode ultrasound imaging for quantifying atherosclerosis of the carotid arteries using the measurement of carotid intima media thickness (IMT), which has shown to be related to age, blood pressure, smoking and elevated lipids (Spence 2004). Although the measurement of carotid IMT is well-validated (Wong et al. 1993; Pignoli et al. 1986; Greenland et al. 2000; Bots et al. 2003), universally accepted and cost effective to implement, other imaging methods may also provide additional, unique and complementary information. The rationale for the development of imaging methods that are 3-D in nature, such as 3-D ultrasound (3DUS), stems from the fact that 3-D imaging provides volumetric data, which by virtue of the extra dimensions may be inherently more sensitive to changes associated with interventions (Ainsworth et al. 2005; Schminke et al. 2002). In addition, 3-D imaging methods such as intravascular ultrasound, 3DUS and magnetic resonance imaging (MRI) can be developed to provide tissue contrast that can be related to plaque or wall composition (Saam et al. 2005; Kern et al. 2004), which are thought to play a role in lesion rupture susceptibility.

To (i) quantify and (ii) visualize wall and plaque changes in the carotid artery that occur during intensive statin treatment, and to try to exploit the inherent advantages of imaging in three dimensions, we applied a novel 3DUS measurement and method—3DUS vessel wall volume (VWV) —that was developed previously in our laboratory (Egger et al. 2007, 2008a) and demonstrated to have low intraobserver variability (intraclass correlation coefficient = 0.95 and coefficient of variation = 4.6% (Egger et al. 2007). To (i) quantify volumetric changes, successive carotid artery vessel wall and lumen segmentation outlines are used to generate a volumetric measurement; and to (ii) visualize volumetric changes, the same segmentations are used to generate carotid thickness and thickness difference maps. We developed 3DUS VWV, which is essentially a 3-D IMT measurement including plaque for the entire common, internal and external carotid artery (Egger et al. 2007), as measurement tool that is complementary to 1-D IMT, 2-D total plaque area (TPA) (Spence et al. 2002) and 3-D total plaque volume (TPV) (Ainsworth et al. 2005; Schminke et al. 2002). Here, we provide quantitative evidence of changes in 3DUS VWV and qualitative evidence of arterial volumetric changes from carotid thickness difference maps (Egger et al. 2008a; Chiu et al. 2006, 2008a) in 35 subjects with carotid stenosis after three months of intensive statin or placebo treatment. Although carotid IMT provides a robust, reproducible and validated measurement of wall thickness changes over time and in response to treatment (Bots et al. 2003; Crouse 3rd et al. 2007), the measurements and methods we describe provide both qualitative and quantitative information on the spatial distribution of atherosclerosis—preserving the regional information inherent in imaging methods and perhaps providing information about how the carotid artery changes over short periods of time.

MATERIALS AND METHODS

Study population

The study population enrolled and analyzed was described previously (Ainsworth et al. 2005). Briefly, 53 subjects with asymptomatic carotid stenosis >60% (defined by carotid Doppler flow velocities), who were participating in a long-term follow-up study to investigate imaging methods that may identify high risk for cardiovascular events, were recruited from The Premature Atherosclerosis Clinic (London, Ontario, Canada) in a consecutive fashion and provided written informed consent to the protocol approved by the University of Western Ontario Standing Board of Human Research Ethics. As described previously, the study commenced in 2001 and ended in 2003, and for those subjects who had been previously administered statin therapy on the basis of baseline cholesterol levels, a six-week washout period was undertaken before randomization. All subjects were randomized to receive placebo or 80 mg atorvastatin daily for the three-month study duration.

Image acquisition

Subjects’ head and neck position were adjusted individually to provide optimal image quality for each subject. 3DUS images were acquired in the axial plane by using a motorized linear mover to translate the ultrasound transducer (L12-5, 50 mm, Philips, Bothell, WA, USA) along the lateral side of the neck of the subject an approximate distance of 4.0 cm while video frames from an US machine (ATL HDI 5000, Philips) were digitized and saved to a computer workstation (Fenster et al. 2001). The transducer was used in compound imaging mode, with a central frequency of 8.5 MHz and translated at a uniform speed of 3 mm/s, without cardiac gating. Throughout translation, the transducer was kept approximately perpendicular to the neck, and the resulting
transverse 2-D images were parallel to each other, with a spatial interval of 0.15 mm to ensure proper sampling. The acquired images were immediately reconstructed into a 3DUS volume and displayed using 3D Quantify, a visualization and quantification software tool developed in our laboratory (Fenster et al. 2001).

