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The Apolipoprotein CI Content of Triglyceride-Rich Lipoproteins Independently Predicts Early Atherosclerosis in Healthy Middle-Aged Men

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OBJECTIVES In this study, we examined the apolipoprotein (apo) CI content of triglyceride-rich lipoproteins (TRLs) in relation to established coronary heart disease (CHD) risk factors and early atherosclerosis.

BACKGROUND In Western society, the postprandial state constitutes a nearly constant stress on the vasculature and the metabolism of lipoproteins. Delayed clearance of postprandial TRL remnants has repeatedly been associated with premature CHD and may include the enrichment of these remnants with apoCI.

METHODS We examined 72 healthy 50-year-old men with an apoE3/E3 genotype who had undergone an oral fat load test and B-mode ultrasound examination of the intima-media thickness (IMT) of the common carotid artery.

RESULTS In the fasting state, plasma, very-low-density lipoprotein (VLDL), and low-density lipoprotein cholesterol, proinsulin, and apoB100-containing intermediate density lipoprotein levels were related to IMT ($p < 0.05$). In the postprandial state, IMT was related to triglycerides at 2 h ($p < 0.01$), large VLDL concentration at 3 h ($p < 0.05$), the apoCI plasma and TRL concentrations at 6 h ($p < 0.05$, $p < 0.05$), and the apoCI content of TRLs at 6 h ($p < 0.002$). Multivariate analysis revealed that the apoCI content of TRLs at 6 h ($p < 0.0001$), plasma triglyceride concentrations at 2 h ($p < 0.006$), and fasting plasma cholesterol concentration ($p < 0.05$) independently predicted IMT. In addition, the apoCI content of postprandial TRLs correlated strongly with the cholesterol content ($r = 0.64$, $p < 0.0001$).

CONCLUSIONS Our results indicate that the apoCI content of postprandial TRLs is a novel independent risk factor for early atherosclerosis in normolipidemic healthy middle-aged men with possible implication for the enrichment of TRL remnant lipoproteins with cholesterol. (J Am Coll Cardiol 2005;45:1013–7) © 2005 by the American College of Cardiology Foundation

In the typical Westerner, the postprandial state influences the metabolism of circulating lipoproteins for more than 20 h per day throughout life. However, the transience of the postprandial state has rendered it difficult to study, and several variables in the fasting state predict alterations of postprandial lipid metabolism (1), suggesting that postprandial studies in relation to coronary heart disease (CHD) might not be necessary. Nevertheless, such studies typically demonstrate postprandial accumulation of triglyceride-rich lipoprotein (TRL) remnants in CHD patients with apparently normal fasting plasma lipoprotein profiles (2–5). Thus, perturbations of lipid metabolism that increase the risk of CHD might be detected earlier in the postprandial state than in the fasting state. If so, certain postprandial features might serve as early markers of CHD risk.

After a meal, newly synthesized TRLs are hydrolyzed by endothelial lipases into smaller remnant particles (6), which are cleared from the circulation mainly by receptor-mediated endocytosis in the liver (7). The metabolic fate of TRL particles is determined by their apolipoprotein (apo) and lipid composition, which governs their access to lipases and receptors (8,9). The structural protein apoB is an integral part of every TRL particle. Of the two forms of apoB—apoB48 (synthesized in the intestine) and apoB100 (synthesized in the liver)—only apoB100 has a specific receptor-binding site. ApoB100 and apoE are important mediators of the clearance of circulating TRLs by receptors (10); apoE-mediated binding of TRLs to receptors is inhibited by members of the apoC family, in particular apoCI and apoCIII (11). Apolipoprotein CII is necessary for lipoprotein lipase-mediated hydrolysis of TRL particles (12), which is blocked by apoCIII (13) but not apoCI (14).

Our studies over the past five years have shown that postprandial alterations in the composition of TRLs and TRL remnant particles appear to favor their clearance from the circulation in healthy people (15,16). However, in patients with coronary artery disease (17) and in healthy subjects with early signs of atherosclerosis (18), TRL remnants are relatively enriched in apoCI and cholesterol. In this study, we evaluated the importance of postprandial

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Abbreviations and Acronyms

apo = apolipoprotein
CHD = coronary heart disease
IMT = intima-media thickness
Sf = Svedberg flotation
TRL(s) = triglyceride-rich lipoprotein(s)
VLDL = very-low-density lipoprotein

enrichment of TRL particles with apoCI in relation to established CHD risk factors and early atherosclerosis, assessed by measuring intima-media thickness (IMT) of the common carotid artery by B-mode ultrasound, in healthy middle-aged men.

