Reduced HDL$_2$ Cholesterol Subspecies and Elevated Postheparin Hepatic Lipase Activity in Older Men With Abdominal Obesity and Asymptomatic Myocardial Ischemia

Leslie I. Katzel, Patricia J. Coon, M. Janette Busby, Sidney O. Gottlieb, Ronald M. Krauss, and Andrew P. Goldberg

Silent myocardial ischemia (SI), an asymptomatic manifestation of coronary artery disease (CAD), was identified in 10% of apparently healthy nonsmoking, nondiabetic older (60±7 years, mean±SD) men with normal plasma cholesterol levels. We hypothesized that in the absence of other major risk factors for CAD, the men with SI would have reduced plasma levels of high density lipoprotein (HDL) and HDL$_2$ subspecies due to an upper-body fat distribution (waist-to-hip ratio [WHR]), hyperinsulinemia, and abnormal postheparin plasma lipoprotein lipase (LPL) and hepatic lipase (HL) activities. Compared with 47 normal control subjects of similar age, obesity, and maximal aerobic capacity, the 18 men with SI had higher plasma triglyceride (TG) (162±71 versus 102±39 mg/dl, p<0.001) and lower HDL-C (33±6 versus 37±7 mg/dl, p<0.02) with no difference in low density lipoprotein cholesterol level. The HDL$_2\alpha$ and HDL$_2\beta$ subspecies measured by gradient gel electrophoresis were also lower in the men with SI (p<0.01). The plasma glucose and insulin responses during an oral glucose tolerance test were the same in both groups. Postheparin plasma HL activity was significantly higher in 12 men with SI than in 41 control subjects (34±8 versus 27±10 µmol/ml·hr$^{-1}$, p<0.03) and was correlated with log insulin area (r=0.36, p<0.05) and WHR (r=0.32, p<0.05) in the control subjects but not in the men with SI. In the control group, the percent HDL$_{2\beta}$ subspecies was correlated inversely with postheparin plasma HL activity (r=-0.46, p<0.01, n=41) as well as WHR (r=-0.49, p<0.001, n=47) and log insulin area (r=-0.37, p<0.05, n=47) but not in the men with SI. Postheparin LPL activity was the same in both groups of men and did not correlate with HDL, WHR, insulin, or plasma TG levels. As the control subjects and men with SI had comparable degrees of abdominal obesity and hyperinsulinemia, these results suggest that the reduced HDL-C levels in men with SI may be related to elevations in HL activity. Thus, abdominal obesity, hyperinsulinemia, elevated TG levels, and low HDL-C and HDL$_2$ subspecies levels may predispose these older men to atherosclerosis.

KEY WORDS • obesity • silent myocardial ischemia • high density lipoproteins • hepatic lipase

Asymptomatic or silent myocardial ischemia (SI) is a manifestation of coronary artery disease (CAD) in which there is a transient alteration in myocardial perfusion, function, and/or electrical activity not accompanied by chest pain or the usual anginal equivalents. Estimates of the prevalence of SI in asymptomatic individuals with no history of angina pectoris or myocardial infarction range from 2-4% in middle-aged men$^{4-6}$ to >15% in healthy octogenarians.$^5$ Prospective studies demonstrate that patients with asymptomatic exercise-induced SI are at a considerably higher risk for the development of angina, myocardial infarction, and death than are healthy control subjects without SI.$^{6-12}$ Known risk factors for SI include age, hypertension, and diabetes mellitus.$^{13,14}$ Abnormalities in triglyceride (TG) and cholesterol levels have not been reported in patients with SI.$^{3,4,9}$ There has not been an extensive evaluation of plasma high density lipoproteins (HDLs) and their subspecies in patients with SI.

