JACC: CARDIOVASCULAR IMAGING
VOL. 4, NO. 9, 2011

ISSN 1936-878X/$36.00
PUBLISHED BY ELSEVIER INC.
DOI:10.1016/j.jcmg.2011.06.013

CVN Interview

MR Imaging of Carotid Plaque Composition During Lipid-Lowering Therapy

A Prospective Assessment of Effect and Time Course

Xue-Qiao Zhao, MD,* Li Dong, MD,† Tom Hatsukami, MD,‡ Binh An Phan, MD,*
Baocheng Chu, MD, PhD,† Andrew Moore, BS,* Trevor Lane, BS,*
Moni B. Neradilek, MS,§ Nayak Polissar, PhD,§ Duane Monick, MD,‖ Colin Lee, MD,¶
Hunter Underhill, MD,† Chun Yuan, PhD†

Seattle and Yakima, Washington; and Boise, Idaho

OBJECTIVES The purpose of this study was to test the lipid depletion hypothesis and to establish the time course of change in carotid plaque morphology and composition during lipid therapy using high-resolution magnetic resonance imaging (MRI).

BACKGROUND Lipid therapy is thought to improve plaque stability and reduce cardiovascular events by targeting the plaque rupture risk features such as large lipid core, thin fibrous cap, and high level of inflammatory infiltrates. However, the plaque stabilizing process during lipid therapy has not been clearly demonstrated in humans and in vivo.

METHODS Subjects with coronary or carotid artery disease, apolipoprotein B $\geq$120 mg/dl, and lipid treatment history $<$1 year, were randomly assigned to atorvastatin monotherapy or to atorvastatin-based combination therapies with appropriate placebos for 3 years. All subjects underwent high-resolution, multicontrast bilateral carotid MRI scans at baseline and annually for 3 years. All images were analyzed for quantification of wall area and plaque composition blinded to therapy, laboratory results, and clinical course.

RESULTS After 3 years of lipid therapy, the 33 subjects with measurable lipid-rich necrotic core (LRNC) at baseline had a significant reduction in plaque lipid content: LRNC volume decreased from 60.4 $\pm$ 59.5 mm^3 to 37.4 $\pm$ 69.5 mm^3 ($p < 0.001$) and %LRNC (LRNC area/wall area in the lipid-rich regions) from 14.2 $\pm$ 7.0% to 7.4 $\pm$ 8.2% ($p < 0.001$). The time course showed that %LRNC decreased by 3.2 ($p < 0.001$) in the first year, by 3.0 ($p = 0.005$) in the second year, and by 0.91 ($p = 0.2$) in the third year. Changes in LRNC volume followed the same pattern. Percent wall volume (100 $\times$ wall/outer wall, a ratio of volumes) in the lipid-rich regions significantly decreased from 52.3 $\pm$ 8.5% to 48.6 $\pm$ 9.7% ($p = 0.002$). Slices containing LRNC had significantly more percent wall volume reduction than those without ($-$4.7% vs. $-$1.4%, $p = 0.02$).

CONCLUSIONS Intensive lipid therapy significantly depletes carotid plaque lipid. Statistically significant plaque lipid depletion is observed after 1 year of treatment and continues in the second year, and precedes plaque regression. (Using Magnetic Resonance Imaging to Evaluate Carotid Artery Plaque Composition in People Receiving Cholesterol-Lowering Medications [The CPC Study]; NCT00715273). (J Am Coll Cardiol Img 2011;4:977–86) © 2011 by the American College of Cardiology Foundation

From the *Department of Medicine, Division of Cardiology, University of Washington, Seattle, Washington; †Department of Radiology, Vascular Imaging Lab, University of Washington, Seattle, Washington; ‡Department of Surgery, Vascular Surgery,

The hypothesis of lipid depletion for plaque and clinical stability has been established on the basis of observations from animal studies (1–4), correlation of histological features in human coronary plaque with unstable clinical episodes (5–9), and angiographic regression and clinical event reduction with lipid-lowering therapy (10,11). In a case-control study of patients randomly allocated to 3 months of pravastatin treatment or placebo before carotid endarterectomy, Crisby et al. (12) provided histological evidence of the efficacy of therapy on plaque lipid content. However, in vivo verification of the lipid depletion hypothesis and establishment of the time course of plaque regression has not been possible until recently (13,14).

See page 987

ABBREVIATIONS AND ACRONYMS

APO = apolipoprotein
CIMT = carotid intima-media thickness
ER = extended release
HDL-C = high-density lipoprotein cholesterol
LDL-C = low-density lipoprotein cholesterol
LRNC = lipid-rich necrotic core
MRI = magnetic resonance imaging
PWV = percent wall volume

Development of high-resolution magnetic resonance imaging (MRI) techniques in recent years has made direct assessment of plaque tissue composition possible. Numerous studies have shown that MRI exhibits high contrast for internal plaque features, and that combined information from multiple contrast weightings is critical for distinguishing all plaque components (15–20). With confirmation by histological studies, high-resolution multicontrast MRI can accurately assess plaque tissue contents (21–23).

We designed and conducted a randomized, double blind, and partial placebo-controlled study entitled The CPC Study (Carotid Plaque Composition by MRI During Lipid-Lowering) to test 2 hypotheses: 1) intensive lipid therapy will deplete plaque lipid content; and 2) low-density lipoprotein cholesterol (LDL-C) lowering plus high-density lipoprotein cholesterol (HDL-C) raising, compared to LDL-C lowering alone, or similar HDL-C raising with further LDL-C lowering will lead to more plaque depletion. The primary endpoint is the carotid plaque lipid content as measured by MRI. The secondary endpoints include plaque burden and other plaque tissue contents. This report describes the study results of the first hypothesis.

