Plasma Levels of HDL Subpopulations and Remnant Lipoproteins Predict the Extent of Angiographically-Defined Coronary Artery Disease in Postmenopausal Women

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Objective — The association of coronary heart disease (CHD) with subpopulations of triglyceride (TG)-rich lipoproteins and high-density lipoproteins (HDL) is established in men, but has not been well characterized in women.

Methods and Results — Plasma HDL subpopulation concentrations, quantified by 2-dimensional gel electrophoresis, and plasma remnant-like particle cholesterol (RLP-C) concentrations were measured in 256 postmenopausal women with established CHD and in 126 CHD-free postmenopausal women. Coronary artery disease was assessed in women with CHD by quantitative coronary angiography. Plasma RLP-C and preβ1 HDL concentrations were higher and α1 and α2 HDL concentrations were lower in CHD than in CHD-free women. After adjustment for conventional CHD-risk factors, plasma levels of RLP-C were positively associated with the degree of coronary artery disease. In similar analyses, plasma preβ1 HDL particle concentrations were positively associated and α2 HDL particle concentrations were inversely associated with the extent of coronary atherosclerosis. Plasma TG, low density lipoprotein cholesterol, and HDL cholesterol levels were not associated with the degree of coronary atherosclerosis.

Conclusions — The degree of coronary atherosclerosis in postmenopausal women is linked to a dysregulation of the TG/HDL metabolism. Subpopulations of TG-rich and HDL lipoproteins are better predictors of disease than TG and HDL cholesterol concentrations. (Arterioscler Thromb Vasc Biol. 2008;28:575-579)

Key Words: women ▪ angiography ▪ lipoproteins ▪ coronary heart disease

Population-based prospective studies have established an association between plasma triglyceride (TG) levels and risk of coronary heart disease (CHD).1 This association is stronger in women than in men.1 Fasting plasma TG concentrations reflect the concentration of both TG-rich very low-density lipoproteins (VLDL) and remnants of VLDL and chylomicrons. Remnant lipoproteins are generated by lipolysis of TG-rich lipoproteins and play a greater role in CHD risk than their precursors. The first evidence that remnant lipoproteins are related to the development of CHD came from studies conducted more than 50 years ago in which remnant lipoproteins with Svedberg flotation rate of 12 to 60 were isolated by ultracentrifugation.2 More recently, case-control studies conducted predominantly in male patients have supported the concept that remnant lipoproteins play an important role in the development of CHD.3-5 Remnant lipoprotein levels have also been shown to be significantly higher in women with CHD as compared with healthy women in the Framingham Offspring Study.6

The association between low levels of high-density lipoprotein (HDL) cholesterol (C) and increased risk of CHD is well documented.7,8 The National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) has defined an HDL-C <40 mg/dL as an independent risk factor for CHD.9 HDL comprise a number of subpopulations differing in apolipoprotein and lipid composition and representing different stages of the continuous HDL remodeling occurring in plasma. These HDL subpopulations exhibit a different ability to protect from CHD.10 Separation of HDL particles by 2-dimensional gel electrophoresis allows the identification of eight major subpopulations: preβ1 to 2, α1 to 3, and preα1 to 3.11 Previous studies conducted in men have established a protective role of α1 and α2 particles against the development of CHD.12,13 These studies have also documented an increased plasma level of α3 and preβ1 particles in men with CHD as compared with healthy men.12 Very little is known about HDL subpopulations and risk of CHD in women.

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The objective of the current study was to assess whether plasma levels of remnant lipoproteins and of HDL subpopulations are significant predictors of the extent of coronary atherosclerosis in a cohort of postmenopausal women with established CHD.

**Subjects and Methods**

**Subjects**
Subjects were postmenopausal women participating in the Estrogen Replacement and Atherosclerosis (ERA) trial, whose design has been described previously. Briefly, postmenopausal women were enrolled if they had established CHD as assessed by quantitative coronary angiography with $\geq 30\%$ stenosis of the luminal diameter at 1 or more epicardial coronary arteries. Women with a previous history of thromboembolism, symptomatic gallstones, uncontrolled diabetes or hypertension, or with plasma TG levels $>400$ mg/dL were excluded from participation in the study. Also, women on hormonal replacement therapy were asked to stop treatment for at least 3 months before starting participation. Approval of the study protocol was obtained from the Institutional Review Boards of the different sites involved in patient recruitment. Study candidates provided informed consent. For the purpose of this study, lipoprotein measurements were performed in 256 women for whom plasma samples obtained at the baseline visit and coronary angiography performed at baseline were available.

