

Indicators of the atherogenic lipoprotein phenotype measured with density gradient ultracentrifugation predict changes in carotid intima-media thickness in men and women

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Objective: Progression of carotid intima-media thickness (CIMT) is a surrogate indicator for the early stages of atherosclerosis.

Methods: The study investigated relationships between baseline lipoprotein cholesterol, triglyceride (TG), and apolipoprotein (Apo) B levels assessed with density gradient ultracentrifugation (DGU) and progression of posterior wall common CIMT in men (45–75 years of age) and women (55–74 years of age) in the control arm of a clinical trial. Participants had baseline posterior wall CIMT 0.7–2.0 mm, without significant stenosis. CIMT was assessed using B-mode ultrasound at baseline, and 12 and ~18 months. A DGU cholesterol panel that assessed the major lipoprotein classes and subclasses, plus triglycerides, lipoprotein (a) cholesterol, low-density lipoprotein (LDL) peak time (inversely related to LDL particle density), and Apo B were performed on fasting baseline samples. Apo B was also measured using an enzyme linked immunosorbent assay.

Results: Baseline CIMT was inversely associated ($P < 0.001$) with CIMT progression. After adjustment for baseline CIMT, significant predictors of posterior wall CIMT progression in linear regression analyses included LDL peak time (inverse, $P = 0.045$), total high-density lipoprotein cholesterol (HDL-C) (inverse, $P = 0.001$), HDL₂-C (inverse, $P = 0.005$), HDL₃-C (inverse, $P = 0.003$), very low-density lipoprotein (VLDL)-C ($P = 0.037$), and VLDL₁₊₂-C ($P = 0.016$).

Conclusion: These data indicate that DGU-derived indicators of the “atherogenic lipoprotein phenotype,” including increased TG-rich lipoprotein cholesterol, lower HDL-C and HDL-C subfractions, and a greater proportion of LDL-C carried by more dense LDL particles, are associated with CIMT progression in men and women at moderate risk for coronary heart disease.

Keywords: carotid intima media thickness, density gradient ultracentrifugation, coronary heart disease risk, lipids, atherosclerosis, lipoprotein subfractions

Introduction

Carotid artery intima-media thickness (CIMT) is a surrogate measure of atherosclerosis that has been shown to correlate with risk factors for atherosclerotic cardiovascular disease.^{1–6} In a recent analysis of patients at moderate risk for coronary heart disease (CHD), non-lipid CHD risk factors were either unrelated to, or weakly associated with, CIMT progression, whereas several indicators of lipoprotein metabolism were significantly associated with CIMT progression.⁵ The strongest individual predictors of CIMT progression were lower baseline CIMT and increased concentrations of triglycerides (TG), non-high-density lipoprotein cholesterol (HDL-C), and apolipoprotein (Apo) B.⁵

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These relationships were based on a standard lipid panel performed using automated chemistry analyzers, including calculation of LDL-C using the Friedewald equation.⁷

While elevated low-density lipoprotein (LDL)-C and non-HDL-C and reduced HDL-C concentrations are acknowledged to be primary CHD risk factors,⁸ the clinical significance of all lipoprotein classes and subclasses is not fully understood. There are several methods available to measure lipoprotein subfractions including Vertical Auto Profile® (VAP; Atherotech Inc, Birmingham, AL), nuclear magnetic resonance, and gradient and modified nongradient gel electrophoresis.⁹ The VAP test directly measures cholesterol concentrations of the lipoprotein classes and subclasses after they are separated in a density gradient, using vertical spin ultracentrifugation.⁹ This study evaluated the associations between density gradient ultracentrifugation (DGU)-derived lipoprotein class and subclass cholesterol concentrations, derived Apo B and the rate of CIMT progression among the subjects at moderate CHD risk based on having CIMT above the US population median of 0.7 mm plus at least one major CHD risk factor or LDL-C ≥ 130 mg/dL.^{5,8}

Methods

Study design

The results described herein are from a sub-analysis of subjects from the control arm of a double-blind, randomized, clinical trial designed to evaluate the effects on CIMT of consumption of pomegranate juice versus a control beverage for ~18 months.¹⁰ Full details of the original study and prior subgroup analyses have been previously published.^{5,10} The trial was conducted in accordance with good clinical practice guidelines, and the protocol was approved by Quorum Review Inc, Seattle, WA. Two clinical research sites, Radiant Research, Chicago, IL and the University of Texas Southwestern Medical Center, Dallas, TX, enrolled subjects in the trial. All subjects provided written informed consent prior to any protocol-specific procedures, and visited the clinic at screening, weeks 0, 13, 26, 38, 52, 65, and once between weeks 78 and 90.