METHODS

Study subjects. Details of the study design have been previously published (24). Briefly, participants were recruited from the University of Washington Medical Center Cardiac Catheterization Laboratory, the Yakima Heart Center Cardiac Catheterization Laboratory, and the St. Luke's Idaho Cardiology Associates in Boise. Subjects who met the following criteria were approached for the study: 1) angiographically confirmed coronary artery disease (defined as having at least 1 50% stenosis or 3 30% coronary lesions, or being post–myocardial infarction, post–percutaneous coronary intervention, or post–coronary artery bypass graft surgery) or carotid disease (defined as having a ≥15% stenosis by ultrasonography); 2) apolipoprotein (Apo) B ≥120 mg/dl; 3) duration of lipid therapy before enrollment <1 year; and 4) no contraindication for gadolinium-based contrast-enhanced carotid MRI. The study procedures and consent forms were approved by the University of Washington institutional review board.

Among the 123 subjects enrolled in the study, mean age was 55 years, mean body mass index was 30 kg/m^2, 73% were male, 43% had a family history of premature cardiovascular disease, 37% had a previous myocardial infarction, 80% had clinically established coronary artery disease, 52% were hypertensive, 12% had diabetes mellitus, 23% were current smokers, and 47% met the criteria for metabolic syndrome. The mean baseline total cholesterol was 238 mg/dl, triglycerides were 202 mg/dl, LDL-C was 163 mg/dl, HDL-C was 41 mg/dl, HDL2 was 7.4 mg/dl, ApoB was 131 mg/dl, ApoA1 was 129 mg/dl, lipoprotein(a) was 69 nmol/l, fasting glucose level was 103 mg/dl, insulin level was 25 μU/dl, and median high-sensitivity C-reactive protein was 1.8 mg/l. The mean (± SD) lipid treatment history before enrollment was 7 ± 4 months.

University of Washington, Seattle, Washington; §The Mountain-Whisper-Light Statistics, Seattle, Washington; ‖Yakima Heart Center, Yakima, Washington; and ¶St. Luke's Idaho Cardiology Associates, Boise, Idaho. This research is supported by a National Institutes of Health grant (R01 HL63895) from the National Heart, Lung, and Blood Institute, and by Pfizer Inc. The study medications are provided by Pfizer Inc., Abbott Laboratories, and Daiichi Sankyo, Inc. The authors have reported they have no relationships relevant to the contents of this paper to disclose.

Manuscript received December 15, 2010; revised manuscript received May 26, 2011, accepted June 8, 2011.

Of the 123 subjects, 33 with acceptable image quality, measurable lipid-rich necrotic core (LRNC) on baseline carotid MRI and LRNC measurements at year 3 were selected to test the hypothesis that intensive lipid therapy will deplete plaque lipid content.

Lipid treatments and clinical follow-up. All subjects were randomly assigned to 1 of 3 treatment groups: 1) single therapy: atorvastatin (10 to 80 mg/day) alone, placebos for extended release (ER) niacin and colesevelam; 2) double therapy: atorvastatin plus ER-niacin (2 g/day), and placebo for colesevelam; 3) triple therapy: atorvastatin, ER-niacin plus colesevelam (3.8 g/day). The treatment target for LDL-C was ≤80 mg/dl for the single-therapy and double-therapy groups and ≤60 mg/dl for the triple-therapy group. The HDL-C target for the 2 niacin-treated groups was to increase 10 mg/dl from baseline.

All subjects were followed up monthly for the first 6 months and then bimonthly for the remaining 30 months of the 36-month protocol. A total of 21 visits per subject was conducted for this study. At these visits, subjects underwent questioning about side effects and symptomatic state as well as a targeted cardiovascular examination. Any adverse events, both cardiovascular and noncardiovascular, were collected. At approximately 50% of the visits, blood samples were drawn (<50 ml). In all treatment arms, dietary counseling was targeted at weight reduction with goals based on the level of obesity, with additional emphasis on the use of monounsaturated fats in a fat-limited (25% to 30%) diet.

As published previously (25), after 1 year of the study therapy, on average, ApoB was reduced by 40%, 45%, and 51% among single-, double-, and triple-therapy groups, respectively; and LDL-C was lowered by 47%, 47%, and 57%, respectively; triglycerides decreased by 25%, 33%, and 42%, respectively; and HDL-C was increased by 12%, 25%, and 29%, respectively.

Carotid MRI scans. High-resolution, multicontrast bilateral carotid MRI scans were performed on a GE 1.5-T Signa whole body scanner using a 5.6 echo speed platform (Waukesha, Wisconsin). The carotid MR scans were obtained at baseline, and at 1, 2, and 3 years. All follow-up MRI scans were repeated using the same imaging protocol and carefully matched for scan coverage using the carotid bifurcation as internal landmark.

Subjects were placed in the supine position in the MRI scanner with the neck extended to bring the carotid arteries into a more superficial location relative to the skin. A custom designed head holder was used to minimize subject movement. Two separate phased-array carotid coils (26) were used for simultaneous bilateral scan. As described in the study methodology (24), 2-dimensional time-of-flight, proton density, and T2-weighted and T1-weighted images were acquired. The longitudinal coverage of this set of images (10 to 12 slices) was centered at the carotid bifurcation and covered the entire most likely diseased region. Then 20 ml (0.1 mmol/kg) of gadolinium contrast material was administrated intravenously though a power injector; the T1 scan was repeated 5 to 7 min after contrast administration. The total scan time for each subject was 40 to 50 min.

MRI analysis for quantification of plaque size and tissue composition. Images were analyzed by experienced reviewers in the Vascular Imaging Laboratory at the University of Washington. A peer-review process was conducted to reach consensus on each of the reviews. The image analyses were blinded to therapy, laboratory results, and clinical course. One experienced reviewer (B.A.P.), blinded to treatment and time points, matched the axial images from different time points according to their distance from the bifurcation of the carotid artery (carotid bifurcation was always used as the landmark for orientation and coverage). The results were peer reviewed by other experienced reviewers (H.U. and B.C.) who were also blinded to therapy and time points. All reviewers separately interpreted each image, and the reviewers conferred to reach a consensus opinion.