Reduced levels of HDL and its HDL$_2$ subspecies are associated with increased cardiac morbidity and mortality.$^{15-17}$ This relation remains strong with aging, whereas...
the positive association between low density lipoprotein cholesterol (LDL-C) levels and CAD weakens. Major causes of reduced levels of plasma HDL-C include abdominal obesity, hyperinsulinemia/diabetes mellitus, cigarette smoking, physical inactivity, male sex, certain drugs, and genetic syndromes. The activity of lipoprotein lipase (LPL) and hepatic lipase (HL), key enzymes in the regulation of HDL and TG metabolism, are often altered in these conditions. LPL is the rate-limiting enzyme in the clearance of TG-rich lipoproteins from plasma and is involved in the formation of HDL2, whereas HL plays a role in the catabolism of TG-rich lipoproteins. Therefore, abnormalities in the regulation of HDL and TG metabolism by LPL and HL may reduce levels of HDL, thereby increasing the risk for CAD.

We reported a 20% incidence of asymptomatic electrocardiographic (ECG) ST-segment depression during treadmill exercise tests in apparently healthy, obese, nonsmoking, normotensive older men who were not diabetic and had normal plasma cholesterol levels. We hypothesized that in the absence of other major CAD risk factors, the men with SI would have reduced plasma levels of HDL and its HDL2 subspecies that were associated with an upper-body fat distribution, hyperinsulinemia, and altered postheparin plasma LPL and HL activities. The results of these metabolic studies suggest that in older men, abdominal obesity, hyperinsulinemia, and elevated postheparin HL activity play a role in lowering the HDL and HDL2 subspecies levels and increasing their risk for CAD.

Methods

Subjects

Healthy male volunteers ranging in age from 46 to 76 years were recruited (Figure 1) through media advertisements in the Baltimore-Washington area. All subjects provided informed consent according to guidelines of the Francis Scott Key Medical Center Human Studies Institutional Review Board.

Subjects had an initial telephone interview followed by the completion of a medical history questionnaire to exclude those with a history of CAD, hypertension (blood pressure >160/90 mm Hg), chronic lung disease, diabetes mellitus, hyperlipidemia, and other comorbid diseases. Physical examinations were performed, and samples for blood chemistries were drawn on 228 men to exclude those with metabolic disease. Exclusion criteria included a fasting plasma glucose level >140 mg/dl or a diabetic response during a 40 g/m² oral glucose tolerance test (OGTT); hyperlipidemia defined as a plasma TG or cholesterol level >90th percentile for age and gender according to Lipid Research Center criteria; anemia; or abnormalities of liver, renal, bone-mineral, or electrolyte metabolism. A urinalysis was performed to exclude subjects with albuminuria and hematuria. There were 39 ineligible men: 16 with diabetes mellitus, 18 with hyperlipidemia, two with anemia, one with cancer, one with liver disease, and one with renal disease. An additional 19 men dropped out because of time constraints imposed by the research, leaving 170 men for further testing.

Noninvasive Cardiac Evaluation

A graded treadmill exercise test to >85% of predicted age-adjusted maximal heart rate (220 minus age) was performed according to the Bruce protocol. Forty-four (26%) of the 170 men had ≥1-mm asymptomatic horizontal or downsloping ST-segment depression for 0.08 second after the J-point (Minnesota Code Criteria) on at least three occasions. The 44 subjects with ischemic treadmill exercise tests were referred for exercise thallium scintigraphy. Four declined, and the other 40 men underwent exercise tomographic thallium scans to >85% of age-adjusted maximal heart rate. All tests were interpreted by a cardiologist blinded to the clinical history of the subjects. Of the 40 men who agreed to this procedure, 23 (57.5%) had ischemic exercise ECGs and reversible perfusion abnormalities on thallium scans, consistent with a diagnosis of SI.