METHODS

Subjects and study protocol. The study included 72 healthy, 50-year-old white men with an apoE3/E3 genotype who were randomly recruited from a population register of the county of Stockholm. The exclusion criteria were chronic disease, a history of CHD or arterial thromboembolic disease, familial hypercholesterolemia, body mass index >32 kg/m^2, alcohol abuse, or current participation in other studies. The study protocol was approved by the Ethical Committee of the Karolinska Hospital, and all subjects gave informed consent to participate. Participants were served a mixed meal (Karlshamns Oil & Fat, Karlshamn, Sweden) that had a total energy content of 1,000 kcal, with 60% of energy from fat, 13% from protein, and 27% from carbohydrates. Blood samples were drawn before the meal and then hourly for 6 h.

TRL subfractionation. Triglyceride-rich lipoproteins were subfractionated by cumulative density gradient ultracentrifugation (19). In brief, the densities of plasma samples obtained before and 3 and 6 h after the meal were increased and subjected to cumulative ultracentrifugation to float lipoprotein fractions with Svedberg flotation (Sf) rates >400, Sf 60 to 400, and Sf 20 to 60, which were aspirated from the top of tube. After the last run, Sf 12 to 20 lipoproteins were isolated 29 mm from the top of the tube; apoB100 and apo48 concentrations were determined in each density fraction; apoCI levels were measured in plasma samples and in the Sf 20 to 400 fraction isolated from the same plasma samples.

Fasting and postprandial measurements. Very-low-density lipoprotein (VLDL), low-density lipoprotein, and high-density lipoprotein cholesterol and triglyceride concentrations were determined by preparative ultracentrifugation, precipitation of apoB-containing lipoproteins, and lipid analyses (20). Plasma triglycerides were measured with a colorimetric assay (450032, Boehringer Mannheim, Indianapolis, Indiana; Wako Chemicals GmbH, Neuss, Germany). Plasma cholesterol concentrations were determined enzymatically (Merck, Darmstadt, Germany); apoB100 and apoB48 concentrations in TRL fractions were determined as described (19). The apoCI concentrations in plasma and in the Sf 20 to 400 TRL fraction were determined with an enzyme immunoassay (21). Fasting insulin and proinsulin levels in fasting, heparinized plasma were measured by ELISA (DAKO Insulin and Intact Proinsulin, DAKO Diagnostics Ltd., Bagsvaerd, Denmark) and glucose by a glucose oxidase measurement (Kodak, Ektachem, Rochester, New York). The apoE genotype was determined as described (22).

Carotid artery ultrasound examination. Carotid artery IMT was measured according to the ultrasound protocol of the European Lacipidine Study on Atherosclerosis (23). The scans were performed with an 8-MHz, high-resolution, annular-array scanner (A2000 II sa, Biosound, Inc., Indianapolis, Indiana), recorded on S-VHS videotape, and evaluated at the Center for Medical Ultrasound, Division of Vascular Ultrasound Research, Wake Forest University, Winston-Salem, North Carolina. The common carotid far-wall IMT (mean of right and left artery registrations) was used as a measure of early atherosclerosis. The examinations were performed by two sonographers. Their coefficients of variation between readings were 3.8% and 5.1%. The coefficient of variation between the sonographers was 4.7%.

Calculations and statistical methods. The apoCI content of TRL particles was calculated by dividing the molarity of apoCI by that of apoB. The molarities were calculated from the fractional (i.e., Sf 20 to 400) concentrations of apoCI and apoB. Variables with skewed distribution were log-normalized before statistical analysis. Univariate associations between clinical or metabolic variables and IMT were assessed by Pearson correlation coefficients; variables that were significantly associated with IMT were included in the multivariate analysis. The multivariate model was generated by multiple stepwise linear regression analysis to identify variables that were independently associated with IMT. A forward approach was used in which significance levels were set at <0.25 to enter the model and at >0.10 to leave the model. All statistical sets were two-sided, and $p < 0.05$ was considered significant.

RESULTS

Characteristics of the study group. The basic characteristics of the study group are summarized in Table 1. Twenty-one of the 72 men were current smokers, and 25 were former smokers. As expected from the entry criteria, very few subjects had a family history of CHD, and the ultrasound examination revealed a fairly normal IMT. Not surprisingly, given the health of the group, IMT did not correlate with blood pressure, hip/waist ratio, or body mass index.