Total vessel area was quantified by placing contours around the outer wall boundaries of the carotid artery using CASCADE (27), a custom-designed image analysis tool. Similarly, lumen area was quantified by placing contours around the lumen boundary, and wall area was calculated by subtracting the lumen area from the total vessel area. Lumen, wall, and total vessel volumes were derived by adding the areas across slices and multiplying by the sum by the slice thickness (2 mm). Percent wall volume (PWV) was calculated using the following formula: (wall volume/total vessel volume) × 100%. The PWV provides a measure of plaque burden that adjusts for variation in artery size, and is similar to percent atheroma volume described in the intravascular ultrasound literature.

Carotid plaque tissue composition (LRNC, fibrous tissue, loose matrix, and calcification) was identified and quantified using previously published

MRI criteria (19,23). Specifically, LRNC was identified using multicontrast weightings plus post-contrast T1-weighted images. LRNC usually appears isointense to hyperintense on the time-of-flight and pre-contrast T1-weighted images and has varied signal intensity on protein density weighted and T2-weighted images, has no or slight contrast enhancement compared with the surrounding tissue on post-contrast T1-weighted images. Plaque component volumes were calculated as described above, and the proportional volume of each component was calculated using the formula: (component volume/wall volume) × 100%.

The plaque LRNC, as the primary end point of the study, was assessed in 2 different ways: 1) total LRNC volume; and 2) percent LRNC (%LRNC) relative to the lipid-containing slices only. As illustrated in Figure 1, the total LRNC volume was measured by multiplying the slice thickness by the sum of the LRNC areas (circled in yellow) on the 8 consecutive images from common carotid through bifurcation to the internal carotid arteries. The %LRNC was calculated based only on the slices containing a LRNC (slices #2 to #7 in this example).

Statistical analysis. In all analyses, the artery was used as the unit of observation. That is, in a given analysis, slice values of the outcome within a given subjects were pooled (as described previously) to produce 1 value per subject. Data from 1 carotid artery (the index artery with more lesion) per subject were used in the analysis. Descriptive statistics are presented as mean ± SD. Changes between carotid scans were annualized because of the variation in time between visits across subjects. Changes over time were compared to zero using the Wilcoxon signed-rank test or the 1-sample *t* test. The *t* test was used for carotid wall measurement data, which were reasonably similar to the normal distribution. Normality of outcomes was assessed by Q-Q plots. The Wilcoxon signed-rank test was carried out for comparisons involving plaque tissue components, which sometimes had non-normal distributions. Change in %LRNC and PWV were

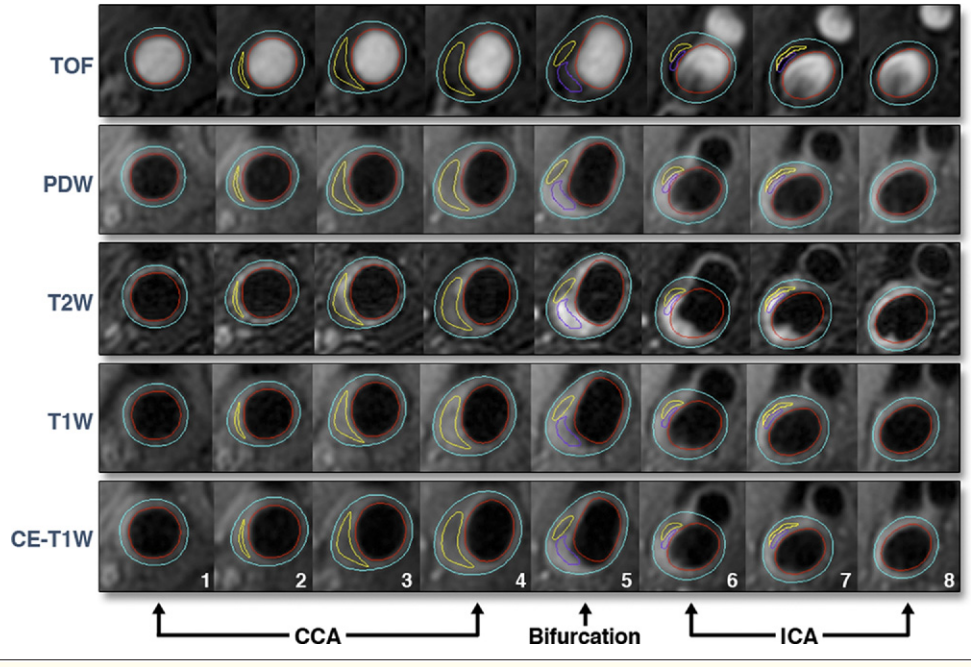

Figure 1. Example of Carotid Artery in Multicontrast MRI

Magnetic resonance imaging (MRI) example of 8 consecutive images of the common carotid artery (CCA) through bifurcation to the internal carotid arteries (ICA) from 5 contrast weightings: time of flight (TOF), proton density weighted (PDW), T2-weighted (T2W), T1-weighted (T1W), and post-contrast T1W. The lumen in **red** and outer wall boundary in **blue** of the carotid artery are identified and outlined. Plaque tissue components within the arterial wall are identified using an established algorithm and quantified (loose matrix in **purple** and lipid content in **yellow**). For example, lipid content in **yellow** appears isointense on the TOF and pre-contrast T1W images and hypointense on PDW and T2W images, and has no contrast enhancement compared with the surrounding tissue on post-contrast T1W images. Total lipid-rich necrotic core (LRNC) volume is calculated by multiplying the slice thickness (2 mm) by the sum of the areas circled in **yellow color** on the 8 consecutive images. Percent LRNC (%LRNC) is the proportion of the wall occupied by the LRNC only in the lipid-containing slices, from slice #2 to #7.

correlated with changes with lipid variables using Spearman correlation. Calculations were carried out in R, version 2.9.0 (28) All p values <0.05 were considered statistically significant.

