Postprandial Enrichment of Remnant Lipoproteins With ApoC-I in Healthy Normolipidemic Men With Early Asymptomatic Atherosclerosis

Johan Björkegren, Angela Silveira, Susanna Boquist, Rong Tang, Fredrik Karpe, M. Gene Bond, Ulf de Faire, Anders Hamsten

Objectives—Recently, we reported that exaggerated postprandial triglyceridemia in normolipidemic patients with coronary artery disease is associated with enrichment of remnant lipoproteins with apolipoprotein C-I (apoC-I). In this study, the number and composition of chylomicron remnants and very low density lipoproteins (VLDLs) were examined in 30 asymptomatic normolipidemic 50-year-old men with and without early carotid atherosclerotic lesions.

Results and Methods—Intima-media thickness of the far wall of the common carotid artery was determined by B-mode ultrasound. Triglyceride-rich lipoproteins were subfractionated by density gradient ultracentrifugation and separated into VLDL and chylomicron remnant fractions by immunoaffinity chromatography. The postprandial triglyceridemia and increase in triglyceride-rich lipoprotein particle number (ie, apolipoprotein B concentrations) were not exaggerated in men with early atherosclerosis. In contrast, their large (Svedberg flotation rate 60 to 400) and small (Svedberg flotation rate 20 to 60) chylomicron remnants and VLDL were greatly enriched with apoC-I, and their small chylomicron remnants and VLDL particles were relatively enriched with cholesterol. Moreover, the number of apoC-I molecules on small chylomicron remnants was strongly associated with the degree of atherosclerosis.

Conclusions—Early asymptomatic atherosclerosis in normolipidemic men without exaggerated postprandial triglyceridemia is associated with the enrichment of postprandial chylomicron and VLDL particles with apoC-I. Therefore, it is conceivable that the apoC-I content of lipoprotein remnants may serve as an early marker of coronary artery disease risk.


Key Words: alimentary lipemia • atherosclerosis • apolipoprotein C-I • cholesterol

Prolonged postprandial triglyceridemia has repeatedly been reported in normolipidemic patients with premature atherosclerosis.1 This exaggerated accumulation of triglycerides, in part, accounts for the postprandial accumulation of intestine-derived triglyceride-rich lipoproteins (TRLs): chylomicrons and chylomicron remnants. In fact, the postprandial accumulation of chylomicron remnants is associated with the presence2,3 as well as the progression4 of coronary artery disease (CAD). In addition, some studies have reported a role for liver-derived VLDLs in coronary atherosclerosis.5,6 These clinical observations have led to interest in the composition and metabolism of chylomicron and VLDL particles in subjects with early atherosclerotic disease.

Normally, the composition of the TRL changes during the metabolic processing of these particles, and these compositional changes facilitate their removal by hepatic lipoprotein receptors.7,8 It is conceivable that perturbations of the postprandial composition of TRLs could contribute to a delay of their clearance and even render them more atherogenic. In support of this theory, a recent study has shown that levels of specific families of TRLs, as defined by their apolipoprotein content, are better predictors of CAD progression than are the total plasma apolipoprotein or lipid levels.9 Furthermore, we recently reported that VLDL particles are enriched with apoC-I during exaggerated postprandial triglyceridemia in normolipidemic CAD patients.10 Despite evidence implicating elevated chylomicron remnants and VLDL in CAD, there have been no attempts to analyze the composition of TRL remnants in early asymptomatic atherosclerosis. In the present study, we tested the hypothesis that perturbation of TRL remnant composition precedes the exaggerated postprandial triglyceridemia commonly observed in normolipidemic CAD patients. To address this hypothesis, we examined the postprandial response in asymptomatic 50-year-old normolipidemic healthy male subjects with or without early carotid atherosclerosis. Despite
normal postprandial triglyceridemia, we identified abnormalities in chylomicron remnant and VLDL composition associated with early lesions in the carotid arteries.