Subjects

Participants in the study included men (ages 45–74 years) and women (ages 55–74 years) at moderate risk for CHD due to at least one of the following: LDL-C ≥ 130 mg/dL and < 190 mg/dL, low HDL-C (< 40 mg/dL), elevated blood pressure ($\geq 140/90$ mmHg), use of medication to treat hypertension, or current cigarette smoking (any cigarette smoking within the past month). Subjects were required to

have a baseline posterior wall common CIMT measurement of > 0.7 mm and < 2.0 mm on either the right or left side, but carotid stenosis $\geq 50\%$ was exclusionary. Subjects were also excluded if they had CHD or a CHD risk equivalent, including diabetes; body mass index (BMI) > 40 kg/m²; hepatic disease or dysfunction; cancer (except non-melanoma skin cancer) in the past two years; human immunodeficiency virus; hepatitis B or C; uncontrolled hypertension (average systolic blood pressure ≥ 160 mmHg and/or average diastolic blood pressure ≥ 100 mmHg); cardiac arrhythmias; untreated hypothyroidism; used β -adrenergic blockers, immunosuppressants, or estrogen and/or progestin therapy; or recently (within 6 weeks prior to screening) used lipid-altering agents other than statins.

Carotid ultrasound measurements

Baseline, 12 month, and end of treatment posterior wall CIMT was measured following the methods described by Mazzone et al.¹¹ Using a high-resolution B-mode carotid artery ultrasound with an HDI® 5000 ultrasound system (Phillips Medical Systems NA, Bothell, WA) longitudinal scans were taken of the blood-intima and media-adventitia interfaces of the right and left common carotid arteries, along a 1 cm segment proximal to the bifurcation.¹⁰ Results were based on the averages of values for the right and left common carotid arteries. Software was used to ensure that all scans were performed in the same artery region. Using end-diastolic electrocardiographic gating scans, images were digitally recorded and the scans were transmitted to a central imaging laboratory where an expert reviewer calculated the mean CIMT using automated lumen-intima and media-adventitia edge detection (Io-QIMT, Synarc-IoDP Medical Imaging Research [Synarc, Paris, France]). All scans were read by a single reader.

Laboratory measurements

Laboratory measurements were conducted by Atherotech Inc (Birmingham, AL) on fasting samples collected at screening and/or baseline (average of two samples) and frozen at -80°C for up to approximately 7.5 years. DGU was used to measure total cholesterol (TC), total LDL-C [$\text{LDL}_{1+2+3+4}$ -C + lipoprotein (a)-C {Lp(a)-C} + intermediate density lipoprotein (IDL)-C], “real” LDL-C ($\text{LDL}_{1+2+3+4}$ -C), LDL_{1+2} -C, LDL_{3+4} -C, Lp(a)-C, LDL peak time, IDL-C, total HDL-C, HDL_2 -C, HDL_3 -C, total VLDL-C, VLDL_{1+2} -C, VLDL_3 -C, TG, and Apo B. Additionally, Apo B concentration was determined using an immunoturbidimetry method [Abbott Architect/C8000 instrument and Architect

Apolipoprotein B reagent (REF# 9D93-21)]. Non-HDL-C was calculated as TC – HDL-C.⁸

Statistical analyses

Statistical analyses were generated using SAS version 9.1.3 (SAS Institute, Cary, NC). Analyses were performed using data collected from subjects who had at least one post-baseline CIMT measurement and for whom DGU analysis results were available. There were no adjustments made for multiple comparisons, and *P*-values <0.05 were considered statistically significant to minimize the chances of a type II statistical error. To determine the CIMT progression rate (mm/year), the slope of the least squares regression line for CIMT on time was calculated for each subject. For subjects who dropped out of the study prior to the final measurement, the progression rate at month 12 was carried forward. Tertile groups were identified according to CIMT progression rate. Chi-square tests (categorical variables) and multivariate regression models, with the characteristic as the dependent variable and tertile group as the independent variable (continuous variables), were used to assess differences in baseline parameters across tertiles. For all models, assumptions of normality of residuals were investigated, and for models where it was determined that the distribution was not approximated by a normal curve, values for independent and/or dependent variables were ranked prior to the final analysis (equivalent to a non-parametric analysis). To further assess the relationships between lipoprotein lipid and Apo B values and CIMT progression rates, multivariate models that also contained the baseline CIMT value were generated to produce adjusted regression coefficients. Sensitivity analyses were also completed to assess possible confounding or effect modification (interaction) by several factors including age (median split), sex, ethnicity (non-Hispanic white and other), and use of lipid-altering agent(s). For these analyses, each model contained terms for baseline CIMT, the predictor variable, the potential effect modifier variable, and an interaction term (predictor x effect modifier variable).