RESULTS

Change in plaque lipid content. Among the 123 subjects enrolled in this study, 33 subjects with measureable LRNC at baseline who completed 3-year follow-up scans and had acceptable image quality were included in this comparison. Six subjects were excluded because of poor image quality. Another 81 subjects had no measurable LRNC at baseline and were excluded from the analysis. Finally, 3 subjects with LRNC at baseline did not complete 3-year follow-up scans and were excluded, too.

After 3 years of intensive lipid therapy, LRNC volume significantly decreased from a mean ± SD of 60.4 ± 59.5 mm^3 to 37.4 ± 69.5 mm^3 ($p < 0.001$) and %LRNC from 14.2 ± 7.0% to 7.4 ± 8.2% ($p < 0.001$), as summarized in Figure 2. An example of a carotid plaque with reduction in LRNC size is shown in Figure 3A.

There were 5 subjects who had new measurable LRNC at some point during the 3 years. Only 1 of these 5 subjects had measurable LRNC at the 3-year scan. Among the 75 study subjects with all 4 annual measurements, the overall number of subjects with measurable LRNC was significantly reduced over the 3 years: 44% at baseline versus 33% at 3 years ($p = 0.03$, McNemar test).

Plaque lipid depletion time course. The year-by-year plaque lipid depletion time course is also illustrated in Figure 2. The mean LRNC volume significantly decreased by 12 mm^3 ($p = 0.007$) in the first year, by 13 mm^3 ($p = 0.004$) in the second year, and increased nonsignificantly by 0.4 mm^3 ($p = 0.3$) in the third year. Similarly, mean %LRNC among lipid-containing slices significantly decreased by 3.2 ($p < 0.001$) in the first year, by 3.0 ($p = 0.005$) in the second year, and reduced nonsignificantly by 0.9 ($p = 0.2$) in the third year. Figure 3B demonstrates an MRI example of plaque lipid depletion and regression in plaque volume over 3 years.

Changes in other plaque tissue components. Figure 4 shows plaque fibrous tissue, calcium, and loose matrix changes among lipid-containing slices over 3 years. Compared to baseline, the volume of fibrous tissue significantly decreased (571 mm^3 vs. 547 mm^3, $p = 0.007$), but percent of plaque composed of fibrous tissue significantly increased (83.4% vs. 90.1%, $p < 0.001$) during the 3 years. The mean volume of fibrous tissue decreased slightly in the first year and significantly in the second year. Mean

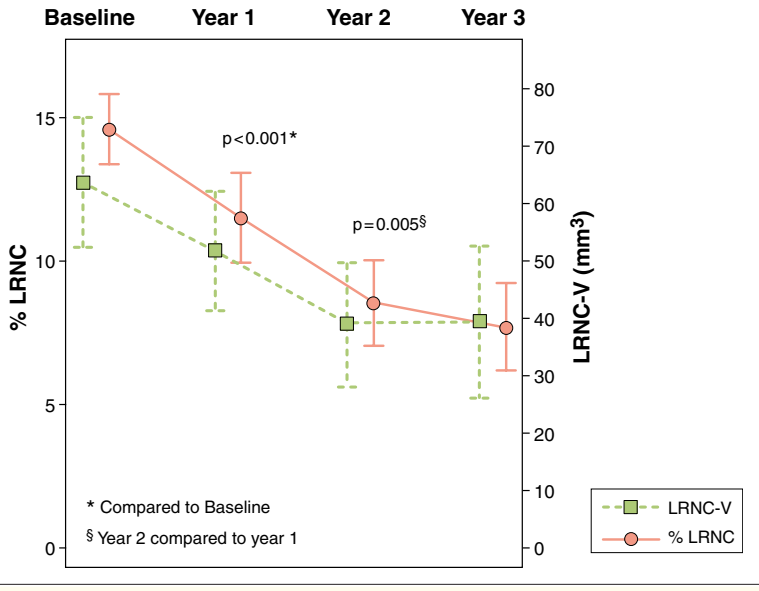

Figure 2. Carotid Plaque Lipid Depletion and Time Course During 3-Year Lipid Therapy

After 3 years of intensive lipid therapy, lipid-rich necrotic core volume (LRNC-V) **(green squares)** significantly decreased from 60.4 mm^3 to 37.4 mm^3. Percent LRNC (%LRNC) **(pink circles)** also significantly decreased from 14.2% to 7.4%. The plaque lipid depletion time course over 3 years showed that %LRNC for pooled slices containing lipid at any time point significantly decreased by 3.2% in the first year, significantly decreased by 3.0% in the second year, and decreased by 0.9% in the third year (the change from year 2 to 3 was not statistically significant). **Bars** around the estimates are standard error bars.