Methods

Human Subjects

Intima-media thickness (IMT) of the far wall of the common carotid artery was determined by ultrasound examination in 96 healthy 50-year-old white men recruited from a population survey in the northern county of Stockholm, Sweden. Carotid ultrasonography was performed according to the European Lacidipine Study on Atherosclerosis (ELSA) ultrasound protocol by using a Biosound 2000 II ultrasound device with an 8-MHz high-resolution annular-array scanner (Biosound Inc). Only subjects who were normolipidemic (LDL cholesterol <4.5 mmol/L and plasma triglycerides <2.0 mmol/L) and had an apoE3/E3 genotype were chosen for the present study. Among these men, the TRL apolipoprotein and lipid composition was assessed only in those who had an IMT above the 75th percentile of 0.92 mm (1.01 ± 0.05 mm; IMT+ group, n = 20) and in those who had an IMT below the 25th percentile of 0.69 mm (0.62 ± 0.05 mm; IMT− group, n = 10). There were no differences in smoking habits, alcohol consumption, or blood pressure between the IMT+ and IMT− groups, as were postprandial responses to the oral fat load (Table 2). Nor were there any statistically significant differences in fasting or postprandial concentrations of TRL triglyceride levels were similar in the 2 IMT groups (Table 2). Nor were there any statistically significant differences in fasting or postprandial concentrations of TRL particle numbers between the IMT+ and IMT− groups, as reflected by the molarity of apolipoproteins and lipids. A enzymatic immunoassay was used to measure fasting concentrations of apoC-I in the plasma. In a subset of the study group, fasting and postprandial plasma apoC-I concentrations were measured by using a commercially available ELISA (Biosdesign International).

Lipid and Apolipoprotein Determination

Triglycerides and phospholipids in the bound and unbound fractions were determined enzymatically (450032, Boehringer-Mannheim; Wako Chemicals GmbH). Cholesterol was determined either enzymatically (Merek) or by a chemical method (if low concentrations were expected). ApoB and apoE were quantified by SDS-PAGE with purified apoB-100 used as a standard, and the contents of apoCs were quantified by agar gel electrophoresis with insulin used as a standard. The plasma lipoprotein concentrations were measured by a combination of preparative ultracentrifugation, precipitation of apoB-containing lipoproteins, and lipid analyses. A enzymatic immunoassay was used to measure fasting concentrations of apoC-I was in the bound fraction and that 81 ± 6% of apoB-100 was recovered from the column. There was no apoB-48 in the bound fraction, ApoB-48-containing particles represented ~80% of total particles in the unbound fraction of both the Sf-60 to -400 and the Sf-20 to -60 fractions.

Calculations and Statistical Methods

To obtain the number of apolipoprotein and lipid molecules per TRL particle, the molarity of apolipoproteins and lipids was divided by the molarity of apoB. The statistical significance of differences in the plasma concentration or composition of TRL particles from men in the IMT+ and IMT− groups was tested by Mann-Whitney test, and within group differences from baseline to postprandial time points were analyzed by Wilcoxon signed rank test. Correlation coefficients were calculated by Spearman rank tests. Values are reported as mean ± SD.

Results

Plasma Triglycerides and ApoB-48 and ApoB-100 Concentrations in TRL Fractions

Fasting plasma triglyceride levels were similar in the 2 IMT groups, as were postprandial responses to the oral fat load (Table 2). Nor were there any statistically significant differences in fasting or postprandial concentrations of TRL particle numbers between the IMT+ and IMT− groups, as reflected by apoB-100 and apoB-48 concentrations in Sf fractions (Table 2). In the Sf > 400 fraction, fasting and postprandial apoB-48 and apoB-100 were low or undetectable, reflecting a low

<table>
<thead>
<tr>
<th>Table 1: Basic Characteristics of the Study Groups</th>
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<tr>
<td>IMT+ (n=20)</td>
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<tr>
<td>BMI, kg/m²</td>
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<tr>
<td>Glucose, mmol/L</td>
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<tr>
<td>Insulin, pmol/L</td>
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<td>Pro-insulin, pmol/L</td>
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<td>Cholesterol, mmol/L</td>
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Values are mean ± SD.

*P < 0.05 vs IMT− subjects.
number of TRL particles. The apoB-48 and apoB-100 concentrations in the Sf-60 to -400 fraction increased transiently in response to the oral fat load, as did the apoB-48 concentrations in the Sf-20 to -60 fraction in the IMT− subjects. In contrast, the apoB-48 concentration in the Sf-20 to -60 fraction in the IMT+ subjects did not return to fasting levels at 6 hours but, instead, remained at the 3-hour postprandial level (P<0.05 versus the fasting level). ApoB-100 concentrations in the Sf-20 to -60 fraction were unaffected by the oral fat load. As expected,17,18 the apoB-100 concentrations in the Sf-60 to -400 fraction increased transiently in response to the oral fat load, as did the apoB-48 concentrations in the Sf-20 to -60 fraction in the IMT− subjects. Values are mean±SEM.