Postmenopausal women ($n=126$) participating in cycle 6 of the Framingham Offspring Study (FOS) were randomly selected as a reference population. These women were free of CHD, and of liver, kidney, or thyroid disease. FOS is a long-term prospective observational study of CHD risk.

**Plasma Lipid, HDL Subpopulation, and Lipoprotein Remnant Determinations**
Plasma total cholesterol (TC) and TG concentrations were measured by automated enzymatic assays. Plasma HDL-C concentrations were measured after heparin-manganese precipitation of apolipoprotein (apo) B–containing lipoproteins.

Plasma remnant-like particle cholesterol (RLP-C) concentrations were measured using an immunonephelometric technique (Polymedco). This technique uses a monoclonal antibody directed against apoA-I to remove HDL particles, and an anti-apoB antibody that does not recognize partially hydrolyzed lipoprotein remnants to remove both large nascent VLDL and LDL particles. The cholesterol content of the remaining remnant lipoproteins was then measured. The inter-assay CV was $<10\%$.

ApoC-III concentrations in plasma and in the supernatant obtained after the precipitation of apoB-containing lipoproteins with polyethylene glycol (PEG) 6000 were measured on a Hitachi 911 autoanalyzer (Hitachi Inc) using an assay and calibrators from Wako Diagnostics.

Plasma apoA-I concentrations were measured using an immunoturbidimetric assay and calibrators from Wako Diagnostics, as previously described. ApoA-I–containing HDL subpopulations in plasma were measured by nondenaturing 2-dimensional gel electrophoresis as previously described. This method allows for the separation of HDL into 8 separate subpopulations (preβ1 to 2, α1 to 3, and preα1 to 3). The concentration of each HDL subpopulation was calculated by multiplying its percentage with total plasma apoA-I concentration and expressed as mg/dL of total apoA-I. The CV was $<10\%$ for α particles, and was $<15\%$ for all other subpopulations.

**Angiographic Assessment of Coronary Arteries**
Quantitative coronary angiography was performed at baseline according to standard methods. A minimum of 3 sets of orthogonal views of the left coronary artery and 1 of the right coronary artery were obtained in each subject. Analysis of angiograms was performed with a previously validated system of cine-projection (SME 3500, Sony). The reference, minimal (the point of greatest narrowing), and average luminal diameters were obtained for 10 proximal epicardial coronary artery segments, as previously described. The mean minimal coronary artery diameter was calculated in each subject as the average of the minimal luminal diameter in the 10 coronary segments.

**Statistical Analysis**
Variables with skewed distribution were log transformed before analyses. Analysis of covariance was used to compare mean lipoprotein and apolipoprotein values between the ERA and FOS groups after adjusting for the following covariates: age, BMI, use of lipid-lowering medications, and diabetes. The association between minimal coronary artery diameter and lipoprotein subfractions (RLP-C and HDL subpopulations) was assessed by mixed-model analysis of covariance with adjustments for location of the segment in the coronary artery tree, age, body mass index (BMI), race, smoking, hypertension, diabetes, use of lipid-lowering medications, and prior percutaneous coronary angioplasty. Because the association between degree of coronary atherosclerosis and lipoprotein subpopulations may not be linear in the models described above, analyses of mean minimal coronary diameter by tertiles of RLP-C and HDL subpopulations, also prespecified, were carried out. These analyses were adjusted for the same CHD-risk factors as listed in the mixed-model analysis. A probability value $\leq0.05$ was set as statistically significant.

**Results**
The baseline characteristics of postmenopausal women participating in the ERA trial are shown in Table 1, together with the characteristics of postmenopausal CHD-free FOS women. ERA women were slightly older, had higher BMI, prevalence of diabetes, and use of lipid-lowering medications than FOS women. ERA women had higher plasma TG and LDL-C and lower HDL-C concentrations than FOS women.

Plasma RLP-C concentrations were significantly higher in ERA than in FOS women (Table 2). Plasma HDL-C, apoA-I, and HDL subpopulation α1, α2, and preα1 and preα2 concen-
trations were significantly lower, whereas HDL subpopulation preβ1 and preα3 concentrations were significantly higher in ERA than in FOS women. Figure 1 shows the HDL subpopulation distribution from representative ERA and FOS subjects.