JACC: CARDIOVASCULAR IMAGING, VOL. 4, NO. 9, 2011
SEPTEMBER 2011:977–86

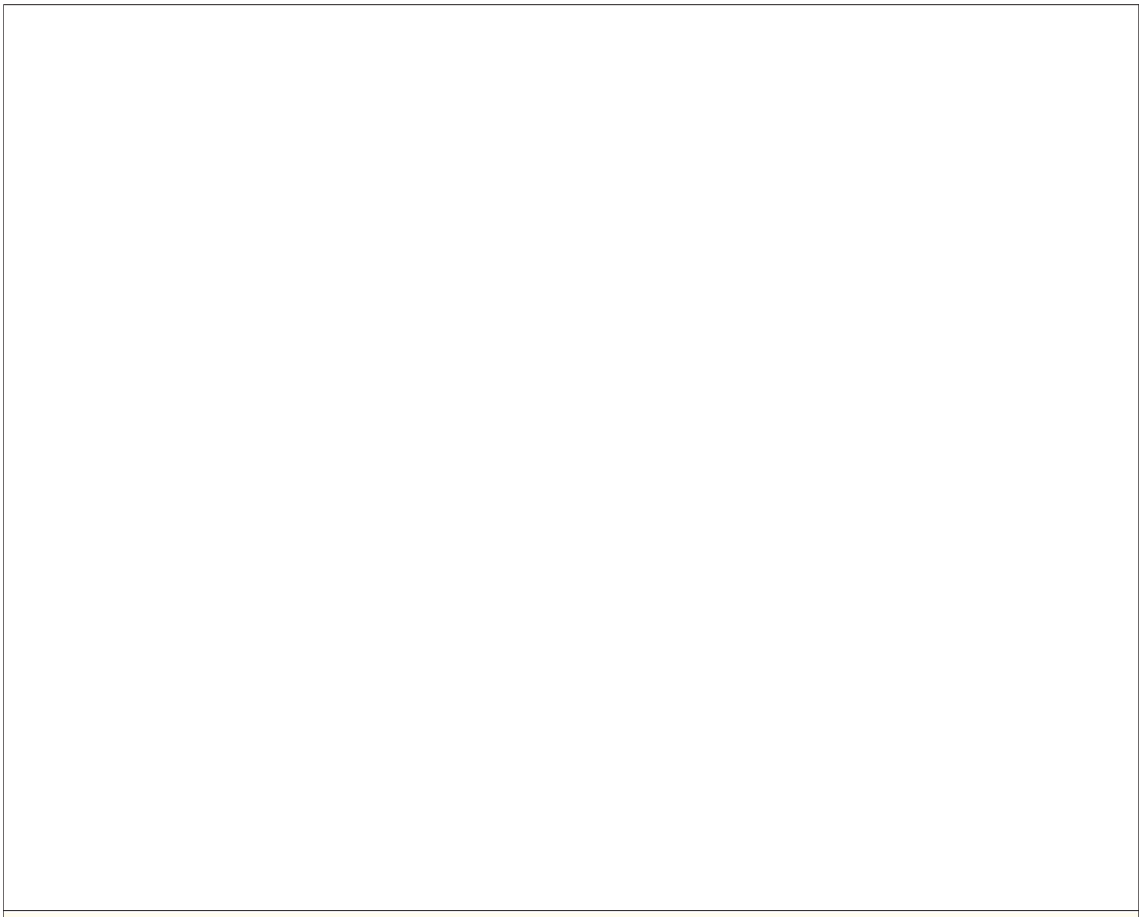

Figure 3. MRI Examples of Plaque Lipid Depletion and Time Course

(A) An example of significant lipid content reduction **(yellow arrows)** and plaque regression at 3 years compared to baseline in the left carotid artery. Overall, 11% of study subjects had completed plaque lipid depletion over 3 years. **(B)** Magnetic resonance imaging (MRI) example of the plaque lipid depletion time course. Regression in lipid-rich necrotic core (LRNC) size was notable between the baseline, 1-year, and 2-year MRI scans. Regression in plaque volume seemed to follow plaque lipid depletion and was most pronounced from years 1 to 3. CE = contrast enhanced; T1W = T1-weighted.

percent fibrous tissue increased significantly during each of the first 2 years. Both volume and percent of fibrous tissue did not change significantly in the third year. Calcium and loose matrix, both in volume and percent composition, remained at a similar level over the 3 years.

Carotid arterial wall burden change and its time course. Three-year and annual changes in total wall volume, PWV, and lumen volume were assessed to describe and test carotid atherosclerotic burden change in subjects with measurable LRNC at baseline. As shown in Table 1, after 3 years of intensive lipid therapy, total wall volume and PWV decreased significantly, but there was no statistically significant change in lumen volume. Time course showed no statistically significant change in wall volume or PWV in the first year, but significant changes during the second year, as wall volume decreased by 42 mm^3 ($p < 0.001$ for 2-year volume compared to baseline; $p < 0.01$ compared to year 1) and PWV was reduced by 2.1% ($p < 0.001$ for 2-year compared to baseline, $p < 0.01$ compared to year 1). The changes in the third year were minimal.

Difference in wall volume change between slices with and without LRNC. As also shown in Table 1, the slices with LRNC showed a significant mean decrease in total wall volume and PWV over 3 years. The reduction in wall burden measurements in slices without LRNC showed the same direction as in slices with LRNC but was smaller and not statistically significant. Furthermore, both for wall volume and PWV, the 2 measures of wall burden, the differences in reduction between the slices with and without LRNC at baseline were statistically significant.

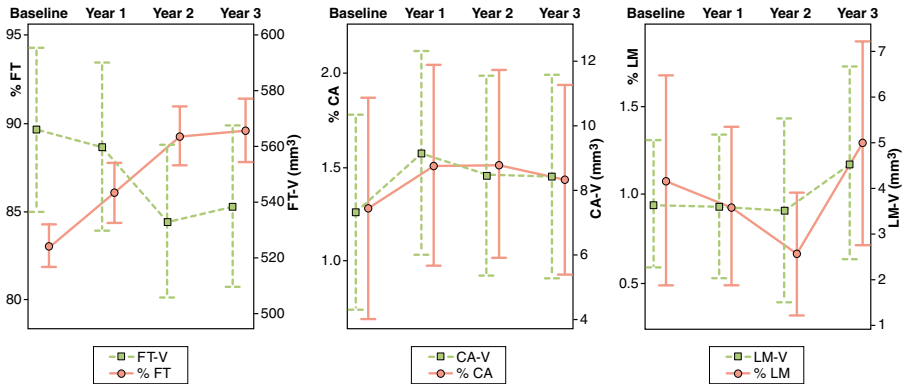

Figure 4. Carotid Plaque Tissue Composition Change Over 3-Year Lipid Therapy

Changes in plaque fibrous tissue (FT), calcium (CA), and loose matrix (LM), both volume (V) **(green squares)** and composition **(pink circles)**, over 3 years. CA = calcium; FT = fibrous tissue; LM = loose matrix. Compared to baseline, volume of fibrous tissue decreased, but percent of fibrous tissue increased. These changes were significant at each of the first 2 years. Calcium and loose matrix did not change significantly over 3 years. Bars around the estimates are standard error bars.