Image analysis

A single observer blinded to subject identity, treatment and time point performed manual segmentation of 3DUS vessel wall volume. Images were viewed simultaneously on dual 19-inch square video monitors to assist in matching bifurcation points (BF), considered to be the flow divider, of the baseline and follow-up images. VWV measurements were quantified using 3-D Quantify as described previously (Egger et al. 2007, 2008a), using manual planimetry. Although a semiautomated segmentation approach has been used previously for the analysis of 3DUS total plaque volume (Fenster et al. 2006), there is currently no automated or semiautomated method for the segmentation of 3DUS VWV. Briefly, to identify the media-adventitia boundary, all 3DUS volumes were viewed simultaneously in the longitudinal and axial views (Fig. 1). This allowed the observer to identify the characteristic double line pattern in the longitudinal view, which was previously histologically validated as representing the intima-lumen and media-adventitia boundaries (Pignoli et al. 1986). In the axial plane, the plane of segmentation, these boundaries were fairly obvious on the artery walls perpendicular to the propagation of the sound waves (the near and far walls); however, unless thick plaques were located on the lateral walls, identifying these boundaries on the lateral walls required some interpretation by the observer. To assist interpretation, the general curvature and thickness of the lateral wall was interpolated from the thickness and curvature of the near and far walls in the same slice, as well as slices proximal and distal to the current slice. The carotid bifurcation was used as a point of reference (Ainsworth et al. 2005) to reduce interscan measurement variability, and the bifurcation was first identified within both follow-up and baseline 3DUS volumes so that all measurements could be initialized with the same 2-D image slice. A tangent of 30 mm was placed parallel to the longitudinal axis of the common carotid artery (CCA) and centered on the coordinates of the carotid bifurcation, with segmentation through the image volume, in the axial plane, using an interslice distance (ISD) of 1 mm to minimize measurement variability (Landry et al. 2004). The CCA was segmented a maximum distance of 15 mm proximal to the bifurcation and the internal and external carotid branches (ICA and ECA, respectively) were segmented 10 mm distal from the bifurcation. The area enclosed by each segmented contour was calculated and multiplied by the ISD to obtain the outer wall volume, defined as the volume enclosed by the media-adventitia boundary, and the lumen volume, defined as the volume enclosed by the lumen-intima boundary. To calculate VWV, the lumen volume was subtracted from the outer wall volume (Egger et al. 2007, 2008a). As described previously, for both 3DUS TPV (Ainsworth et al. 2005) and VWV (Egger et al. 2007), the left and right VWV were summed for each subject. The observer performed all segmentations using a Wacom Intuos3 professional pen tablet (Wacom Technology Corporation, Vancouver, WA, USA) that was digitally connected to the two monitors. Percent atheroma volume (PAV), which is a measure of atherosclerotic lesion burden developed previously for use in intravascular ultrasound (IVUS) studies (Nissen et al. 2003) was also derived from the manually segmented contours. PAV was calculated as described previously (Nissen et al. 2003) as the ratio of 3DUS VWV and the entire outer wall volume.

Generation of 3-D and 2-D carotid maps

VWV segmentation allows for the generation of vessel wall, plaque thickness maps (Chiu et al. 2006, 2008a) and plaque thickness difference maps. Briefly, and as described previously (Chiu et al. 2008a), for each carotid artery, a 3-D carotid thickness map was generated by establishing corresponding points of the vessel wall and lumen segmentation surfaces, with the resultant thickness of the vessel wall and plaque considered to be the distance between each pair of corresponding points. To map the 3-D thickness map onto a 2-D plane, the carotid map was bisected and flattened using arc-preserving...
(Chiu et al. 2006) and area-preserving (Chiu et al. 2008a, 2008b) algorithms. To generate carotid thickness difference maps (Egger et al. 2008a), carotid maps from baseline and three-month follow-up were registered (Chiu et al. 2008a, 2008b) and digitally subtracted.

**Statistical methods**

SPSS version 15.0 (SPSS Inc., Chicago, IL, USA) was used for all data analyses. Mean VWV and PAV were evaluated using a repeated measures analysis of variance (ANOVA) to determine any significant differences between the placebo and atorvastatin group over time. A Student’s t-test was also used to determine the significance in VWV and PAV changes over time between the two treatment groups. In all statistical analyses, results were considered significant when the probability of making a Type I error was <.05% (p < .05).