Measurement of Maximal Aerobic Capacity (VO₂max)

After a 2-minute warm-up, the speed and incline of the treadmill were set at a work load to produce 75% of the subject's previously measured peak heart rate. Treadmill speed and/or grade was increased every 2 minutes until the subject was exhausted and could not continue or until the subject developed >2-mm ST-segment depression on ECG. Ventilation was collected through a low-resistance, low-dead-space, three-way mixing valve (Otis McKeon) into a mixing chamber and a 120-l Tissot spirometer. The expired gas concentrations were measured using Beckman CO2 and O2 analyzers (Beckman Industries, Fullerton, Calif.). The oxygen consumption (VO2), carbon dioxide production (VCO2), and respiratory exchange ratio (R) were then calculated. In three men the VO₂max tests were stopped prematurely because of an ST-segment depression >2 mm during exercise, and a peak VO2 was obtained as a measure of the ischemic threshold; this "peak" VO2 is an underestimate of their "true" VO₂max. Other VO₂max tests fulfilled at least two of the following three criteria: 1) heart rate at maximal exercise was >85% of the age-predicted maximal heart rate (220 minus age); 2) R was >1.10; and 3) a plateau in oxygen uptake was achieved with a change in VO2 <0.2 l/min during the final two gas collections. The VO₂max is
expressed in milliliters per kilogram per minute but in milliliters per kilogram fat-free mass (FFM) per minute when it became necessary in the regression analyses to control for the effects of obesity.

**Selection**

Two groups of subjects were selected for study, control subjects and men with exercise-induced SI.

**Controls.** Because exercise thallium scintigraphy was not performed in men with a normal exercise ECG, there exists the possibility of a “false-negative” exercise test in this group. To minimize the frequency of this occurrence, we required that control subjects included in this analysis have at least three normal exercise tests at baseline and remain without evidence of ischemic heart disease by exercise ECG for at least 3 years after the initial evaluation. On this basis, four men were excluded because they progressed to symptomatic CAD and two others were excluded because they had ST-segment depression on exercise tests at follow-up 3 years later. We report data on the first 47 men who completed metabolic testing at least 3 years previously and had normal treadmill exercise tests at both baseline and follow-up evaluation.

**SI (asymptomatic exercise-induced ST-segment depression on exercise treadmill tests and myocardial perfusion abnormalities on thallium scanning).** We report data on 18 of the 23 men with SI who completed metabolic testing.

**Dietary Stabilization**

The men were instructed by registered dietitians about the principles of an American Heart Association Phase I diet. Adherence was monitored by review of 7-day food records. After 4 weeks of dietary and weight stabilization on ad libitum diets consisting of >50% carbohydrate, 30–35% fat, 15% protein, and 300–400 mg/day cholesterol with a polyunsaturated to saturated fat ratio of 0.6–0.8, subjects were provided weight-maintaining American Heart Association Phase I diets from the General Clinical Research Center metabolic kitchen for 6 days of metabolic testing.

**Body Composition**

Body mass index (BMI) was calculated as body weight in kilograms divided by the height in meters squared. Hydrostatic weighing was performed to determine body density, and percent body fat was calculated using the Siri equation corrected for residual lung volume measured by helium dilution. FFM was calculated as kilograms of weight minus kilograms of body fat. The waist-to-hip ratio (WHR), an index of the regional distribution of body fat, was determined as the ratio of the minimal abdominal circumference divided by the circumference at the maximal gluteal protuberance.

**Metabolic Testing**

Blood samples for the measurement of fasting lipoprotein lipid levels were drawn into chilled tubes containing EDTA (1 mg/ml blood) after a 12–14-hour overnight fast on days 4 and 6 of the controlled metabolic diet. The reported lipoprotein lipid values were the mean of two determinations. On day 4 of the metabolic diet, an OGTT was performed after ingestion of 40 g glucose per square meter of body surface area. Blood samples were drawn every 30 minutes over a 2-hour period. Plasma glucose level was measured by the glucose oxidase method using a Beckman Glucose Analyzer (Beckman Instruments). Plasma immunoreactive insulin (in microunits per milliliter) was measured by radioimmunoassay. Glucose and insulin areas during the OGTT were calculated using a trapezoidal model.

After the lipid samples were drawn on day 6, a standardized dose (2,280 units per square meter of body surface area) of beef intestinal mucosa heparin (Organon Teknika, Rockville, Md.) was injected into all eligible subjects, and postheparin blood samples were drawn 10 minutes afterward into iced EDTA-containing tubes. The blood was spun immediately at 4°C, and triplicate aliquots of postheparin plasma were stored at −70°C for analysis at a later time. Heparin was not administered to 12 subjects because of a history of peptic ulcer disease, allergy to amines, or refusal to have the test performed.