Relation between plaque and lipid changes. Spearman correlation analysis showed no statistically significant association between change in %LRNC and PWV as assessed by MRI and change in lipid variables over 3 years among the 33 subjects with measurable LRNC at baseline in this report. Observed Spearman correlations ranged in magnitude from 0.00 to 0.31. The lipid variables include changes in total cholesterol, very low density lipoprotein cholesterol, intermediate-density lipoprotein cholesterol, LDL-C, HDL-C, HDL-C2, HDL-C3, ApoB, ApoA1, and triglycerides.

DISCUSSION

The most widely accepted hypothesis is that lipid-lowering therapy targets the plaque rupture risk features such as large lipid core, thin fibrous cap, and high level of inflammatory infiltrates and activity; therefore, the plaque stability is improved and cardiovascular events are reduced. The evidence from diet-induced atherosclerosis animal models (1–4) showed that lowering cholesterol can decrease foam cells and cholesterol ester content at 6 months, diminish cholesterol crystals and necrosis at 12 months, and reduce plaque size at 24 to 48 months. The CPC study, 1 of the first studies testing the lipid depletion hypothesis in humans and in vivo, demonstrated that intensive lipid therapy significantly depletes plaque lipid content over 3 years. We found a 6.8% absolute reduction in lipid-containing segment (14.2% at baseline vs. 7.4% at 3 years) with intensive lipid therapy and 11% lower frequency of subjects with measurable LRNC (44% at baseline vs. 33% at 3 years). These reductions were statistically significant.

Histological studies of human coronary plaques have demonstrated an association between features such as the presence of a large lipid core, thin fibrous cap, and high concentration of inflammatory infiltrates with prior unstable clinical events (5–9). In the carotid artery, MRI has provided an essential tool for identifying potential high-risk

Table 1. Carotid Wall Measurements Change Over 3 Years and Differential Changes Between Slices With and Without LRNC

	Comparison: Baseline vs. 3 Yrs				Annual Changes*				Comparison: Change Over 3 Yrs Slices LRNC (+) vs. LRNC (−)†			
	n	Baseline	3 Yrs	p Value‡	n	1st Yr	2nd Yr	3rd Yr	n	LRNC (+)	LRNC (−)	p Value‡
Wall volume, mm³	33	641 ± 200	597 ± 198	<0.001	30	−16 ± 62	−42 ± 57§‖	3 ± 34	31	−45 ± 55¶	−11 ± 32	0.004
Percent wall volume, %	33	46.5 ± 7.1	44.3 ± 7.2	0.006	30	−0.6 ± 2.9	−2.1 ± 3.2§‖	−0.2 ± 2.5	31	−4.7 ± 7.1¶	−1.4 ± 3.2	0.020
Lumen volume, mm³	33	750 ± 249	764 ± 258	0.110	30	2 ± 50	18 ± 52	5 ± 42	31	12 ± 41	7 ± 40	0.300

Values are mean ± SD for the comparison of baseline versus 3 years and mean ± SE for each annual change during the 3 years. *Changes between the scans were annualized because of different times between the visits for different subjects. The calculation of changes was limited to patients with all 4 annual visits. Thus, the sample size is smaller than for the comparison of baseline versus 3 years. †The smaller sample size (n = 31) was the results of 2 patients not having both lipid-rich necrotic core (LRNC) positive (+) and LRNC negative (−) slices. ‡Paired *t* test. §$p < 0.001$ when compared to null hypothesis of zero change. ‖$p \leq 0.01$ when compared to year 1. ¶$p < 0.001$ when compared to zero change.

plaque features associated with more rapid progression and future ischemic events. In a recent prospective natural history study of carotid atherosclerosis, Underhill et al. (29) showed that carotid plaques with larger LRNC at baseline were associated with a significantly higher risk of plaque surface disruption on 3-year follow-up carotid MRI. Furthermore, in a prospective study of subjects with initially asymptomatic 50% to 79% carotid stenosis, Takaya et al. (30) found that plaques with larger LRNC were associated with a higher risk of cerebrovascular events during a mean follow-up of 3 years (hazard ratio for every 10% increase in LRNC size: 1.6, $p = 0.004$). The plaque lipid depletion demonstrated in this study suggest a potential mechanism by which aggressive lipid therapy results in plaque stabilization and a lower risk for future ischemic events, and provides compelling evidence to justify larger prospective clinical trials to this hypothesis.

This study also demonstrated that significant plaque lipid depletion can be achieved after 1 year of intensive treatment, continues with a similar rate of change during the second year, and begins to plateau during the third year. The time course for plaque lipid depletion demonstrated in this study provides a vascular biological explanation for the onset and persistence of clinical benefit seen in the placebo-controlled lipid-lowering trials (10). The cardiovascular event reduction in these trials began at 1 to 2 years after initiation of lipid-lowering therapy, which corresponds with the timing of significant plaque lipid depletion and increase in percent fibrous tissue at 1 and 2 years observed in this study. The temporal relationship between the plaque biological changes and clinical event reduction during lipid therapy will be further examined in our on-going studies.