Results

Study population

The original study randomized 383 subjects to either pomegranate juice (*n* = 192) or control (*n* = 191) groups. The results herein are from 110 subjects in the control arm who had at least one post-baseline posterior wall CIMT measurement and for whom DGU analyses results were available. Mean baseline ± standard error of the mean (SEM) baseline, 12 month, and end of study posterior wall CIMT values were

0.78 ± 0.01, 0.79 ± 0.01, 0.78 ± 0.01 mm, respectively. The mean progression rate in the control group at the end of the trial was 0.0070 ± 0.0034 mm/year, respectively.

Subject characteristics according to tertiles of CIMT progression

Demographic and baseline clinical characteristics of the subjects according to tertiles of posterior wall CIMT progression at the end of the treatment period are shown in Table 1. Univariate analyses for CIMT progression as an ordinal (tertile) variable yielded a significant trend for increasing CIMT progression tertile with greater concentrations of fasting glucose (*P* = 0.006) and a significant trend for less CIMT progression higher baseline CIMT (*P* < 0.001). A significant difference among tertiles was also shown for lipid-altering medication use (*P* = 0.032), however there was no clear pattern, as the greatest numbers of subjects taking lipid-altering medications (primarily statins) were in the lowest and highest tertiles of CIMT progression. Analyses completed for the subset of subjects who did not use lipid-altering medications yielded parameter estimates that were not materially different from those in the entire sample (data not shown).

Baseline DGU lipids and Apo B measurements according to tertiles of posterior wall CIMT progression at the end of the treatment period are shown in Table 2. Significant relationships were detected for increasing CIMT progression tertile with greater concentrations of LDL₃₊₄-C (*P* = 0.008), non-HDL-C (*P* = 0.028), TG (*P* < 0.001), Apo B measured by DGU (*P* = 0.005), and Apo B measured by immunoassay (*P* = 0.002). A significant relationship for lower LDL peak time (*P* = 0.001) with increasing CIMT progression tertile was also shown. While there were statistically significant *P*-values for HDL₂-C (*P* = 0.028), total VLDL-C (*P* = 0.010), VLDL₁₊₂-C (*P* = 0.002), and VLDL₃-C (*P* = 0.022), the relationships did not monotonically increase or decrease across CIMT progression tertiles for these variables.

Predictors of CIMT progression as a continuous variable

Linear regression analyses for lipoprotein lipid and Apo B parameters with posterior wall CIMT progression rate (mm/year) as the dependent variable, adjusted for baseline CIMT, are shown in Table 3. Statistically significant and near-significant predictors of the posterior wall CIMT progression rate included LDL peak time (*P* = 0.045), HDL-C (*P* = 0.001), HDL₂-C (*P* = 0.005), HDL₃-C (*P* = 0.003), VLDL-C (*P* = 0.037), VLDL₁₊₂-C (*P* = 0.016), VLDL₃-C (*P* = 0.056), and TG (*P* = 0.079). Sensitivity analyses evaluating age,

Table 1 Baseline demographic and clinical characteristics of subjects according to tertiles of posterior wall common carotid artery intima media thickness progression