**Analytical Methods**

Plasma TG and cholesterol levels were measured enzymatically on an Abbott ABA 200 series bichromatic analyzer. Because none of the subjects had a plasma TG >400 mg/dl, HDL-C was measured in the supernatant after precipitation of apolipoprotein B–containing lipoproteins with dextran sulfate. LDL-C was calculated as

\[
\text{Total cholesterol} - (\text{TG}/5 + \text{HDL-C})
\]

A second precipitation with high-molecular-weight dextran sulfate was performed on the supernatant HDL to separate the HDL₂ and HDL₃ subspecies.

In addition to the standard measurements of lipoprotein lipids and HDL subspecies by precipitation, gradient gel electrophoresis was performed to separate the subspecies of HDL. The gels were stained for protein using Coomassie blue and then scanned at 603 nm with a model RFT densitometer (Transidyne Corp., Ann Arbor, Mich.). The scanner was interfaced with a PDP8/E minicomputer (Digital Equipment Corp., Maynard, Mass.). The optical density of HDL (concentration of HDL protein) was plotted as the relative mobility of HDL protein from the origin compared with that of bovine serum albumin. Five HDL subspecies were separated using this method and quantified as the areas in each reference interval as a percentage of the total area of the HDL scan.

To validate the precipitation and gradient gel measurements of the HDL subfractions, we measured HDL₁ and HDL₃ mass by analytic ultracentrifugation for 25 of these obese sedentary men and eight lean active older men recruited from other metabolic studies. There were significant correlations between HDL₁-C and HDL₂ mass (by analytic ultracentrifugation) for 25 of these obese sedentary men and eight lean active older men recruited from other metabolic studies. There were significant correlations between HDL₁-C and HDL₂ mass (by analytic ultracentrifugation) for 25 of these obese sedentary men and eight lean active older men recruited from other metabolic studies. 

Because of these highly significant relations, all of the correlations between the HDL₂ subspecies with other parameters will only be reported for percent HDL₂-C.
Subject Characteristics

Table 1. Subjects’ Physical Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects (n=47)</th>
<th>Asymptomatic Ischemia (n=18)</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>59±8</td>
<td>62±7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29±3</td>
<td>29±3</td>
</tr>
<tr>
<td>Percent body fat</td>
<td>28±5</td>
<td>28±6</td>
</tr>
<tr>
<td>Vo2max (ml/kg · min⁻¹)</td>
<td>30±6</td>
<td>28±6</td>
</tr>
<tr>
<td>WHR</td>
<td>0.96±0.05</td>
<td>0.98±0.06</td>
</tr>
</tbody>
</table>

BMI, body mass index; Vo2max, maximal aerobic capacity; WHR, waist-to-hip ratio. Data are mean±SD.

The assay for the measurement of postheparin plasma LPL and HL activities was adapted from the assay of Krauss et al with modifications according to Huttunen et al. This permitted the use of immunochromatographic techniques to test the validity of protamine inhibition of LPL and the calculation of LPL activity as the difference between the total postheparin lipolytic activity and HL activity. The measurement of HL activity after protamine inhibition of LPL in postheparin plasma was validated using an antibody to human HL to measure LPL activity directly in postheparin plasma after antibody inhibition of HL. This measure of postheparin LPL activity correlated directly with LPL activity calculated as the difference between total postheparin lipolytic activity and HL activity remaining after protamine inhibition of LPL activity in postheparin plasma (r=0.98, p<0.0001). The interassay coefficient of variation for this assay was 7% for total postheparin lipolytic activity, 10% for LPL, and 7% for HL. The combination of anti-HL antibody and protamine resulted in the inhibition of >95% of the total postheparin lipolytic activity. Postheparin HL and LPL activities are expressed as micromoles of fatty acids hydrolyzed per milliliter per hour.