In addition to reduction in lipid core volume, regression in overall plaque burden was observed, primarily after 2 to 3 years of intensive lipid therapy, consistent with the observations from animal studies (1–4), and consistent with plaque burden regression seen in other lipid-lowering studies in humans (13,31,32). We believe that the regression observed in plaque burden was primarily induced by lipid depletion because:1) substantial and statistically significant carotid wall burden reduction only occurred in the slices containing a LRNC; and 2) the time course of wall burden reduction seemed to follow plaque lipid depletion, with the largest changes occurred during the second year.

The temporal relationship between plaque lipid and plaque burden regression, and the observed regression only at sites with a LRNC may also help to explain the inconsistent results seen in studies using carotid intima-media thickness (CIMT) as a marker for regression during LDL-C–lowering therapy. In the METEOR (Effect of Rosuvastatin on Progression of Carotid Intima-Media Thickness in Low-Risk Individuals With Subclinical Atherosclerosis) study (33), where relatively statin-naïve subjects with thick CIMTs were recruited (baseline CIMT of 1.16 mm, and hence more likely to have lipid-rich plaques), statistically significant differences between treatment groups were observed (0.014 mm/year). In contrast, the ENHANCE (Simvastatin With or Without Ezetimibe in Familial Hypercholesterolemia) study (34), where subjects had been treated for an extended period of time, and had a relative normal CIMT at baseline (0.68 mm and more likely to have lipid-poor plaques) showed a very small difference (0.005 mm per 2 years) between groups. Once atherosclerotic plaque lipid has been depleted and regression has achieved with therapy, further treatment is certainly necessary to keep plaque stable without significant progression, but maybe unlikely to induce further regression.

Although there are no statistically significant correlations between the plaque changes, in both %LRNC and PWV, and the lipid variables, this does not indicate there is no differential treatment effect among the 3 different lipid therapies, because only 33 subjects were included in the current correlation analysis. The differential treatment effect will be investigated fully when the CPC study is closed, and it will be further examined in the carotid MRI substudy in the AIM-HIGH (Atherothrombosis Intervention in Metabolic syndrome with low HDL/high triglycerides: Impact on Global Health outcomes) trial in approximately 200 subjects (35).

The effects of the 3 treatment therapies may have introduced some heterogeneity into the responses. This increase in variability, if it occurred, would tend to decrease power to detect changes and identify correlates of change. Thus, statements of statistical significance in this paper may be conservative. It is unlikely that statistically significant differences noted here are artifacts. Statistically significant differences and associations observed in this study—with a sample size of <40 subjects—can be attributed to several aspects of the study, including: 1) a relatively treatment-naive population; 2) potent lipid modification on both LDL-C and HDL-C, and; 3) highly accurate assessment of plaque change by MRI.

CONCLUSIONS

In conclusion, intensive lipid therapy significantly depletes carotid atherosclerotic plaque lipid in humans. Statistically significant plaque lipid depletion is observed after 1 year of treatment and continues in the second year. Regression in overall plaque burden was observed primarily at locations with a LRNC, and its time course follows plaque lipid depletion. These findings suggest a potential mechanism for plaque stabilization associated with intensive lipid therapy. Furthermore, these results highlight the importance of selection of subjects with a LRNC in future trials examining the effect of therapy on plaque regression.

Reprint requests and correspondence: Dr. Xue-Qiao Zhao, University of Washington, Cardiology, Box 354800, 1107 Northeast 45th Street, Suite 114, Seattle, Washington 98105. *E-mail: xueqiao@u.washington.edu.*