**RESULTS**

Baseline demographics for the 35 evaluated subjects are provided in Table 1. As previously described (Ainsworth et al. 2005), 53 subjects were scanned at baseline, with a total of six patients who withdrew because of intercurrent illness (1 death, 2 carotid endarterectomies, and 3 transient ischemic attacks or stroke). In addition, seven patients withdrew from treatment and therefore did not return for a second US measurement, and five patients were judged to have inadequate image quality specifically for VWV segmentation (difficulty identifying vessel boundaries or the vessel itself because of shadowing).

There were no significant differences in risk factors between the two groups. For the analysis described here, one of the baseline or follow-up images for three subjects was judged by the observer to have inadequate image quality for quantitative evaluation (single subject in placebo group) or inadequate length of CCA (single subject in each of the atorvastatin and placebo groups) and so could not be used for this analysis. For the 35 subjects evaluated in this study, carotid images clearly showed at least 10 mm of the CCA, which was segmented manually. Measurements of the ECA were not included in this study because of inconsistent image quality and the increased variability associated with its segmentation (Egger et al. 2007).

The results of the analysis of VWV and PAV for both treatment groups are provided in Fig. 2 and Table 2. Baseline VWV (mean ± SD) was 1330 ± 300 mm³ for the atorvastatin treatment group and 1510 ± 450 mm³ for the placebo group, and the difference between treatment groups was not significant (p = 0.19). After three months, subjects in the atorvastatin treatment group demonstrated a mean VWV change (mean ± SD) of −30 ± 110 mm³, whereas for subjects in the placebo treatment group, a mean VWV increase of 70 ± 140 mm³ and this difference between groups was statistically significant (p < 0.05). Ultrasound volumes demonstrating changes in selected subjects are seen in Fig. 3. As shown in Table 2, the change in PAV (mean ± SD) was 0.2 ± 3.2 % for the atorvastatin treatment group and 1.9 ± 3.8 % for subjects in the placebo treatment group, and this difference was not significant (p > 0.05). In addition, repeated-measures ANOVA demonstrated a significant interaction of time and treatment for VWV (p < 0.05) but not for PAV. However, for PAV, ANOVA also detected a significant treatment effect (p = 0.045).

The carotid artery thickness difference maps shown in Fig. 4 demonstrate localized spatial vessel wall and plaque thickness changes over three months for six representative subjects from the atorvastatin (Fig. 4a–c) and placebo (Fig. 4d–f) treatment groups, respectively. Thickness difference maps for six additional subjects are provided in Fig. 4g–i for the atorvastatin treatment group and Fig. 4h–j for the placebo treatment group, respectively.

The resultant quantification of plaque and wall thickness is represented by color gradients, normalized for all maps according to the measured minimum and maximum thickness changes observed for all 12 subjects. Representative maps are provided for two subjects with the largest VWV increases in the atorvastatin and placebo groups (Fig. 4a and 4d), as well as for two subjects representing the average amount of VWV change measured (Fig. 4b and 4e), and for two subjects with the largest VWV decreases in both the atorvastatin (Fig. 4b and 4c) and placebo group (Fig. 4f). The maps show spatial changes in vessel wall and plaque thickness, which, for subjects in the atorvastatin treatment group shown in Fig. 4a–c, can be observed as regions of decreased thickness in the CCA and ICA for two subjects (Fig. 4b and 4c), and a region of increased thickness in the CCA for the single subject in that treatment group that showed the corresponding
The greatest increase in VWV. Thickness difference maps for two representative patients in the placebo treatment group provided in Fig. 4d and 4e show large increases in vessel wall thickness within the CCA and ICA. For the single subject in the placebo treatment group that showed the greatest overall decrease in VWV, there was a corresponding small focal area of decreased vessel wall and plaque thickness in the CCA.