Statistical Methods

Data were entered into a data base designed on a VAX 70 computer for this study and transferred to an SAS data set for analysis. Distributions were examined for skewness and kurtosis. Plasma TG, postheparin LPL activity, and insulin areas were not normally distributed. Plasma TG and the insulin areas were normalized to log_{10} before performance of the parametric analyses. Pearson product-moment correlation coefficients (r) were calculated. Multiple regression with interaction correlation coefficients

Results

Subject Characteristics

The control subjects and the men with SI were of comparable age, percent body fat, BMI, WHR, and Vo2max (Table 1). As a group, they were sedentary with a mean Vo2max of 29±6 ml/kg · min⁻¹, moderately obese with a mean percent body fat of 28±5%, and had a mean WHR of 0.97±0.05, consistent with an upper-body or abdominal distribution of body fat. Fasting plasma glucose and insulin levels and their responses during the OGTT were comparable in the two groups (Figure 2). Plasma insulin levels were directly related to both percent body fat (r=0.43, p<0.001, n=65) and WHR (r=0.31, p<0.02, n=65). Similar relations were present in control subjects and men with SI.

Lipoprotein Lipids and HDL Subspecies

Total cholesterol and LDL-C levels were in the normal range and similar in the men with SI and the control subjects. The men with SI had significantly higher plasma TG levels (162±71 versus 102±39 mg/dl, p<0.001) and lower plasma levels of HDL-C (33±6 versus 37±7 mg/dl, p<0.02) than the control subjects (Table 2). The difference in HDL-C levels was due to a reduction in the HDL₃-C subfraction, as HDL₄-C levels were comparable in both groups (p<0.11). There was

Table 2. Fasting Plasma Lipoprotein Lipids

<table>
<thead>
<tr>
<th>Lipoprotein Lipid</th>
<th>Control Subjects (n=47)</th>
<th>Asymptomatic Ischemia (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG</td>
<td>102±39</td>
<td>162±71*</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>178±24</td>
<td>192±35</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>121±22</td>
<td>128±29</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>37±7</td>
<td>33±6*</td>
</tr>
<tr>
<td>HDL₃ cholesterol</td>
<td>4±3</td>
<td>2±2*</td>
</tr>
<tr>
<td>HDL₄ cholesterol</td>
<td>33±5</td>
<td>31±6</td>
</tr>
</tbody>
</table>

TG, triglyceride; LDL, low density lipoprotein; HDL, high density lipoprotein. Data are mean±SD, with lipid values in milligrams per deciliter.

* p<0.001, † p<0.05.
an inverse relation between plasma TG levels and HDL-C ($r=-0.54, p<0.001, n=65$).

To further characterize the differences in HDL levels between the two groups of men, the distribution of HDL subspecies was measured by gradient gel electrophoresis. As shown in Figure 3, there was a marked decrease of the characteristic HDL$_2$ subspecies peak in subjects with SI, with a shift in the distribution toward the HDL$_3$ subfractions. Significant inverse relations existed between percent HDL$_{3b}$ and both percent HDL$_{2b}$ ($r=-0.88, p<0.0001, n=65$) and percent HDL$_{1b}$ subclasses ($r=-0.87, p<0.001, n=65$). Although percent HDL$_{2a}$ was not different between the two groups of men (Table 3), both percent HDL$_{3a}$ and percent HDL$_{2a}$ were significantly lower ($p<0.004$) while both percent HDL$_{2b}$ and percent HDL$_{3b}$ were significantly lower ($p<0.01$) in the men with SI. Therefore, the HDL$_2$ subspecies measured by both precipitation and gradient gel electrophoresis were significantly lower in the men with SI than in the control subjects.

