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Letter to the Editor

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To the Editor:

A strong relationship exists between cholesterol and atherosclerosis,[1] with low density lipoprotein (LDL) being a major risk factor.[2] However, 50% of patients with acute coronary events have "normal" cholesterol, and 75% patients with premature coronary heart disease (CHD) have normal LDL.[3] Thus, contribution of other lipoproteins has been explored. High density lipoprotein (HDL), comprised primarily of 2 subfractions, HDL_2 (large buoyant) and HDL_3 (small dense), has a protective role in CHD.[4,5] LDL can be dense or buoyant, and dense LDL is highly atherogenic, associated with 4-fold increased CHD risk.[6] $Lipoprotein_a$ [Lp(a)], is a strong risk factor for CHD and stroke.[7]

MRI can noninvasively visualize arterial wall remodelling and atherosclerotic plaque components (lipid core, fibrous cap, and calcium).[8,9] However, in vivo relationships between plaque components and cholesterol subfractions have not been demonstrated in humans. We examined in vivo relationship between cholesterol subfractions and atherosclerotic plaque, measured by MRI, in internal carotid arteries (ICA) of atherosclerotic patients.

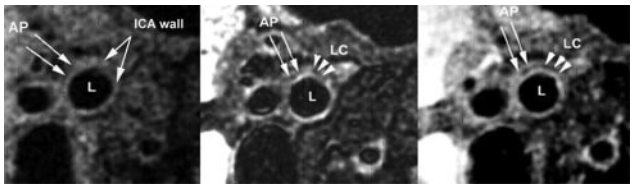
This was an exploratory cross-sectional study of consecutively enrolled initial 28 patients who were part of an ongoing randomized trial testing the effects of extended release Niacin versus placebo station top of baseline therapy on carotid plaque regression, analyzed at baseline. All patients signed written informed consent and had documented atherosclerosis in at least one vascular territory: >3.9 mm aortic atherosclerosis on transesophageal echocardiography, >50% lesion in one coronary artery at cardiac catheterization, >50% carotid lesion on ultrasound or peripheral arterial disease (PAD). We excluded patients with pacemakers, defibrillators, aneurysm clips, elevated liver transaminases (>2 X normal), significant medical event within 3 months, decompensated heart failure, or inability to consent. The types and dosages of statins, alcohol intake, and abdominal girth were recorded. To account for different statins, a dose standardization model was developed (10 mg Atorvastatin=20 mg Simvastatin=40 mg Pravastatin=80 mg Fluvastatin).[10]

After an overnight fast, patients had chemistries and lipid profile drawn. Subsequently, serum was separated, frozen at −70°C, and analyzed by ultracentrifugation for cholesterol subfractions [HDL_2, HDL_3, Dense LDL, Lp(a)] using the validated[11,12] vertical auto profile II technique by a certified laboratory (Atherotech, Inc).

On the same day, an MRI was performed on a 1.5-T magnet (GE Medical Systems) using a dual 3-inch immobilized surface coil.[9] Five double-oblique slices through proximal ICA were acquired at the level of thickest carotid plaque, perpendicular to long axis of the lumen at 2 weightings: T1 (TR=1 R-R, TE=minimum) and T2 (TR=2 R-R, TE=69 ms) before and after 0.1 mmol/kg intravenous injection of gadodiamide (GE Healthcare).

Vessel wall volume was calculated (cm^3) by drawing regions of interests (ROI's) at luminal and the external edges of ICA on precontrast T1 images (Figure A). ROI's were drawn around lipid core identified on postcontrast T1 images, as reported[9,13–15,16] (Figure C). Images from 5 randomly selected patients were reanalyzed by 2 observers for intra- and interobserver variability. Data were analyzed using customized software (University of Leiden, The Netherlands). Because of intrapatient carotid interdependence, the higher of 2 measurements from one ICA was used per patient (14 each on left and right).

Univariate and multivariate regression analysis was used to test association between vessel wall/lipid core volume and cholesterol subfractions. Confounding variables considered included: age, sex, hypertension, diabetes mellitus, smoking, alcohol intake, physical activity, serum creatinine, and type of statin used. Subsequently, only significant confounding variables (sex, diabetes, and abdominal girth) were used for multivariate analysis. Reproducibility was determined using intraclass correlation coefficients (ICC). A probability value <0.05 was considered statistically significant.



High resolution MR images of the right internal carotid artery (ICA) of a 70-year-old man with advanced atherosclerosis. T1-weighted (A), T2-weighted (both precontrast; B) and T1 weighted (postcontrast; C) fast spin echo images of the right internal carotid artery demonstrating arterial wall remodeling caused by atherosclerotic plaque (AP). Note the lipid core (LC) within the atherosclerotic plaque on T2-weighted and postcontrast T1-weighted images. L indicates lumen

The study population consisted of 21 males and 7 females (mean age 73±4 years, 77% hypertensives, 27% diabetics, 20% smokers, 34% with a history of stroke, 31% with history of myocardial infarction, 12% with PAD, and 70% with regular alcohol intake). Twenty four patients took baseline statins (12 Atorvastatin, 5 Simvastatin, 4 Pravastatin and 3 Fluvastatin). Mean abdominal girth was 39±5 inches, systolic blood pressure 131±17 mm Hg, and heart rate was 69±17 beats. Mean cholesterol subfraction values (mg/dL) were: total HDL, 49±11; HDL_2, 11±5; HDL_3, 37±7; LDL, 86±25; Dense LDL, 49±15; Lp(a), 6.4±4; and triglycerides, 137±104. Mean vessel wall and lipid core volume of ICA were 0.45±0.11 cm^3 and 0.03±0.03 cm^3, respectively.

Univariate regression analyses relating vessel wall volume and serum dense LDL, triglycerides, Lp (a), HDL_2, HDL_3, and HDL were not significant (r=0.19, 0.21, −0.05 to 0.2, −0.13 and −0.2, respectively). A significant relationship existed between ICA lipid core volume and total HDL (r=−0.5, P=0.01) as well as HDL_3 (r=−0.57, P=0.003). The association between lipid core and HDL_2 was borderline significant (r=−0.35, P=0.08) and not significant for dense LDL, Lp(a), or triglycerides (r=−0.14, −0.002, and −0.13). Multivariate analysis revealed significant relationships between lipid core and total HDL (adjusted R^2=58% for the model, *P* for HDL <0.05) and HDL_3 (adjusted R^2=63% for the model, *P* for HDL_3<0.001) and nonsignificant with HDL_2 (adjusted R^2=50% for the model, *P* for HDL_2=0.29) and other cholesterol subfractions. The ICCs for intraobserver and interobserver concordances for vessel wall volume (0.93 and 0.83, respectively) and lipid core volume (0.90 and 0.93, respectively) by MRI were high.

Our preliminary findings demonstrate a significant inverse association between lipid core measured in vivo by MRI and serum HDL and HDL_3 in patients with advanced atherosclerosis. There was no association between lipid core and other cholesterol subfractions, including HDL_2. HDL_3 is thought to be more effective than HDL_2 in inhibiting LDL oxidation, a major determinant of atherosclerosis progression and lipid core.[16] However, it is possible that some findings were affected by small sample size and baseline statin usage. Further longitudinal studies are needed to elucidate the role of cholesterol subfractions in atherosclerosis.

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