Parameter	CIMT progression <−0.0035 mm (n = 36)	CIMT progression −0.0035 and <0.0214 mm (n = 37)	CIMT progression ≥0.0214 mm (n = 37)	P-value ^a
Mean (SEM)				
Age, years	61.0 (8.8)	60.4 (8.3)	60.8 (6.7)	0.790 ^b
BMI, kg/m ²	27.5 (4.9)	29.3 (3.4)	29.1 (4.9)	0.136
Systolic BP, mmHg	131.1 (15.7)	127.8 (17.1)	130.8 (21.6)	0.955
Diastolic BP, mmHg	70.5 (12.3)	70.7 (9.3)	72.2 (8.9)	0.480
Fasting glucose, mg/dL	92.1 (8.2)	95.0 (9.0)	97.7 (9.2)	0.006 ^b
Vitamin D (25-OH), ng/mL	19.4 (12.6, 29.7)	19.1 (13.7, 26.3)	23.5 (16.7, 31.4)	0.150 ^b
Baseline CIMT, mm	0.84 (0.11)	0.75 (0.07)	0.73 (0.06)	<0.001 ^b
Framingham 10-yr risk ^c	7.7 (5.9)	7.8 (4.9)	9.8 (5.9)	0.063 ^b
Number (%)				
Men	18 (50.0)	23 (62.2)	21 (56.8)	0.577
Age intervals				0.573
≤64 years	22 (61.1)	24 (64.9)	26 (70.3)	
≥65 years	14 (38.9)	13 (35.1)	11 (29.7)	
Race/ethnicity				0.404
White	21 (58.3)	27 (73.0)	25 (67.6)	
Black	10 (27.8)	7 (18.9)	9 (24.3)	
Asian	2 (5.6)	3 (8.1)	1 (2.7)	
Hispanic/latino	1 (2.8)	0 (0.0)	2 (5.4)	
BMI ≥ 30 kg/m ²	10 (27.8)	15 (40.5)	15 (40.5)	0.426
Medication use				
Antihypertensive	14 (38.9)	7 (18.9)	14 (37.8)	0.117
Aspirin	10 (27.8)	5 (13.5)	8 (21.6)	0.323
Lipid-altering ^d	10 (27.8)	2 (5.4)	9 (24.3)	0.032
Major CHD risk factor				
Smoker	5 (13.9)	7 (18.9)	8 (21.6)	0.686
BP ≥ 140/90 mmHg or use of antihypertensive agents	20 (55.6)	16 (43.2)	20 (54.1)	0.515
HDL-C < 40 mg/dL	18 (50.0)	21 (56.8)	27 (73.0)	0.119
Family history of CHD	4 (11.1)	1 (2.7)	1 (2.7)	0.190

Notes: ^aP-values for continuous variables were for the slope (test for trend) derived by linear regression analysis, and by chi-square test for categorical values; ^bvalues were not normally distributed, and were ranked prior to the final analysis; ^c10-year % risk of a CHD event; ^dof the 24 subjects taking lipid-altering medications, 17 were on a statin.

Abbreviations: BMI, body mass index; BP, blood pressure; CHD, coronary heart disease; CIMT, carotid artery intima media thickness; HDL-C, high-density lipoprotein cholesterol; SEM, standard error of the mean.

sex, or ethnicity as confounders or effect modifiers yielded parameter estimates that were not materially different from those in the entire sample (data not shown).

Discussion

Numerous previous investigations have examined the cross-sectional relationships between cardiovascular disease risk markers and CIMT, however relatively few have evaluated predictors of CIMT progression rate.^{5,12,13} As previously reported in this population of subjects at moderate CHD risk,⁵ baseline CIMT was a strong inverse predictor of posterior wall CIMT progression, but non-lipid CHD risk factors such as blood pressure, age, and body mass index were not significantly associated with CIMT progression. Among lipoprotein-related variables, the strongest predictors of CIMT progression were higher levels of VLDL-C

and VLDL₁₊₂-C, reduced levels of HDL-C and cholesterol carried by HDL subfractions (HDL₂-C and HDL₃-C), and higher LDL peak time (an indicator of greater average LDL density). Thus, the “atherogenic lipoprotein phenotype,”⁸ also sometimes referred to as the “lipid triad” of elevated TG, reduced HDL-C and a predominance of small, dense LDL particles was more closely associated with CIMT progression in the present study than other indicators of atherosclerosis risk such as LDL-C, non-HDL-C, and Apo B.