Fasting plasma lipid and lipoprotein concentrations and glucose-insulin homeostasis. Intima-media thickness correlated significantly with the fasting plasma concentrations of proinsulin, cholesterol, and VLDL, and low-density lipoprotein cholesterol ($r = 0.30$, 0.28, 0.25, and 0.27, respectively, $p < 0.05$) (Table 2). Fasting triglyceride and insulin levels also correlated positively, but not significantly,

Table 1. Basic Characteristics of the Study Group

No. of subjects	72
Family history of CHD*, %	8
Smoker, n	46
Current	21
Former	25
Alcohol intake, g/day	18 ± 15
BMI, kg/m^2	25.2 ± 2.5
Waist/hip ratio	0.93 ± 0.06
Blood pressure, mm Hg	
Systolic	122 ± 12
Diastolic	79 ± 7.8
Common carotid artery IMT, mm	
Far wall	0.85 ± 0.15
Near wall	0.86 ± 0.13

*Diagnosis of CHD in a first-degree relative <60 years old. Values are mean ± SD.
BMI = body mass index; CHD = coronary heart disease; IMT = intima-media thickness.

with IMT (r = 0.19 and 0.21, p > 0.05); IMT did not correlate with high-density lipoprotein cholesterol, VLDL triglycerides, or glucose.

Postprandial plasma TRL concentrations. Plasma triglyceride concentrations during the first 4 h after the meal correlated significantly with IMT (Fig. 1); a particularly strong correlation was seen at 2 h (r = 0.34, p < 0.01). Apart from the concentration of large apoB100-containing VLDL particles (i.e., the apoB100 concentration in the Sf 60 to 400 fraction) at 3 h (r = 0.23, p < 0.05), neither the fasting nor the postprandial TRL particle concentrations (i.e., apoB concentrations in the Sf 20 to 60 or Sf 60 to 400 fractions) correlated with IMT (Table 3). In contrast, a correlation was seen between fasting plasma intermediate-density lipoprotein concentration and IMT (r = 0.24, p < 0.05).

apoCI. Intima-media thickness correlated significantly with postprandial, but not fasting, apoCI concentrations (Table 4). Most striking was the correlation between IMT and the TRL apoCI content (r = 0.37, p < 0.002, Table 4). The TRL apoCI concentration also correlated strongly with the VLDL cholesterol concentration (Fig. 2).

Multivariate analysis. Multivariate analysis demonstrated significant independent associations between IMT and fasting plasma cholesterol (p < 0.05), plasma triglycerides at 2 h (p < 0.006), and the TRL apoCI content at 6 h (p < 0.0001). The fasting plasma cholesterol concentrations accounted for 5% of the variation in IMT, whereas postprandial plasma triglyceride and the TRL apoCI concentrations accounted for 14% each.

Table 2. Fasting Plasma Glucose, Insulin, Cholesterol, and Triglyceride Concentrations in Plasma and Major Lipoprotein Fractions and Relationships to the Common Carotid Artery IMT

	Mean ± SD	Correlation With IMT, r*
Glucose, mmol/l	4.7 ± 0.5	0.02
Insulin, pmol/l	41 ± 34	0.19
Proinsulin, pmol/l	3.7 ± 3.4	0.30†
Cholesterol, mmol/l		
Plasma	5.36 ± 0.92	0.28†
VLDL	0.69 ± 0.81	0.25†
LDL	2.96 ± 1.45	0.27†
HDL	1.36 ± 0.51	−0.03
Triglycerides, mmol/l		
Plasma	1.29 ± 1.28	0.21
VLDL	0.64 ± 0.42	0.02
LDL	0.42 ± 0.33	0.10
HDL	0.11 ± 0.03	−0.06

*Pearson correlation coefficient; †p < 0.05.
HDL = high-density lipoprotein; IMT = intima-media thickness; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein.

