Journal of the American College of Cardiology
© 2005 by the American College of Cardiology Foundation
Published by Elsevier Inc.

Vol. 45, No. 7, 2005
ISSN 0735-1097/05/$30.00
doi:10.1016/j.jacc.2004.12.049

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

Anders Hamsten, MD, PHD,* Angela Silveira, PHD,* Susanna Boquist, MD, PHD,† Rong Tang, MD,‡ M. Gene Bond, PHD,‡ Ulf de Faire, MD, PHD,§ Johan Björkegren, MD, PHD*

Stockholm, Sweden; and Winston-Salem, North Carolina

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

From the *Atherosclerosis Research Unit, King Gustaf V Research Institute, Karolinska Institutet, Stockholm, Sweden; the †Cardiology Unit, Karolinska Hospital, Stockholm, Sweden; the ‡Division of Ultrasound, Department of Neurobiology and Anatomy, Wake Forest University, Winston-Salem, North Carolina; and the §Division of Cardiovascular Epidemiology, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden. This study was supported by the Swedish Medical Research Council, the Swedish Heart-Lung Foundation, the Hans and Loo Ostermans Foundation, and the Professor Nanna Svartz Foundation.

Manuscript received October 6, 2004; revised manuscript received December 1, 2004, accepted December 6, 2004.

JACC Vol. 45, No. 7, 2005
April 5, 2005:1013–7

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,