**DISCUSSION**

This study evaluated the potential of a novel 3DUS measurement of arterial intima, media and plaque in carotid atherosclerosis to try to better understand how to exploit the potential advantages of imaging atherosclerosis in three dimensions and to better track arterial changes that occur over short periods of time. Accordingly, 3DUS-derived VWV, which is essentially a 3D IMT measurement, was evaluated in a small group of subjects randomized to either intensive statin therapy or placebo. The clinical details for this study were published previously (Ainsworth et al. 2005). Unfortunately for this imaging substudy, cardiovascular risk scores were not available; however, as described previously, patients with a previous history of angina or myocardial infarction were excluded from the study. Baseline study characteristics were published previously; however, follow-up clinical parameters were not acquired during the scanning visit. We note that this was a stable group of patients for whom a placebo-controlled statin study was ethically acceptable according to Canadian Consensus guidelines at the time of the study. However, with a mean follow-

![Fig. 2. Transverse and longitudinal 3DUS of carotid atherosclerosis. Both longitudinal (L) and axial (A) views are shown. Arrows indicate regions corresponding to regions-of-interest in Fig. 4. All scale bars represent 2 mm. (a) A representative atorvastatin subject with mean negative change (decrease) in VWV between baseline (i) and follow-up (ii). (b) A representative placebo subject with mean positive change (increase) in VWV between baseline (i) and follow-up (ii).](image-url)

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<th>Table 2. Three-dimensional ultrasound atherosclerosis measurements</th>
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<td>Atorvastatin treatment group ( n = 16 )</td>
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VWV is expressed as the sum of both left and right carotid arteries ± standard deviation. PAV is expressed as the percent atheroma volume of both left and right carotid arteries ± standard deviation.
TPV decrease of –90% in the placebo group compared with a measured mean TPV increased 17% in the same subjects using 3DUS TPV (Ainsworth et al. 2005). VWV was in part because of a previous finding in a subgroup.

LDL cholesterol higher at follow-up than in the atorvastatin group, systolic blood pressure was lower and 2.38 mmol/L. These results together suggest that in the atorvastatin subgroup reported high-density lipoprotein (HDL)/low-density lipoprotein (LDL) of 1.55/1.87 mmol/L, and the placebo group reported 1.31/2.38 mmol/L. These results together suggest that in the placebo group systolic blood pressure was lower and LDL cholesterol higher at follow-up than in the atorvastatin subgroup.

The need for the development and application of 3DUS VWV was in part because of a previous finding in the same subjects using 3DUS TPV (Ainsworth et al. 2005). In this previous analysis (Ainsworth et al. 2005), TPV increased 17 ± 74 mm$^3$ (+2.3 ± 10%) for subjects in the placebo group compared with a measured mean TPV decrease of –90 ± 85 mm$^3$ (–13 ± 12%) for subjects in the atorvastatin group (p < 0.0001). However, although 3DUS TPV provides a sensitive measure of atherosclerotic lesion changes in this patient group with advanced disease and significant carotid stenosis, the intraobserver variability appears to increase as plaque burden decreases (Landry et al. 2004), potentially limiting the utility of TPV in other vulnerable patient groups with less plaque. Because it is critical that both measurement sensitivity and precision be considered along with subject characteristics in studies using imaging measurements, we felt it was necessary to develop a method that could be used in a wide variety of subjects with high sensitivity, specificity and precision. The measurement of 3DUS VWV to quantify plaque burden was proposed previously (Egger et al. 2007) because, unlike TPV, VWV measurements incorporate the volume of plaque, intima and media layers and require manual segmentation of the lumen-intima/plaque and the media-adventitia boundaries. Because of the high contrast afforded by these boundaries in 3DUS images, there is also an opportunity to develop semi-automated algorithms (Abolmaesumi et al. 2000; Mao et al. 2000) for 3DUS VWV, which has the potential to both decrease measurement variability and the time required to make these measurements. The potential for semi-automation of the 3DUS VWV measurement and the fact that even manually segmented 3DUS VWV has lower intraobserver variability than TPV, as indicated by higher coefficients of variation (COV) and intraclass correlation coefficients (ICC), (TPV: COV = 22.7%, ICC = 0.85; VWV: COV = 4.6%, ICC = 0.95) (Egger et al. 2007), may allow for this measurement to be applied broadly in a greater number of studies and sites.

Several important observations were made in this study. First, we found that 3DUS VWV measured over the CCA and ICA over a fixed 2.5-cm distance demonstrated statistically significant effects of high-dose statin therapy in three months. These results are in agreement with previous results obtained from TPV measurements of the same population (Ainsworth et al. 2005), although, as might be expected, because VWV incorporates both plaque, the intima and media, the sensitivity of 3DUS VWV (1.4% decrease in statin group) was less than previously observed for TPV (12% decrease in same subjects) but greater than observed for PAV. In comparing volumetric measurements of plaques and artery walls with other studies, it is noted that there exist a number of terms and methods of assessing the volume and any changes that occur. The most straightforward measurement is a volumetric assessment, which in this study and others has been called 3DUS VWV (Egger et al. 2007, 2008b) and can be considered a 3-D IMT + plaque measurement. The same concept is used in IVUS studies of the coronary artery; however, it is stated as “atheroma volume” (Nissen et al. 2003). Volumes may also be normalized to the arterial volume, as described in the Methods section, and in this paper, in an attempt to ease comparisons between studies, we have used the IVUS convention of labeling it as PAV.