The relations of TG, HDL-C, and HDL$_2$ subspecies levels to WHR, percent body fat, $V_{\text{O}_{2}\text{max}}$, and plasma insulin levels were examined in the whole population and separately in the control subjects and men with SI. In the control group, abdominal obesity, hyperinsulinemia, and low $V_{\text{O}_{2}\text{max}}$ were associated with higher plasma TG and lower HDL and HDL$_2$ subspecies levels (Table 4). However, in men with SI there was no significant relation between WHR and either HDL-C or percent HDL$_{2a}$ (Figure 4). Both the slopes and the intercepts of these relations were significantly different ($p<0.01$) between the control subjects and the men with SI. The men with SI had lower HDL levels independent of WHR. Therefore, because the percent body fat, WHR, $V_{\text{O}_{2}\text{max}}$, and plasma glucose and insulin levels in the men with SI were comparable to those of control subjects, the low HDL levels in men with SI could not be explained by differences in these parameters.

**Postheparin Plasma Lipase Activities**

To determine whether abnormalities in the enzyme activities of LPL and HL could account for the group differences observed in HDL-C and HDL$_2$, and for the altered relation between HDL levels and WHR in men with SI, postheparin plasma activities of LPL and HL were measured in 41 control subjects and 12 men with SI. The physical characteristics and lipoprotein lipids of these subgroups did not differ from the larger sample.

Postheparin plasma HL activity was significantly higher in men with SI than in the control subjects (34±8 versus 27±10 $\mu$mol/ml • hr$^{-1}$; $p<0.03$). Ten of 12 men with SI had postheparin HL activity above the mean HL activity of the control subjects (Figure 5, upper panel). In the control subjects, postheparin HL activity was directly related to both WHR ($r=0.32, p<0.05, n=41$; Figure 6, upper left panel) and insulin area ($r=0.36, p<0.05, n=41$; Figure 6, lower left panel). In contrast, in the men with SI there were no significant relations between postheparin HL activity and either WHR ($r=0.01, p=\text{NS}, n=12$; Figure 6, upper right panel) or plasma insulin levels ($r=0.20, p=\text{NS}, n=12$; Figure 6, lower right panel) because HL activity was elevated in men with SI compared with control subjects of similar WHR and insulin levels. The activity of postheparin LPL did not differ between the control subjects and men with SI ($13±7$ versus $13±4$ $\mu$mol/ml • hr$^{-1}$; $p=\text{NS}$; Figure 5, lower panel). There were no significant relations between LPL activity and either WHR or insulin area in the control subjects or the patients with SI.

The relation of plasma HDL and TG levels to the postheparin plasma activities of HL and LPL was examined in the control subjects and patients with SI (Table 5). There were significant negative correlations between HDL levels and postheparin HL activity in the control subjects but not the patients with SI because of their low HDL levels and high HL activity (Figure 7). The activity of postheparin plasma LPL did not correlate with plasma TG or HDL levels in the control subjects or the patients with SI. Thus, these results suggest that men with SI have lower HDL levels than do control subjects of comparable abdominal obesity and hyperinsulinemia due to elevated HL activity.

**Discussion**

In this study, 10% of presumed healthy, normotensive, nondiabetic older men with no history of CAD had...
asymptomatic exercise-induced ST-segment depression and perfusion abnormalities on exercise thallium scintigraphy. This is consistent with the reported 2–15% prevalence of SI in middle-aged and older men in other studies.\textsuperscript{2–5} Compared with the nonischemic control subjects, the men diagnosed with SI had higher plasma TG levels, lower HDL-C and HDL\textsubscript{2} subspecies levels, and elevated postheparin HL activity despite similar degrees of abdominal obesity and plasma glucose and insulin responses during an OGTT. Furthermore, the HDL\textsubscript{2} subspecies levels were negatively correlated with postheparin plasma HL activity, WHR, and plasma insulin levels in the normal control subjects but not in the men with SI. This suggests that elevated HL activity not only reduced HDL levels but also eliminated the relations between HDL and body composition in the men with SI. These associations have not been previously reported in a select population of older men in whom SI is the only manifestation of CAD. Furthermore, these men with SI were free of comorbid diseases and were not taking medications that affect glucose and cholesterol metabolism, thereby avoiding the confounds that are often present in metabolic studies of patients with symptomatic CAD. Nevertheless, these findings warrant cautious interpretation because of the small sample size of subjects with SI and the multicollinearity among the independent variables. Thus, abdominal obesity, hyperinsulinemia, and elevated postheparin HL activity seem to reduce HDL-C and HDL\textsubscript{2} subspecies and increase TG levels in these older men and thereby increase their risk for CAD.