It should be noted that while CIMT is a surrogate marker for the early stages of atherosclerosis, variables that are related to the initiation and progression of early atherosclerosis may not always be the best predictors of atherosclerotic cardiovascular disease event risk. Cardiovascular events result from processes that involve not only atherosclerosis, but also inflammation, thrombosis, and plaque instability.¹⁴

Table 2 Baseline density gradient ultracentrifugation lipid measurements according to tertiles of posterior wall carotid intima media thickness progression

Parameter (mg/dL except LDL peak time which is seconds)	CIMT progression <−0.0035 mm (n = 36)	CIMT progression −0.0035 and <0.0214 mm (n = 37)	CIMT progression ≥0.0214 mm (n = 37)	P-value ^a
Mean (SEM) or median (IQL)				
TC	156.3 (4.6)	158.7 (4.8)	165.8 (5.7)	0.181
Total LDL-C ^b	97.2 (3.6)	99.4 (3.7)	106.2 (4.5)	0.107
“Real” LDL-C ^b	78.8 (3.6)	80.7 (3.6)	86.3 (4.0)	0.156
LDL ₁₊₂ -C	29.9 (19.1, 39.4)	30.2 (17.7, 38.2)	24.2 (17.5, 32.6)	0.115 ^c
LDL ₃₊₄ -C	47.7 (3.2)	49.5 (2.4)	59.3 (3.5)	0.008
Lp(a)-C	4.0 (3.0, 8.5)	5.0 (4.0, 7.0)	6.0 (4.0, 8.0)	0.301 ^c
LDL peak time	115.6 (0.6)	114.6 (0.7)	112.5 (0.6)	0.001
IDL-C	12.0 (10.0, 14.0)	10.0 (10.0, 15.0)	12.0 (10.0, 17.0)	0.502 ^c
Total HDL-C	39.0 (1.8)	37.7 (1.7)	34.9 (1.7)	0.095
HDL ₂ -C	8.5 (6.0, 12.0)	9.0 (5.0, 11.0)	7.0 (6.0, 9.0)	0.028 ^c
HDL ₃ -C	29.5 (1.3)	29.1 (1.1)	27.3 (1.2)	0.188
Total VLDL-C	19.0 (15.0, 23.5)	18.0 (15.0, 23.0)	23.0 (18.0, 29.0)	0.010 ^c
VLDL ₁₊₂ -C	7.4 (5.5, 9.6)	6.8 (6.0, 9.6)	9.9 (7.3, 14.6)	0.002 ^c
VLDL ₃ -C	11.5 (10.0, 13.5)	11.0 (10.0, 13.0)	13.0 (11.0, 16.0)	0.022 ^c
Non-HDL-C	117.2 (4.0)	121.0 (4.2)	130.9 (4.9)	0.028
TG	63.0 (43.0, 87.5)	70.0 (53.0, 101.0)	103.0 (72.0, 127.0)	<0.001 ^c
Apo B DGU	81.5 (2.2)	84.3 (2.2)	91.3 (2.8)	0.005
Apo B immunoassay	79.5 (2.7)	82.0 (2.4)	91.7 (3.0)	0.002

Notes: ^aP-values derived by linear regression model analysis; ^btotal LDL-C = LDL₁₊₂₊₃₊₄-C + Lp(a)-C + IDL-C and “Real” LDL-C = LDL₁₊₂₊₃₊₄-C; ^cvalues were not normally distributed, and were ranked prior to the final analysis.

Abbreviations: Apo, apolipoprotein; CIMT, carotid intima media thickness; DGU, density gradient ultracentrifugation; HDL-C, high-density lipoprotein cholesterol; IDL-C, intermediate density lipoprotein cholesterol; IQL, interquartile limits; LDL-C, low-density lipoprotein cholesterol; Lp(a)-C, cholesterol carried by lipoprotein(a); SEM, standard error of the mean; TC, total cholesterol; TG, triglycerides; VLDL-C, very low density lipoprotein cholesterol.

Thus, variables that are only weakly associated or unrelated to CIMT progression (such as blood pressure, cigarette smoking, and LDL-C in the present investigation) may still be highly clinically relevant as predictors of cardiovascular event risk and are important targets for therapy. Carotid atherosclerosis, and specifically CIMT, has been shown to be highly heritable.^{15,16} Thus, differences in relationships between risk factors and CIMT may be influenced by genetics. Several genetic variants for carotid atherosclerosis have been identified, including Apo E genotype and angiotensin converting enzyme and methylene tetrahydrofolate reductase polymorphisms, but larger studies are needed to confirm their associations with CIMT and CIMT progression rates.¹⁶ To date, the examinations of polymorphic associations have primarily focused on the presence of carotid atherosclerosis, and not on changes in CIMT over time.