To provide some context for these results, two previous exploratory studies using 3DUS (Schminke et al. 2000, 2002) found changes in plaque ulceration and plaque progression over 17.6 ± 6.3 and 15.1 ± 4.5 months, respectively. A study of recombinant ApoA-1 Milano demonstrated a -1.29 ± 3.48 % (mean ± SD) change in PAV in a five-week period (Nissen et al. 2003). Similarly, the ASTEROID trial demonstrated a -0.98 ± 3.15% (mean ± SD) change in PAV in a one-year period (Nissen et al. 2006) with rosuvastatin therapy. The changes measured over three months using 3DUS VWV are in good agreement with the magnitude of change demonstrated in these previous studies. A subanalysis of the EUROPA study found that over a median follow-up of 3 years, subjects treated with perindopril had a –0.48 ± 8.1 % decrease in PAV (Rodriguez-Granillo et al. 2007).
and concluded perindopril was associated with constrictive remodeling, without affecting the lumen.

We were surprised that the 3DUS VWV measurements in this study were statistically significantly different after follow-up, even with a relatively small number of subjects. The fact that this result was statistically significant is likely indicative of the sensitivity of 3DUS VWV to both plaque and vessel wall changes. We note that we previously showed (Ainsworth et al. 2005) significant results in the same patient group using the measurement of carotid 3DUS total plaque volume (TPV). Because of this previous result and the apparent differences in measurement sensitivity among US measurement tools, we compared and reported 3DUS TPV, VWV, B-mode IMT and MRI-derived VWV measurement precision (Egger et al. 2008b), providing sample sizes required for clinical trials based on the relative precision of the measurements. This work suggests that some of the differences in sensitivity to change between this study and the previously mentioned studies that focused on coronary IVUS (Nissen et al. 2003, 2006) is not likely because of the sample size differences between the studies. Rather,

Fig. 4. 3DUS thickness difference maps. Regions-of-interest on maps correspond to regions-of-interest demonstrated in Fig. 3a. Carotid thickness difference map for a representative subject from the atorvastatin treatment group, with largest positive change (increase) in VWV measured over three-month follow-up period. (b) Carotid thickness difference map for a representative subject from the atorvastatin treatment group, with mean VWV change measured over three-month follow-up period. (c) Carotid thickness difference map for a representative subject from the atorvastatin treatment group, with greatest negative change (decrease) in VWV measured over three-month follow-up period. (d) Carotid thickness difference map for a representative subject from the placebo treatment groups, with largest positive change (increase) in VWV measured over three-month follow-up period. (e) Carotid thickness difference map for a representative subject from the placebo treatment group, with mean change in VWV measured over three-month follow-up period. (f) Carotid thickness difference map for a representative subject from the placebo treatment group, with greatest negative change (decrease) in VWV measured over three-month follow-up period. (g–l) Carotid thickness difference maps for six additional representative subjects from the atorvastatin (g, h, i) and placebo (j, k, l) groups.
our previous experience suggests a number of likely factors including (i) differences in measurement precision, (ii) differences in measurement sensitivity, (iii) differences in the treatments evaluated (80 mg/day oral atorvastatin in the current study compared with intravenous Apo-A1 Milano or 40 mg/day oral rosuvastatin) and (iv) differences in vascular bed response to treatment and background disease. Therefore, although all these previous studies conclusively showed that these treatments resulted in statistically significant changes in specific surrogate measurements, different measurement sensitivity (between intravascular ultrasound of the coronaries and carotid 3DUS), as well as response of the vascular bed interrogated may have resulted in some differences.

Although the current study showed that 3DUS VWV had decreased sensitivity to plaque-specific changes compared with TPV, we previously showed that VWV has increased precision compared with TPV (Egger et al. 2007).