Plasma RLP-C concentrations were significantly and positively associated with plasma TG and apoC-III levels (Spearman \( r = 0.625 \) and \( r = 0.702 \), respectively; \( P < 0.0001 \)) and inversely associated with plasma HDL-C and apoA-I levels (\( r = -0.436 \) and \( r = -0.194 \), respectively; \( P < 0.001 \)) in ERA subjects. However, the association of RLP-C with HDL subpopulations was more complex, showing a positive association with preβ1 and α3 particles (\( r = 0.210, P < 0.001 \); and 0.322, \( P < 0.0001 \), respectively), and an inverse association with α1, α2, preα1, and preα2 particles (\( r = -0.523, -0.301, -0.475, \) and \( -0.297, \) respectively; \( P < 0.0001 \)).

In the ERA population, plasma concentrations of RLP-C were associated with minimal coronary artery diameter at baseline (\( P = 0.05 \); Table 3). No association of minimal coronary artery diameter with plasma TG or apoC-III concentrations was observed (Table 3). Plasma HDL-C and apoA-I concentrations were not significantly associated with the degree of coronary atherosclerosis. However, a significant and inverse association between preβ1 HDL particle concentrations and minimal coronary artery diameter \( (P < 0.01) \) and a significant and positive association of plasma concentration of α2 HDL particles with minimal coronary diameter were observed \( (P < 0.03) \; Table 3). \n
To further validate the association of the degree of coronary atherosclerosis with plasma RLP-C and HDL subpopulation concentrations, ERA subjects were divided into tertiles according to their plasma lipoprotein concentrations. Regression analyses showed only a trend toward an association between tertiles of RLP-C concentrations and mean minimal coronary artery diameter \( (P = 0.07) \). A significant association was observed between mean minimal coronary artery diameter and tertiles of preβ1 \( (P < 0.0001) \), α1 \( (P < 0.03) \), α2 \( (P < 0.0001) \), and α3 \( (P = 0.05) \) HDL particle concentrations (Figure 2B). Postmenopausal women in the highest tertile for α1 and α2 particle concentrations had larger mean minimal coronary diameter than women in the lowest tertile, whereas the reverse was true for preβ1 and α3 tertiles.

**Discussion**

Our study is the first to report an association, although weak, between remnant lipoprotein concentrations and angiographically-defined CHD in postmenopausal women. Previous angiographic studies had shown an association of TG and TG-rich lipoproteins with presence of coronary plaques in women,\textsuperscript{22,23} but no measurement of remnant lipoproteins was

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**Table 2. Plasma Concentrations of RLP-C, ApoC-III, and ApoA-I–Containing HDL Subpopulations in Postmenopausal ERA and FOS Women**

<table>
<thead>
<tr>
<th></th>
<th>ERA mg/dL</th>
<th>FOS mg/dL</th>
<th>( P ) Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>RLP-C</td>
<td>13.0 (0.7)</td>
<td>10.1 (1.02)</td>
<td>0.023</td>
</tr>
<tr>
<td>Plasma apo C-III</td>
<td>14.0 (0.33)</td>
<td>22.3 (0.52)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL apo C-III</td>
<td>9.8 (0.26)</td>
<td>11.2 (0.41)</td>
<td>0.0062</td>
</tr>
<tr>
<td>Apo A-I</td>
<td>126.9 (1.4)</td>
<td>154.7 (2.03)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL-C</td>
<td>44.7 (0.89)</td>
<td>59.2 (1.29)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

HDL subpopulations† expressed as mg/dl of plasma apoA-I.

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**Table 3. Association of Minimal Coronary Artery Diameter With RLP-C, ApoC-III, and ApoA-I–Containing HDL Subpopulations at Baseline**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RLP-C</td>
<td>-0.0041</td>
<td>0.05</td>
</tr>
<tr>
<td>TG</td>
<td>-0.0001</td>
<td>0.51</td>
</tr>
<tr>
<td>LDL-C</td>
<td>-0.0007</td>
<td>0.19</td>
</tr>
<tr>
<td>Plasma apo C-III</td>
<td>-0.0026</td>
<td>0.56</td>
</tr>
<tr>
<td>HDL apo C-III</td>
<td>-0.0019</td>
<td>0.45</td>
</tr>
<tr>
<td>Apo A-I</td>
<td>0.0009</td>
<td>0.39</td>
</tr>
<tr>
<td>HDL-C</td>
<td>0.0020</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Mix-model analysis adjusted for location in the coronary tree, age, BMI, race, smoking, hypertension, diabetes, prior PTCA, and use of lipid-lowering medications.