Population studies have consistently shown that non-HDL-C is a stronger correlate of CHD event risk than LDL-C in those with and without hypertriglyceridemia.^{17–21} Investigators have expressed differing views on the explanation for the superiority of non-HDL-C. The view expressed in the National Cholesterol Education Program Adult Treatment Panel III report is that remnants of TG-rich lipoproteins (remnants of VLDL, IDL, and chylomicron particles) are

atherogenic, and that VLDL-C is a strong correlate of TG-rich remnant particles.⁸ Thus, non-HDL-C is a better indicator of the total burden of cholesterol carried by atherogenic lipoproteins (“real” LDL + IDL + Lp(a) + VLDL + chylomicron remnants).

Others have suggested that the superiority of non-HDL-C does not result from the atherogenicity of TG-rich lipoprotein remnants, but instead reflects the relationship between non-HDL-C and the number of circulating LDL particles.^{22,23} They point out that approximately 90% of Apo B is found in LDL particles, whether or not an individual has hypertriglyceridemia. In those with an elevated TG concentration, the average LDL particle size is typically smaller, and thus the LDL particle concentration is often higher in such individuals than would be predicted based on the level of LDL-C.

The present results cannot resolve this controversy, but can be interpreted as consistent with a role for TG-rich lipoproteins in early atherogenesis since VLDL-C and VLDL₁₊₂-C concentrations were more strongly associated with CIMT progression than levels of LDL-C or Apo B. However, caution is warranted since elevated levels of VLDL-C are generally associated with other lipoprotein abnormalities including increased levels of TG; a predominance of small,

Table 3 Linear regression analysis for progression rate (mm/year) after adjustment for baseline posterior wall carotid intima media thickness

Variable	Regression coefficient (SE)	P-value
TC	0.0000 (0.0001)	0.842
Total LDL-C ^a	0.0001 (0.0001)	0.359
"Real" LDL-C ^a	0.0001 (0.0001)	0.451
LDL ₁₊₂ -C	-0.0001 (0.0001)	0.562
LDL ₃₊₄ -C	0.0001 (0.0001)	0.147
Lp(a)-C	0.0000 (0.0001)	0.601
LDL peak time	-0.0002 (0.0001)	0.045
IDL-C	0.0001 (0.0001)	0.557
Total HDL-C	-0.0003 (0.0001)	0.001
HDL ₂ -C	-0.0003 (0.0001)	0.005
HDL ₃ -C	-0.0003 (0.0001)	0.003
Total VLDL-C	0.0002 (0.0001)	0.037
VLDL ₁₊₂ -C	0.0002 (0.0001)	0.016
VLDL ₃ -C	0.0002 (0.0001)	0.056
Non-HDL-C	0.0001 (0.0001)	0.173
TG	0.0002 (0.0001)	0.079
Apo B DGU	0.0001 (0.0001)	0.133
Apo B immunoassay	0.0002 (0.0001)	0.067

Notes: ^aTotal LDL-C = LDL₁₊₂₊₃₊₄-C + Lp(a)-C + IDL-C and "Real" LDL-C = LDL₁₊₂₊₃₊₄-C.

Abbreviations: Apo, apolipoprotein; CIMT, carotid intima media thickness; DGU, density gradient ultracentrifugation; HDL-C, high-density lipoprotein cholesterol; IDL-C, intermediate density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Lp(a)-C, cholesterol carried by lipoprotein(a); SE, standard error; TC, total cholesterol; TG, triglycerides; VLDL-C, very low density lipoprotein cholesterol.

dense LDL particles; and reduced levels of HDL-C. While speculative, it may be true that different lipoprotein particles are more important at different stages in the atherothrombotic process. For example, remnants of TG-rich lipoproteins may be particularly important in the early stages of development whereas LDL particles may be more important regarding progression to advanced lesions and/or for promoting plaque instability. Despite considerable advances in understanding the pathophysiology, at present it is unclear whether reducing levels of TG-rich lipoprotein cholesterol, in the absence of improvements in other lipoprotein parameters, will be associated with less progression of atherosclerosis or lower cardiovascular event rates.