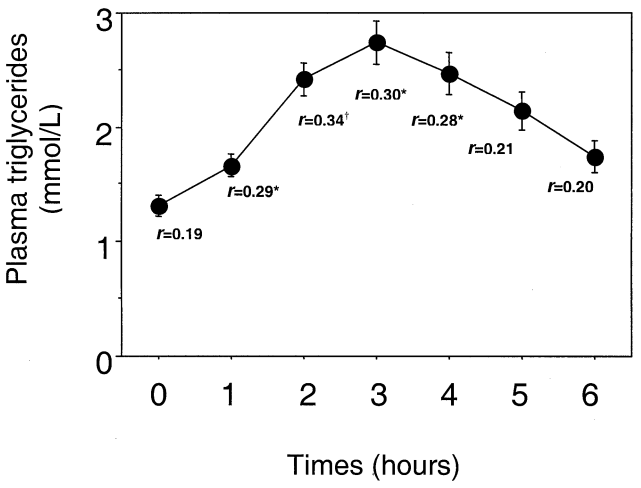

Figure 1. The plasma triglyceride concentrations before and after a standardized mixed meal containing 1,000 kcal (60% of energy from fat) in 72 healthy 50-year-old men. Pearson correlation coefficient (r) to intima-media thickness and *p < 0.05; †p < 0.01.

Table 3. Fasting and Postprandial ApoB100 and ApoB48 Concentrations in Lipoprotein Subfractions

	Time (h)		
Fraction	0	3	6
Sf >400			
ApoB100	0.03 ± 0.06	0.21 ± 030	0.16 ± 0.33
	r = 0.13	r = 0.09	r = 0.02
ApoB48	0.01 ± 0.04	0.23 ± 0.22	0.12 ± 0.22
	r = −0.05	r = 0.14	r = 0.03
Sf 60 to 400			
ApoB100	19.4 ± 17.1	28.9 ± 18.9	21.6 ± 20.9
	r = 0.11	r = 0.23*	r = 0.17
ApoB48	0.34 ± 0.49	1.32 ± 0.98	0.78 ± 0.93
	r = 0.10	r = 0.06	r = 0.06
Sf 20 to 60			
ApoB100	30.3 ± 16.9	25.8 ± 12.4	30.1 ± 16.6
	r = 0.01	r = 0.07	r = 0.00
ApoB48	0.51 ± 0.38	0.74 ± 0.40	0.51 ± 0.37
	r = 0.13	r = 0.02	r = 0.00
IDL			
ApoB100	48.0 ± 23.0	ND	ND
	r = 0.24*		
ApoB48	0.14 ± 0.21	ND	ND
	r = 0.02		

Values are mean ± SD (mg/dl). r values indicate Pearson correlation coefficients with intima-media thickness. *p < 0.05.
Apo = apolipoprotein; IDL = intermediate-density lipoprotein; ND = not determined; Sf = Svedberg flotation.

Table 4. Fasting and Postprandial ApoCI Concentrations in Plasma, TRL Subfractions, and TRL Particles

	Time (h)	
	0	6
Plasma		
ApoCI (mg/dl)	170 ± 66.8 r = 0.17	170 ± 63.7 r = 0.27*
Sf 20 to 400		
ApoCI (mg/dl)	18.6 ± 15.7 r = 0.03	17.0 ± 13.5 r = 0.24*
Sf 20 to 400		
ApoCI per TRL particle (mol. apoCI/mol. apoB)	28.0 ± 22.3 r = −0.04	29.7 ± 17.8 r = 0.37†

Values are mean ± SD. The r values indicate Pearson correlation coefficients with intima-media thickness. *$p < 0.05$; †$p < 0.002$.

ApoCI = apolipoprotein CI; mol. = molecules; Sf = Svedberg flotation; TRL = triglyceride-rich lipoprotein.

DISCUSSION

This study shows that the postprandial plasma triglyceride concentration at 2 h and the apoCI content of TRL particles at 6 h are independent predictors of early atherosclerosis. The TRL apoCI content also correlated strongly with the TRL cholesterol concentration. Because the latter (i.e., VLDL cholesterol) was not an independent marker of early atherosclerosis, we suspect that the postprandial transfer of apoCI from high-density lipoprotein to TRL particles occurs before cholesterol enrichment, supporting the notion that TRL apoCI concentration governs the cholesterol enrichment of remnants and contributes to the development of early atherosclerosis.

To identify early atherosclerosis, we used B-mode ultrasound to measure the IMT of the common carotid artery. The validity of this surrogate measurement is supported by several observations. Common carotid IMT measured by ultrasound correlates well with that measured by light microscopy (24) and with many risk factors for atherosclerotic disease, including smoking, hypertension, and age (25). It also reflects the severity of atherosclerotic coronary artery disease (26,27) and predicts future cardiac events (28). However, whether IMT can be used to predict future atherosclerosis risk remains unproven. Age, gender, and apoE genotype are strong determinants of common carotid IMT (26,27,29,30). Therefore, we included only 50-year-old men with an apoE3/E3 genotype.