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**Figure 1.** ApoA-I–containing HDL subpopulation distribution in a representative woman with CHD (ERA) (a) and a healthy normolipidemic woman (FOS) (b). c, schematic representation of HDL subpopulations by 2-dimensional gel electrophoresis.
performed in those studies. Some cross-sectional studies have shown plasma levels of RLP-C to be significant independent predictors of CHD risk.\(^6\)\(^{–}\)\(^{24}\) Plasma RLP-C concentrations were also found to be significant and independent predictors of the risk of recurrent events in subjects with CHD.\(^{24}\) Similarly, a measure of remnant lipoproteins, but not TG levels, was significantly correlated with CHD progression in a study of 272 men and 68 women.\(^{25}\) Remnant lipoproteins play a role in the development of CHD through different mechanisms. Remnant lipoproteins have been shown to induce endothelial cell dysfunction\(^{26,27}\) and to enter the subendothelial layer, where they interact with receptors on the surface of macrophages, alter the microenvironment, and promote their own uptake by macrophages and plaque formation.\(^{28}\)

Plasma apoC-III levels were highly correlated with plasma TG and RLP-C concentrations in the ERA population, indicating the contribution of this apolipoprotein to the metabolism of TG-rich lipoproteins. However, apoC-III levels in plasma and in HDL were not related to the degree of coronary atherosclerosis in ERA. This observation suggests that other factors, in addition to apoC-III, contribute to plasma remnant lipoprotein concentrations in this population. Although some studies have found apoC-III concentration in HDL or in the non-HDL fraction to be a predictor of CHD in men,\(^{29–31}\) no significant association was found in women.\(^{31}\) This is in agreement with the results of our study. The gender difference in the relationship of apoC-III with CHD should be further investigated.

The inverse association between plasma HDL-C concentrations and CHD risk is well established.\(^7\)\(^–\)\(^9\) In ERA women, significant associations between minimum luminal coronary diameter and plasma levels of HDL preβ1 (inverse) and α2 (positive) were observed. These findings were further confirmed by the observation of significant differences in mean minimal coronary diameter by tertiles of plasma preβ1 and α2 concentrations. No association between HDL-C or apoA-I concentrations and degree of coronary atherosclerosis was observed. Our findings confirm and extend to postmenopausal women recent evidence obtained in men indicating that the profile distribution of HDL subpopulations is more informative than HDL-C concentrations in the assessment of CHD risk.\(^{12,13,32–34}\) Moreover, our results are consistent with observations in men participating in the Veterans Affairs HDL Intervention Trial (VA-HIT), who had established CHD and low HDL-C levels (\(\leq 40\) mg/dL), similarly to women in the ERA study.\(^{33}\) When VA-HIT subjects were divided into quartiles of different HDL subpopulations, subjects in the upper quartile for preβ1 had a significantly greater risk of recurrent CHD events than subjects in the lowest quartile. In addition, VA-HIT subjects with higher α2 concentrations had a significantly lower risk of recurrent CHD than subjects with lower concentrations, and the value of α2 in predicting recurrent CHD was greater than that of α1.\(^{33}\) We have not noticed a significant association between coronary lumen diameter and plasma α1 particle levels in the ERA population. This is likely explained by the fact that women in the ERA study had homogeneously low HDL-C and low α1 particle levels.

HDL particles are involved in different steps in reverse cholesterol transport (RCT), a metabolic pathway responsible for the removal of free cholesterol from peripheral cells and its transport back to the liver for catabolism. Preβ1 HDL particles are small, lipid-poor, apoA-I–containing only particles that interact with the cholesterol transporter ABCA1 (ATP binding cassette A1) to promote cell free-cholesterol efflux. After preβ1 particles pick up cell free-cholesterol they mature into large HDL particles, such as α1 and α2 via enzymatic esterification by lecithin:cholesterol acyl transferase (LCAT).\(^{35}\) It has been proposed that increases in preβ1 concentrations reflect an impairment in HDL maturation.\(^{35}\)

A limitation of our study is the use of coronary angiography for the assessment of the extent of CHD. When the ERA trial was initiated, coronary angiography was accepted as the gold standard for the assessment of coronary disease lesions. More recently, other diagnostic tools, including intravascular ultrasound (IVUS) and 64-slice computed tomography (CT), have shown to have equal or greater accuracy in the assessment of coronary lesions.\(^{36,37}\)

Our results strongly support the notion that alterations in plasma lipoprotein levels other than LDL, which is the current target of the NCEP recommendations,\(^7\) play an important role in the development of CHD in women. Our findings indicate that the degree of coronary atherosclerosis in postmenopausal women is linked to a dysregulation of the TG-HDL metabolism: increases in remnant lipoprotein levels may be associated with increased lipid deposition in the arterial wall, plaque formation, and the generation of HDL subpopulations that are less efficient in the reverse cholesterol removal from the arterial wall.

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Disclosures

None.

References

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