In this study, there was an inverse relation between HDL-C and HDL\textsubscript{2} subspecies levels with HL in the control subjects but not in the men with SI, in whom high HL activity was associated with low HDL levels. This is consistent with the results of Kuusi et al,\textsuperscript{27} who reported elevated postheparin HL activity in men with low levels of HDL-C. Similarly, Patsch et al\textsuperscript{28} and Applebaum-Bowden et al\textsuperscript{29} found an inverse relation between HDL-C levels and postheparin HL activity. In contrast, other investigators have reported reduced HL activity in individuals with dyslipoproteinemia\textsuperscript{48} or CAD.\textsuperscript{49} In a recent study, postheparin HL activity measured 24 hours after the ingestion of a high-fat meal was reported to be low in men with CAD and reduced levels of HDL-C.\textsuperscript{50} However, the existence of hypercholesterolemia in some of the patients with severe CAD and the use of medications that affect lipid metabolism may account for these disparate results. In the current study, the normocholesterolemic older men with SI received no medications at the time of study. Thus, because of the purported role of HL in the catabolism of HDL\textsubscript{2},\textsuperscript{24,25,27} the heightened postheparin HL activity seems the most likely mechanism for the reduced HDL\textsubscript{2} subspecies levels in this select population of men with SI.

Postheparin plasma HL activity is influenced by the level of percent body fat,\textsuperscript{51} plasma insulin levels,\textsuperscript{52} and androgenicity.\textsuperscript{20,53} In this study, the relation of HL activity to WHR and insulin in the normal subjects suggests that individuals with abdominal obesity and

### Table 4. Relations of Body Composition, Maximal Aerobic Capacity, and Insulin to Triglyceride, HDL Cholesterol, and HDL\textsubscript{2} Subspecies in Control Subjects and Men With Silent Myocardial Ischemia

<table>
<thead>
<tr>
<th></th>
<th>HDL cholesterol</th>
<th>Percent HDL\textsubscript{2}</th>
<th>Log TG</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Controls SI</td>
<td>Controls SI</td>
<td>Controls SI</td>
</tr>
<tr>
<td>n</td>
<td>47</td>
<td>18</td>
<td>47</td>
</tr>
<tr>
<td>Percent body fat</td>
<td>-0.44\textsuperscript{*}</td>
<td>-0.50\textsuperscript{*}</td>
<td>-0.42\textsuperscript{t}</td>
</tr>
<tr>
<td>WHR</td>
<td>-0.53\textsuperscript{t}</td>
<td>-0.08</td>
<td>-0.49\textsuperscript{t}</td>
</tr>
<tr>
<td>V\textsubscript{O}\textsubscript{max} (ml/kg FFM \cdot min\textsuperscript{-1})</td>
<td>0.40\textsuperscript{t}</td>
<td>0.05</td>
<td>-0.33\textsuperscript{*}</td>
</tr>
<tr>
<td>IRI\textsubscript{0} (munits/ml)</td>
<td>-0.32\textsuperscript{*}</td>
<td>-0.51\textsuperscript{*}</td>
<td>-0.21</td>
</tr>
<tr>
<td>Log insulin area</td>
<td>-0.44\textsuperscript{t}</td>
<td>-0.32</td>
<td>-0.37\textsuperscript{t}</td>
</tr>
</tbody>
</table>

-\textsuperscript{p}<0.05; \textsuperscript{t}p<0.01; \textsuperscript{t}p<0.001.

HDL, high density lipoprotein; SI, silent myocardial ischemia; TG, triglyceride; WHR, waist-to-hip ratio; V\textsubscript{O}\textsubscript{max}, maximal aerobic capacity; FFM, fat-free mass; IRI\textsubscript{0}, fasting plasma insulin concentration. Log insulin area is in microunits \cdot minute per milliliter during a 2-hour oral glucose tolerance test.