Several years ago, we used this technique in a study of fasting and postprandial risk factor profiles in relation to early atherosclerosis in 96 healthy 50-year-old men with an apoE3/E3 genotype (31). In the current study, we reanalyzed 72 of these men to assess the role of apoCI plasma and the TRL concentrations. As in this previous study, several established risk factors of CHD were associated with early atherosclerosis—plasma cholesterol, low-density lipoprotein cholesterol, proinsulin, plasma triglyceride concentration at 2 h, apoB100 in VLDL at 3 h, and baseline level of intermediate-density lipoproteins (31). Notably, however, the postprandial TRL apoCI concentration was an even stronger predictor of early atherosclerosis in these men. These results suggest that apoCI-rich TRLs are atherogenic.

Apolipoprotein CI is probably the least studied member of the apoC family. This is surprising because apoCI has properties that clearly distinguish it from other family members (11). For instance, in contrast to CIII, apoCI does not block the hydrolysis of TRLs by lipoprotein lipase but, like apoCIII, it does block apoE-mediated clearance of TRLs by receptors. In the fasting state, about 90% of apoCI is associated with high-density lipoprotein, whereas apoCII and apoCIII are equally distributed between TRLs and high-density lipoprotein. In the postprandial state, the transfer of apoCI from high-density lipoprotein to TRLs is greater than that of apoCII and CIII (32). Thus, the metabolic properties of apoCI are consistent with an increased half-life of TRL remnants in the circulation, which, in turn, would increase its cholesterol ester/triglyceride ratio. Consistent with this notion, cholesterol-rich remnants accumulate in the plasma of mice over-expressing apoCI (14).

In addition to the apoCI content of TRL particles, fasting plasma cholesterol and the postprandial plasma triglyceride concentration independently predicted IMT. The apoCI content of TRL particles did not correlate with early postprandial triglyceride concentration. In contrast, there was a strong correlation between the concentrations of apoCI and cholesterol in TRL particles (Fig. 2), suggesting that the apoCI on TRL particles is associated with particularly atherogenic cholesterol or cholesterol that is more likely to end up in the arterial wall. However, whether apoCI in itself is atherogenic or whether apoCI is atherogenic by increasing TRL cholesterol ester content cannot be distinguished in this study. In fact, it is an inherent difficulty to exclude noncausal relationships when using a study design as the one chosen for this study.

We had speculated that the strong association between postprandial triglycerides and IMT in our previous study (31) might be explained by the effects of apoCI on postprandial triglyceride metabolism. The lack of association between the apoCI content of TRL particles and postprandial triglyceride concentration does not support this hypoth-

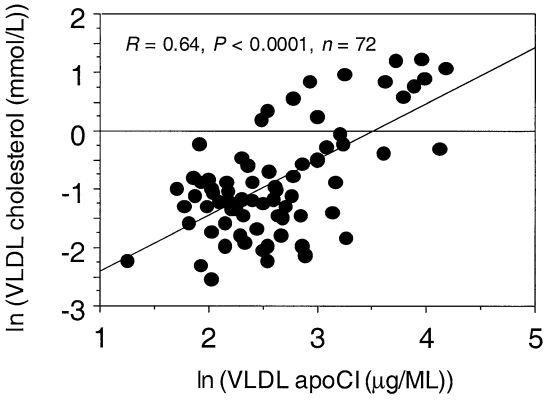

Figure 2. Regression plot of the log-normalized (ln) cholesterol and apolipoprotein (apo) CI concentrations of the Svedberg flotation 20 to 400 fraction. *$p < 0.0001$. VLDL = very-low-density lipoprotein.

esis. Further studies are required to better understand whether these two variables are independent risk factors for early atherosclerosis or merely reflect a more general perturbation of postprandial TRL metabolism.

Potential clinical implications. The current study adds the TRL content of apoCI to the list of potential risk factors for premature development of atherosclerosis. Longitudinal studies are warranted to assess the usefulness of the apoCI TRL content as a marker of individuals with increased CHD risk. For future clinical use, we foresee the development of a clinical test device containing two immunoabsorbent steps: the first step with antibodies retaining apoB100-containing TRLs from postprandial plasma, which then should be passed on to a second step retaining and apoCI-containing particles from the apoB100-containing TRLs. Interestingly, because postprandial plasma is required, it might be valuable to ask patients to visit the clinic nonfasted. According to the results of the current study, the cholesterol/protein ratio of the retained fraction from this absorbent (the apoB100- and apoCI-containing TRL fraction retained in the second step) should potentially indicate the level of CHD risk.

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