**TABLE 2. Concentrations of Plasma Triglycerides and of Apo-B48 and Apo-B100 in Sf-60 – 400, Sf 60 – 400, and Sf 20 – 60 Fractions in the IMT− and IMT+ Groups**

<table>
<thead>
<tr>
<th>Time After Test Meal, h</th>
<th>Groups</th>
<th>Plasma Triglycerides, mmol/L</th>
<th>Sf−400</th>
<th>Sf 60 – 400</th>
<th>Sf 20 – 60</th>
</tr>
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<tr>
<td></td>
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<td>ApoB-48, mg/L</td>
<td>ApoB-100, mg/L</td>
<td>ApoB-48, mg/L</td>
<td>ApoB-100, mg/L</td>
</tr>
<tr>
<td>0</td>
<td>IMT+ (n=20)</td>
<td>1.2±0.3</td>
<td>ND</td>
<td>0.9±0.6</td>
<td>1.5±1.1</td>
</tr>
<tr>
<td></td>
<td>IMT− (n=10)</td>
<td>1.4±0.6</td>
<td>ND</td>
<td>1.2±1.0</td>
<td>1.3±0.9</td>
</tr>
<tr>
<td>3</td>
<td>IMT+ (n=20)</td>
<td>2.7±1.1†</td>
<td>0.4±0.3</td>
<td>2.4±1.6‡</td>
<td>2.0±1.5*</td>
</tr>
<tr>
<td></td>
<td>IMT− (n=10)</td>
<td>2.7±1.0‡</td>
<td>0.5±0.7</td>
<td>3.0±1.4*</td>
<td>1.9±1.1*</td>
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<tr>
<td>6</td>
<td>IMT+ (n=20)</td>
<td>1.6±0.8*</td>
<td>0.2±0.2</td>
<td>1.4±1.3*</td>
<td>2.0±1.0*</td>
</tr>
<tr>
<td></td>
<td>IMT− (n=10)</td>
<td>1.8±0.8*</td>
<td>0.2±0.2</td>
<td>1.6±0.8</td>
<td>1.5±0.8</td>
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Values are mean±SD. ND indicates not detectable; Sf, Svedberg flotation rate.

*P<0.05, †P<0.005, ‡P<0.0005 vs the fasting determinations in the two groups. Differences between IMT groups were not statistically significant by Mann-Whitney test (P>0.05).

Composition of Fasting and Postprandial Chylomicron Remnants

The number of triglyceride molecules per large (Sf-60 to -400) chylomicron remnant particle in both groups was unaffected by the oral fat load. However, the number of triglyceride molecules per large chylomicron particle was 2-fold higher in the IMT+ subjects than in IMT− subjects at all time points (please see online Figure I, available at http://www.atvb.ahajournals.org). The number of apoC-II and apoC-III molecules on large chylomicron particles was also 2-fold higher in the IMT+ group (P<0.005 and P<0.05, respectively; see online Figure I). In contrast, the number of apoC-I molecules on large chylomicron remnant particles at 6 hours was 4-fold higher in IMT+ subjects than in IMT− subjects (P<0.005), indicating that large chylomicron remnants from IMT+ subjects were enriched with apoC-I (Figure 1). In addition, the apoE and cholesterol contents of large chylomicron remnant particles returned to fasting levels at 6 hours in the IMT− subjects but remained elevated in the IMT+ subjects (P<0.05 and P<0.05, respectively, versus baseline; see online Figure I).

In contrast to large chylomicron remnant particles, the triglyceride content of small chylomicron remnant particles (Sf 20 to 60) was similar in both groups (see online Figure I). Despite similar particle size, the number of apoC-I molecules on small chylomicron remnant particles at 6 hours was 2-fold higher in the IMT+ than in the IMT− subjects (Figure 1, P<0.05). Furthermore, the number of apoC-I molecules on small chylomicron remnant particles at 6 hours was positively associated with the IMT score in the IMT+ group (r=0.47, P<0.005; n=20) and in the IMT− group (r=0.65, P<0.01; n=10). In addition, the average cholesterol/triglyceride ratio of small chylomicron remnant particles increased considerably from baseline to the 6-hour time point in response to the oral fat load.
test meal in IMT+ subjects (from 56±13% to 75±18%, P<0.05; n=20) but remained unchanged or even decreased in IMT− subjects (54±14% versus 48±22%, P=NS; n=10).