All LDL particles, independent of size, are likely atherogenic, but smaller, more dense LDL particles have been proposed to possess enhanced atherogenicity due to longer residence time in circulation, greater ease of entry into the arterial wall, increased binding to subendothelial proteoglycans, and greater susceptibility to oxidative modification.^{24–26} Although studies have shown a link between a predominance of small, dense LDL particles and atherosclerotic cardiovascular disease,^{25–32} adjustment for the number of LDL particles, as indicated by the

Apo B or LDL particle concentration, generally attenuates the relationship.^{25,32} Thus, controversy exists regarding whether a gradient of atherogenicity exists across Apo B containing lipoproteins and, if so, whether the gradient is steep enough to have clinical relevance. In the present study the sample size was not large enough to allow meaningful investigation of the predictive ability of multiple correlated variables. Accordingly, it is uncertain whether the association between LDL peak time as a continuous variable reflecting LDL subclass distribution was associated with greater CIMT progression because of a gradient of atherogenicity across Apo B-containing lipoprotein particles, or because it correlates with other predictive variables such as higher levels of TG-rich lipoproteins or reduced HDL-C.

DGU-assessed HDL-C concentration was inversely associated with CIMT progression, which agreed with results reported previously for traditional HDL-C measurements.⁵ The results were similar for total HDL-C and the HDL₂-C and HDL₃-C subfractions. Thus, the present results do not support the view that cholesterol carried by the major HDL subfractions is superior to total HDL-C concentration for predicting CIMT progression.

As demonstrated in a previous examination of these data,⁵ there was a significant trend for increasing CIMT progression tertile with greater concentrations of fasting glucose. Several studies have reported an association between fasting and postprandial glucose levels and CIMT.^{33–35} However, it is unclear whether the relationship is causal, ie, hyperglycemia acting directly on the arterial wall to initiate thickening, or whether it is confounded by other cardiovascular risk factors. Using the principles of Mendelian randomization, a recent analysis demonstrated a significant association between a fasting glucose genetic risk score (created by weighing the strength of associations of several glucose-associated genetic variants) and CIMT, supporting a causal hypothesis.³⁵ However, this analysis does not preclude the possibility that other cardiovascular risk factors may also be associated with these genetic polymorphisms.

While use of lipid-altering medications (primarily statins) differed significantly across tertiles of CIMT progression, there was no clear pattern, with the lowest frequency of lipid-altering medication use in the middle tertile. In the present study the components of the atherogenic lipoprotein phenotype including increased cholesterol carried by TG-rich lipoproteins, lower HDL-C, and a predominance of cholesterol carried by more dense LDL particles, was associated with CIMT progression. This observation suggests that it would be of interest to compare

the effects of medications that primarily act to reduce TG-rich lipoproteins, raise HDL-C, and shift the LDL subclass distribution, such as fibrates and omega-3 fatty acids, to those of statins, which primarily act to reduce LDL-C and have smaller effects on TG-rich lipoproteins, HDL-C, and LDL subclass distribution. Statin and fibrate treatment have each been shown to slow the rate of CIMT progression.^{36–38} The authors are not aware of any randomized trial that has directly compared a statin with a fibrate or omega-3 fatty acids to evaluate effects on CIMT progression. In one study, where consecutive dyslipidemia patients were treated with either a statin or fibrate, fibrate treatment was associated with significantly greater CIMT and a steeper CIMT-time relationship than statin treatment, and these differences were not explained by differences in LDL-C concentrations.³⁹ However, such results are difficult to interpret because patients treated with fibrates may have differed with regard to the dyslipidemia present at the time treatment was initiated, thus additional research will be required to address this question in patients with the atherogenic dyslipidemia phenotype.

Limitations of the present analyses include: (1) limited generalizability of the results due to the exclusion of subjects with CHD and diabetes, and the restriction of the sample to those with baseline CIMT value >0.7 and <2.0 mm; (2) the possibility of type I statistical errors because a relatively large number of variables were evaluated; (3) the potential for variability in CIMT scanning at the two clinical research sites, although utilization of a single reader at a central location, and the employment of masking software to insure replication of the carotid region of interest,¹⁰ improved the reliability of the measurements; and (4) a relatively short follow-up period.

Conclusion

The results from these analyses indicate that higher levels of TG-rich lipoprotein cholesterol, lower levels of HDL-C and HDL-C subfractions, and a greater proportion of LDL-C carried by more dense LDL particles were each associated with CIMT progression in men and women at moderate risk for CHD. Notably, these variables were more strongly associated with CIMT progression than LDL-C, non-HDL-C, and Apo B concentrations, suggesting that the atherogenic lipoprotein phenotype, including elevated TG (and TG-rich lipoprotein cholesterol), reduced HDL-C and a predominance of smaller, more dense LDL particles may have an important role in the initiation and progression of early atherosclerosis.

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