Second, we provide visual quantitative and qualitative evidence of the spatial changes in carotid artery VWV thickness distance maps that identify exactly where changes in the vessel intima, media and plaque occur over short periods of time. To our knowledge, this method provides the first in vivo evidence of the regional heterogeneity of carotid artery changes that has apparently occurred over three months. Previous attempts to demonstrate regional and specific changes in arterial volume have used IVUS measurements of the most diseased 10-mm subsegments in coronary artery images (Nissen et al. 2003). Carotid artery thickness maps generated in this study reveal thickness changes localized to the bifurcation, which agrees with previous findings that regions of low shear stress contribute to the formation and stability of plaques (Cheng et al. 2006). When comparing regions of thickness change and the corresponding regions in the 3DUS axial images, thickness differences are coincident with hyperechoic contrast in the axial images, raising the possibility that at least some of the changes are occurring as a result of the disruption of fibrous thrombi.

There were a number of limitations of this study, the first of which is the fact that image quality was inadequate for the analysis of three subjects, limiting the analysis to 35 of 38 subjects who completed the study. A second important limitation was the fact that the established measurement of carotid atherosclerosis, measured using B-mode ultrasound (IMT) was not acquired, which limits the comparison of our results to other studies where IMT was measured. Comparisons of IMT with other measurements such as TPA, TPV and VWV have demonstrated that although IMT provides lower interscan and intraobserver variability (8.9% and 3.4%, respectively) (Egger et al. 2008b), in some cases VWV is more sensitive to temporal changes in carotid atherosclerosis compared with IMT and TPA (Mallett et al. 2009).

Another limitation is that although manual segmentation is yet the most reliable method for 3DUS VWV segmentation, it is still labor intensive and time consuming, which limits translation of this phenotyping tool until a semiautomated method is validated. Another shortcoming of our approach is that currently, VWV thickness difference maps do not display an image textural component to allow for visual discrimination between intima/media and plaque changes. Finally, although the maps and volumetric changes measured using VWV provide a new in vivo window with which to view carotid artery changes in response to therapy, the results here may only be considered applicable to the special subject group with carotid stenosis and significant plaque burden. In the current clinical care context, whereby most patients at risk are administered statins, it is difficult to predict and translate the current result. Although no intraobserver variability measurements were performed with this particular set of subjects, the significant plaque burden would be expected to minimize the variability of VWV measurements (Landry et al. 2004), possibly less than the coefficient of variation of ~4%, previously reported in a VWV study in which subjects only had moderate atherosclerosis and no stenosis (Egger et al. 2007).

Currently, the most cost effective and widely used imaging measurement of carotid atherosclerosis is IMT (Smilde et al. 2001; Crouse 3rd et al. 2007). Unfortunately, significant differences in IMT between treatment groups in the ENHANCE, RADIANCE1 and RADIANCE2 studies was not demonstrated, even while significant decreases in LDL levels and increased HDL were observed (Kastelein et al. 2007, 2008; Bots et al. 2007) possibly because of inadequate sensitivity or specificity or perhaps because no difference in IMT occurred. Comparisons of IMT with other measurements such as TPA, TPV and VWV have demonstrated that although IMT provides lower interscan and intraobserver variability (8.9% and 3.4%, respectively) (Egger et al. 2008b), in some cases VWV is more sensitive to temporal changes in carotid atherosclerosis when compared with IMT and TPA (Mallett et al. 2009). 3DUS VWV provides, essentially, a 3-D IMT measurement including any plaque and encompassing the entire carotid artery, including the near, far and side walls, and as such it can be considered an indicator of global change (Egger et al. 2007). Another advantage provided by 3DUS VWV is that VWV thickness maps and thickness difference maps can be generated for the visualization of atherosclerotic changes (Egger et al. 2008a). We propose 3DUS VWV as an alternative and complementary measurement to both IMT and TPV, essentially making use of the histologically validated boundaries provided in IMT measurements (Pignoli et al. 1986; Wong et al. 1993) and applying this in three dimensions.
SUMMARY

Carotid 3DUS VWV is a quantitative measure of atherosclerosis burden including the intima, media and plaque, with sensitivity to detect changes over short periods of time. Quantitative in vivo VWV thickness difference maps provide visual evidence of the spatial and temporal dynamics of carotid artery changes in response to therapy.

Acknowledgements—We gratefully acknowledge financial support from the Ontario Research and Development Challenge Fund, the Canadian Institutes of Health Research, the Heart and Stroke Foundation of Ontario and Pfizer Canada Inc. We also thank Chris Blake, M.Sc., Shanya McKay and Maria Cicciro for technical support. Helpful discussions regarding the results and analysis with Drs. Beverly Bauer, Brad Wyman, Dan Hackam and Richard Rankin are also gratefully acknowledged.

REFERENCES