REFERENCES

1. Armstrong ML, Megan MB. Lipid depletion in atheromatous coronary arteries in rhesus monkeys after regression diets. Circulation Res 1972; 30:675–80.
2. Armstrong MC, Megan MB. Arterial fibrous protein in cynomolygous monkeys after atherogenic and regression diets. Circulation Res 1975;36:256–61.
3. Clarkson TB, Bond MG, Bullock BC, Marzetta CA. A study of atherosclerosis regression in Macaca mulatta. IV. Changes in coronary arteries from animals with atherosclerosis induced for 19 months and then regressed for 24 or 48 months at plasma cholesterol concentrations of 300 or 200 mg/dl. Exp Mol Pathol 1981;34:345–68.
4. Small DM, Bond MG, Waugh D, Prack M, Sawyer JK. Physiochemical and histological changes in the arterial wall of nonhuman primates during progression and regression of atherosclerosis. J Clin Invest 1984;73:1590–605.
5. Davies MJ. Stability and instability: two faces of coronary atherosclerosis. The Paul Dudley White Lecture 1995. Circulation 1996;94:2013–20.
6. Van der Wal AC, Becker AE, Van der Loos CM, Das PK. Site of intimal rupture or erosion of thrombosed coronary atherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology. Circulation 1994;89:36–44.
7. Moreno PR, Falk E, Palacios IF, Newell JB, Fuster V, Fallon JT. Macrophage infiltration in acute coronary syndromes: implications for plaque rupture. Circulation 1994;90:775–8.
8. Davies MJ, Richardson PD, Woolf N, Katz DR, Mann J. Risk of thrombosis in human atherosclerotic plaques: role of extracellular lipid, macrophages, and smooth muscle cell content. Br Heart J 1993;69:377–81.
9. Libby P, Geng YJ, Aikawa M, et al. Macrophages and atherosclerotic plaque stability. Curr Opin Lipidol 1996;7:330–5.
10. Brown BG, Zhao X-Q. Lipid-Lowering Therapy. In: Hennekens CH, editor. Clinical Trials in Cardiovascular Disease (A Companion to Braunwald's Heart Disease). Philadelphia, PA: WB Saunders, 1999:199–216.
11. Brown BG, Zhao XQ, Chait A, et al. Simvastatin and niacin, antioxidant vitamins, or the combination for prevention of coronary disease. N Engl J Med 2001;345:1583–92.
12. Crisby M, Nordin-Fredriksson G, Shah PK, Yano J, Zhu J, Nilsson J. Pravastatin treatment increases collagen content and decreases lipid content, inflammation, metalloproteinases, and cell death in human carotid plaques: implications for plaque stabilization. Circulation 2001;103:926–33.
13. Zhao XQ, Chun Y, Hatsukami TS, et al. Effects of intensive lipid-lowering therapy on carotid atherosclerotic plaque composition in vivo by MRI: a case-control study. Arterioscler Thromb Vasc Biol 2001;21:1623–9.
14. Underhill HR, Yuan C, Zhao XQ, et al. Effect of rosuvastatin therapy on carotid plaque morphology and composition in moderately hypercholesterolemic patients: a high-resolution magnetic resonance imaging trial. Am Heart J 2008; 155:584.e1–8.
15. Shinnar M, Fallon JT, Wehrli S, et al. The diagnostic accuracy of ex vivo MRI for human atherosclerotic plaque characterization. Arterioscler Thromb Vasc Biol 1999;19:2756–61.
16. Yuan C, Mitsumori LM, Ferguson MS, et al. In vivo accuracy of multispectral magnetic resonance imaging for identifying lipid-rich necrotic cores and intraplaque hemorrhage in advanced human carotid plaques. Circulation 2001;104:2051–6.
17. Mitsumori LM, Hatsukami TS, Ferguson MS, Kerwin WS, Cai J, Yuan C. In vivo accuracy of multisequence MR imaging for identifying unstable fibrous caps in advanced human carotid plaques. J Magn Reson Imaging 2003;17:410–20.
18. Cappendijk VC, Cleutjens KB, Kessels AG, et al. Assessment of human atherosclerotic carotid plaque components with multisequence MR imaging: initial experience. Radiology 2005;234:487–92.
19. Saam T, Cai JM, Cai YQ, et al. Carotid plaque composition differs between ethno-racial groups: an MRI pilot study comparing mainland Chinese and American Caucasian patients. Arterioscler Thromb Vasc Biol 2005;25:611–6.
20. Clarke SE, Hammond RR, Mitchell JR, Rutt BK. Quantitative assessment of carotid plaque composition using multicontrast MRI and registered histology. Magn Reson Med 2003;50: 1199–208.
21. Cai JM, Hatsukami TS, Ferguson MS, Small R, Polissar NL, Yuan C. Classification of human carotid atherosclerotic lesions with in vivo multicontrast magnetic resonance imaging. Circulation 2002;106: 1368–73.
22. Takaya N, Cai J, Ferguson MS, et al. Intra- and interreader reproducibility of magnetic resonance imaging for quantifying the lipid-rich necrotic core is improved with gadolinium contrast enhancement. J Magn Reson Imaging 2006;24:203–10.
23. Cai JM, Hatsukami TS, Ferguson MS, et al. In vivo quantitative measurement of intact fibrous cap and lipid rich necrotic core size in atherosclerotic carotid plaque: a comparison of high resolution contrast enhanced MRI and histology. Circulation 2005; 112:3437–44.
24. Zhao XQ, Phan BA, Chu B, et al. Testing the hypothesis of atherosclerotic plaque lipid depletion during lipid therapy by magnetic resonance imaging: study design of Carotid Plaque Composition Study. Am Heart J 2007;154:239–46.

25. Moore AB, Phan BA, Challender CM, Williamson J, Marcovina S, Zhao XQ. Effects of adding extended-release niacin and colesevelam to statin therapy on lipid levels in subjects with atherosclerotic disease. J Clin Lipidol 2007;1:620–5.
26. Hayes CE, Mathis CM, Yuan C. Surface coil phased arrays for high resolution imaging of the carotid arteries. J Magn Reson Imaging 1996;1:109–12.
27. Kerwin W, Xu D, Liu F, et al. Magnetic resonance imaging of carotid atherosclerosis: plaque analysis. Top Magn Reson Imaging 2007;18:371–8.
28. R: A Language and Environment for Statistical Computing. Version 2.9.0. Vienna, Austria: The R Foundation for Statistical Computing, 2009.
29. Underhill HR, Yuan C, Yarnykh VL, et al. Predictors of surface disruption with MR imaging in asymptomatic carotid artery stenosis. AJNR Am J Neuroradiol 2009;31:487–93.
30. Takaya N, Yuan C, Chu B, et al. Association between carotid plaque characteristics and subsequent ischemic cerebrovascular events: a prospective assessment with MRI—initial results. Stroke 2006;37:818–23.
31. Corti R, Fuster V, Fayad ZA, et al. Lipid lowering by simvastatin induces regression of human atherosclerotic lesions: two years' follow-up by high-resolution noninvasive magnetic resonance imaging. Circulation 2002;106:2884–7.
32. Corti R, Fuster V, Fayad ZA, et al. Effects of aggressive versus conventional lipid-lowering therapy by simvastatin on human atherosclerotic lesions: a prospective, randomized, double-blind trial with high-resolution magnetic resonance imaging. J Am Coll Cardiol 2005;46:106–12.
33. Crouse JR III, Raichlen JS, Riley WA, et al., for the METEOR Study Group. Effect of rosuvastatin on progression of carotid intima-media thickness in low-risk individuals with subclinical atherosclerosis: the METEOR trial. JAMA 2007;297:1344–53.
34. Kastelein JJ, Akdim F, Stroes ES, et al., for the ENHANCE Investigators. Simvastatin with or without ezetimibe in familial hypercholesterolemia. N Engl J Med 2008;358:1431–43.
35. Carotid Plaque Characteristics by MRI in AIM-HIGH. Available at: http://clinicaltrials.gov/show/NCT01178320. Accessed August 3, 2011.

Key Words: atherosclerosis ■ carotid artery ■ lipids ■ magnetic resonance imaging.