**Figure 4.** Scatter plots showing that the relation between percent HDL\textsubscript{2} and WHR was significant in the control subjects (r=-0.49, p<0.001; left panel) but not in men with silent myocardial ischemia (SI) (r=0.13, p=NS; right panel). HDL, high density lipoprotein; WHR, waist-to-hip ratio.
hyperinsulinemia have higher postheparin HL activity and lower levels of HDL-C. However, these relations were altered in the men with SI because they had high HL activity and low HDL-C levels despite plasma insulin levels and WHRs comparable with those of the control subjects. Furthermore, the relations of both HDL-C and HDL₃ subspecies levels to WHR differed significantly in the men with SI, suggesting that factors other than abdominal obesity and hyperinsulinemia contributed to their high HL activity and low HDL-C and HDL₃ levels. This raises the possibility that there may be a genetic basis for increased HL activity and reduced HDL levels in some of the men with SI. This is supported by studies in twins that indicate that the activity of HL is influenced by genetic factors. Family studies of the probands with SI are in progress to test this hypothesis.

Reduced plasma levels of HDL-C and HDL₃ subspecies, elevated TG, and normal LDL-C are often seen in patients with symptomatic CAD; thus, this dyslipoproteinemia is not unique to older men with SI. The HDL-C concentration was <10th percentile in >60% of patients with atherosclerotic lesions on cardiac catheterization while LDL-C was >90th percentile in only 15%. In a study of 1,000 consecutive patients undergoing cardiac catheterization, 244 of 351 patients with angiographically documented CAD had total cholesterol levels <200 mg/dl. Their mean HDL-C levels were 6 mg/dl lower than in patients without angiographically documented lesions. Case-control and prospective studies also show an association between elevated TG levels and CAD. Indeed, in our study plasma TG levels were significantly higher in the men with SI than

**Figure 5.** Upper panel: Distribution of postheparin hepatic lipase (HL) activity in control subjects and men with silent myocardial ischemia (SI). Mean activities are indicated by straight lines. Lower panel: Distribution of postheparin lipoprotein lipase (LPL) activity in the control subjects and men with SI. Controls; •, men with SI. Postheparin lipolytic activity is in micromoles free fatty acids hydrolyzed per milliliter per hour.

**Figure 6.** Scatter plots showing that postheparin hepatic lipase (HL) activity is correlated positively with waist-to-hip ratio (WHR) in controls (r=0.32, p<0.05) (upper left panel) but not in men with silent myocardial ischemia (SI) (r=0.01, p<0.99; upper right panel). In controls (lower left panel), postheparin HL activity correlated positively with the log of the insulin area (IRI) (r=0.36, p<0.05), but there was no significant relation in men with SI (r=0.20, p<0.53; lower right panel). Postheparin HL activity is in micromoles of free fatty acids per milliliter per hour; insulin area is in microunits · minute per milliliter.
in the control subjects. However, because of the strong inverse relation between TG and HDL levels, there is no consensus as to whether hypertriglyceridemia is an independent risk factor for CAD or a marker for other metabolic abnormalities that increase risk for CAD.

In summary, in this study the older men with SI had higher TG, lower HDL, and lower HDL\(_2\) subspecies levels and higher postheparin HL activity than healthy control subjects of comparable age, body composition, and \(V_{\text{max}}\) with no evidence of CAD. The cross-sectional relations suggest that abdominal obesity, physical deconditioning, hyperinsulinemia, and elevated postheparin HL activity reduce HDL and raise TG levels to increase the risk for CAD. However, in the men with SI, the relations of WHR to HDL-C and HDL\(_2\) subspecies levels were altered because of the high postheparin HL activity. Prospective intervention studies designed to raise HDL and lower TG levels through aerobic exercise, weight loss, or medications will be necessary to determine the causality of these relations. These studies are currently in progress.

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Reduced HDL2 cholesterol subspecies and elevated postheparin hepatic lipase activity in older men with abdominal obesity and asymptomatic myocardial ischemia.
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