Composition of Fasting and Postprandial VLDL
As observed for chylomicron remnant particles, the number of apoC-I molecules on large and small VLDL particles was 2-fold higher at 6 hours in the IMT+ subjects than in the IMT− subjects (P<0.005 and P<0.05 for large and small VLDL, respectively; Figure 2). In addition, the apoE and cholesterol contents of small VLDL particles remained elevated at 6 hours in the IMT+ group but returned to fasting levels in the IMT− group (P<0.005 and P<0.05, respectively, versus baseline; please see Figure II, available at http://www.atvb.ahajournals.org). The cholesterol/triglyceride ratio of the small VLDL particles in the IMT+ group increased substantially from baseline to the 6-hour time point (from 49±22% to 68±41%, P<0.05; n=20) but remained unchanged or even decreased in the IMT− group (from 57±23% to 55±19%, P=NS; n=10).

Discussion
Prolonged postprandial triglyceridemia associated with delayed clearance of chylomicron remnants has been consistently noted in patients with premature atherosclerosis. However, the underlying metabolic mechanism for this perturbed handling of postprandial triglycerides has not been defined. In the present study, we examined the TRL apolipoprotein and lipid composition in response to a fatty meal in healthy normolipidemic men with or without early asymptomatic atherosclerosis. In subjects with early atherosclerosis, large and small chylomicron remnants and VLDL particles were enriched with apoC-I, and small chylomicron and VLDL particles were relatively enriched with cholesterol. In addition, the number of apoC-I molecules per small chylomicron remnant particle was strongly associated with the degree of atherosclerosis in both groups. In contrast, there were no differences in fasting and postprandial plasma triglyceride levels or fasting and postprandial TRL particle numbers between men with and without early atherosclerosis.

The apoC-I enrichment of postprandial TRL remnants could be an important mechanism for the delayed clearance of lipoprotein remnants repeatedly observed in patients with premature CAD. Unlike apoC-II and apoC-III, apoC-I does not affect the conversion of large TRLs to smaller TRLs by lipoprotein lipase–mediated hydrolysis.20 However, apoC-I does affect chylomicron remnant and VLDL binding to receptors.21 More specifically, apoC-I inhibits apoE-mediated binding of TRL particles to the LDL receptor and to the LDL receptor–like protein.22,23 Hence, the apoC-I enrichment of large and small chylomicron and VLDL particles (observed in the present study in the IMT+ group) has the potential to affect the clearance of these particles by receptor-mediated pathways but should not affect the conversion of larger remnant particles to smaller remnant particles by lipoprotein lipase. Furthermore, an apoC-I–mediated delay in the receptor-clearance would predominantly affect apoB-48–containing remnants (ie, chylomicron remnants), considering their dependence on apoE (as opposed to VLDL, which also can be cleared through apoB-100–mediated LDL receptor binding). In support of this notion, postprandial concentrations of apoC-I–enriched small chylomicron remnants in the IMT+ subjects remained at the 3-hour level throughout the entire postprandial period, whereas the postprandial concentration of small chylomicron remnants in the IMT− subjects increased transiently (Table 2). Previously, we have shown that the apoC-I enrichment of VLDL particles that occurs in CAD patients is also associated with exaggerated postprandial triglyceridemia.10 The absence of exaggerated postprandial triglyceridemia and the tendency to perturbed handling of small chylomicron remnants in healthy normolipidemic subjects with asymptomatic atherosclerosis in the present study indicate that apoC-I enrichment of postprandial TRL remnants precedes perturbations in the postprandial metabolism that are sensed by alterations in postprandial triglyceride concentrations. However, other mechanisms (even secondary) for the postprandial apoC-I enrichment of VLDL and chylomicron particles are possible, and the phenomenon merits further investigation. For instance, apoC-I–enriched lipoproteins in diabetic mice have recently been suggested to be a secondary phenomenon to decreased remnant clearance and heparan sulfate proteoglycan production.24 On the other hand, the fact that apoC-I plays a primary role in regulating remnant metabolism is supported by the finding of a functional polymorphism in the human apoC-I promoter that has been shown to be associated with the profile of plasma lipoproteins.25

Studies of human apoC-I transgenic mice also support a role for apoC-I in the metabolism of postprandial TRLs.26 In addition, the human apoC-I transgenic mouse was found to be lean.27 The same authors also unexpectedly found that overexpression of human apoC-I protects against obesity and
insulin resistance in the ob/ob mouse, a leptin-deficient mouse model that develops obesity due to hyperphagia. Another related observation has also recently been made in the VLDL receptor–knockout mouse, in which the absence of the VLDL receptor also has a protective effect against obesity. In consideration of the repeated observation that apoC-I interferes with the binding of VLDL to the VLDL receptor (apoE mediated) but not with the binding of VLDL to the LDL receptor (apoB-100 mediated), the mechanism of the protection against obesity in the setting of high levels of apoC-I and in the setting of absent VLDL receptor could be similar. It is possible that apoC-I enrichment of VLDL and chyomicron remnants interferes with the binding of these particles to the VLDL receptor in white adipose tissue. This interference would cause a reduction in the metabolism of TRLs in the adipose tissue, which would reduce white adipose tissue mass and, potentially, the body weight. From this perspective, it is noteworthy that the IMT+ individuals with postprandial apoC-I enrichment of TRLs had a body mass index that was significantly lower than that of age-matched IMT− individuals without this postprandial perturbation (Table 1).

The fact that apoC-I was uniquely elevated in the postprandial VLDL and chyomicron particles in men with early asymptomatic atherosclerosis and the fact that the apoC-I content of small postprandial chyomicron remnants was strongly related to the degree of atherosclerosis in these individuals suggest that the apoC-I enrichment of these remnants is pivotal in causing early atherosclerotic lesions. Small postprandial chyomicrons and VLDL particles from subjects with early atherosclerosis were, in addition to apoC-I, enriched with cholesterol relative to their triglyceride content. It is reasonable to assume that this relative cholesterol enrichment reflects cholesterol esters that have been transferred from HDL in exchange for triglycerides via cholesterol ester transfer protein (CETP). The grounds for this assumption are as follows: (1) apoC-I enrichment of these particles is likely to have a depleting effect on the apoC-I content of HDL, which would increase CETP activity, and (2) apoC-I enrichment is likely to extend the half-life of remnant particles in the circulation, which would increase the time during which CETP-mediated cholesterol-triglyceride exchange with HDL could occur. The formation of small relatively cholesterol-rich VLDL and chyomicron remnants could be very relevant to the development of early atherosclerosis in the IMT+ subjects because these small remnants, like LDL, are thought to be prone to retention within the arterial intima. Prospective studies are warranted to determine whether the apoC-I content of lipoprotein remnants constitutes a general marker of CAD risk.

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References


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Fig. 1. Line plots of changes in postprandial VLDL composition (number of apolipoprotein or lipid molecules (mol) per particle (mol apo-B100)) in Sf 60 to 400 and Sf 20 to 60 lipoproteins in IMT− (n = 10) and IMT+ (n = 20) subjects. Blood samples were drawn before and 3 and 6 h after intake of the test meal. VLDL were isolated from chylomicron remnants by immunoaffinity chromatography with the human apo-B100–specific monoclonal antibodies 4G3 and SE11. □, VLDL from IMT+ subjects; ▄, VLDL from IMT− subjects. Values are mean ± SEM. There were no significant differences between IMT groups (P > 0.05). †, P < 0.05 vs. baseline within each group; ‡, P < 0.005 vs. baseline within each group.
Fig. 1. Line plots of changes in postprandial chylomicron composition [number of apolipoprotein or lipid molecules (mol) per particle (mol apo-B48)] in Sf 60 to 400 and Sf 20 to 60 lipoproteins in IMT− (n = 10) and IMT+ (n = 20) subjects. Blood samples were drawn before and 3 and 6 h after intake of the test meal. Chylomicron remnants were isolated from VLDL by immunoaffinity chromatography with the human apo-B100–specific monoclonal antibodies 4G3 and 5E11: ■, chylomicron remnants from IMT+ subjects; ○, chylomicron remnants from IMT− subjects. Values are mean ± SEM.

*, P < 0.05 vs. IMT− subjects; **, P < 0.005 vs. IMT− subjects.
†, P < 0.05 vs. baseline within each group; ††, P < 0.005 vs. baseline within each group.