HDLs and Alimentary Lipemia
Studies in Men With Previous Myocardial Infarction at a Young Age

Fredrik Karpe, Jean-Marie Bard, George Steiner, Lars A. Carlson, Jean-Charles Fruchart, and Anders Hamsten

The plasma concentration, particle size, and chemical composition of high density lipoproteins (HDLs) are associated with the metabolism of triglyceride-rich lipoproteins (TGRLs). During alimentary lipemia there is active exchange of lipids and apolipoproteins between HDL and apolipoprotein B-containing lipoproteins. Whereas HDL has been assigned a protective role against the development of atherosclerosis, alimentary lipemia has been proposed to represent a potentially atherogenic state. We examined plasma HDL concentration, particle size, and composition and their relations to postprandial TGRLs in 32 postinfarction patients and 10 healthy control subjects after intake of a standardized oral fat load of a mixed-meal type. All patients had undergone coronary angiographies in connection with the myocardial infarction and around 5 years thereafter. The plasma HDL cholesterol concentration decreased significantly in response to the oral fat load, particularly in hypertriglyceridemic patients, with a concomitant increase of HDL triglycerides. A limited and reversible yet consistent increase of HDL particle size (1–2%) was seen 6 hours after intake of the oral fat load on nonnondenaturing gradient gel electrophoresis (GGE) in both patients and control subjects. Virtually no changes in the plasma concentration of HDL GGE subspecies, lipoproteins containing apolipoprotein A-I but no apolipoprotein A-II (LpA-I), or lipoproteins containing both apolipoproteins A-I and A-II (LpA-I:A-II) were induced in the postprandial state despite massive increases of large very low density lipoprotein (VLDL) and large chylomicron remnant levels (determined as apolipoproteins B-100 and B-48 on sodium dodecyl sulfate–polyacrylamide gel electrophoresis). Strong inverse correlations with fasting plasma HDL cholesterol and the larger HDL GGE subspecies were found for large postprandial VLDL and large chylomicron remnants, whereas the corresponding relations for small VLDL and small chylomicron remnants were weaker. The relations of both large and small VLDL and chylomicron remnants to HDL cholesterol were confined to subjects in the lower fasting plasma HDL cholesterol range (<1.2 mmol/l). None of the HDL parameters measured, either in the fasting or in the postprandial state (HDL cholesterol, HDL triglycerides, HDL GGE subspecies, LpA-I, and Lp A-I:A-II), were related to the development of coronary atherosclerosis, whereas the postprandial plasma levels of small chylomicron remnants, which showed weak negative correlations with HDL, related positively to the progression of coronary atherosclerosis. The data suggest that the link between postprandial plasma levels of small chylomicron remnants and the progression of coronary artery disease is not accounted for by close metabolic interrelations with the HDL system, a concomitant low HDL cholesterol concentration, or inefficient reverse cholesterol transport, but is instead explained by a direct atherogenic action of the small chylomicron remnants per se. (Arteriosclerosis and Thrombosis 1993;13:11–22)

KEY WORDS • HDL subclasses • postprandial lipoproteins • alimentary lipemia • coronary atherosclerosis

Most prospective epidemiological studies have shown an independent inverse relation between the high density lipoprotein (HDL) cholesterol concentration and the risk of future coronary heart disease (CHD). The mechanisms underlying this negative association as well as the seemingly protective effect of HDL against the development of coronary atherosclerosis are not well defined. It has been hypothesized either that HDL exerts a direct protective effect by facilitating cholesterol transport from peripheral tissues, including the arterial wall, or that a low plasma HDL level merely reflects metabolic processes that are associated with the development of atherosclerosis. The processes involved in the delivery of cholesterol are not well defined. It has been hypothesized either that HDL exerts a direct protective effect by facilitating cholesterol transport from peripheral tissues, including the arterial wall, or that a low plasma HDL level merely reflects metabolic processes that are associated with the development of atherosclerosis. The processes involved in the delivery of cholesterol are not well defined. It has been hypothesized either that HDL exerts a direct protective effect by facilitating cholesterol transport from peripheral tissues, including the arterial wall, or that a low plasma HDL level merely reflects metabolic processes that are associated with the development of atherosclerosis.
least esterified cholesterol from HDL to the liver remain incompletely understood. Specific membrane-bound receptors for HDL, transfer of cholesteryl esters to apolipoprotein B–containing lipoproteins that are later taken up by the liver, or both may be involved. It is not possible, using purely statistical approaches, to separate the coronary risk effect of a low HDL cholesterol concentration from that of high plasma levels of triglyceriderich lipoproteins (TGRLs, chylomicrons, chylomicron remnants, very low density lipoproteins [VLDLs], and their remnants). This is because two such highly correlated parameters cannot be adequately separated by multiple regression analysis.

The early phase of the catabolism of TGRLs occurs at the endothelial surface through a process involving the interaction of lipoprotein lipase (LPL) and its activator, apolipoprotein C-II, which is transferred from HDL. During the lipolytic process apolipoprotein A-I and the surface components of chylomicrons are delivered to HDL. Furthermore, alimentary lipemia leads to active exchange of lipids and apolipoproteins between plasma lipoproteins, whereby HDL is thought to undergo triglyceride enrichment through the net transfer of triglycerides from apolipoprotein B–containing lipoproteins in exchange for cholesteryl ester. Subsequently, triglyceride-rich, large HDL particles are converted to smaller ones by the action of hepatic lipase (HL). Postprandial hyperlipidemia has been suggested to be a potentially atherogenic state. Interestingly, a number of studies have indicated that efficient removal of TGRLs from the plasma is associated with high plasma levels of HDL, particularly the larger HDL subspecies. Accordingly, the fractional catabolic rates of TGRLs have been shown to correlate positively with HDL cholesterol concentrations in humans. Hence, low HDL cholesterol concentrations may reflect a diminished capacity to eliminate the possibly atherogenic postprandial TGRLs from the plasma. Thus, the coronary risk associated with low HDL levels may be due to the accumulation of postprandial TGRLs and not because of the low HDL cholesterol concentration per se.

New techniques for HDL separation have enabled identification of a number of subpopulations of particles of varying size and composition. Gradient gel electrophoresis (GGE) has illustrated the particle size heterogeneity of HDL. By using immunoaffinity chromatography, Cheung and Albers have demonstrated the existence of two apolipoprotein-specific HDL populations: HDL with apolipoprotein A-I but not apolipoprotein A-II (LpA-I) and HDL with both apolipoprotein A-I and A-II (LpA-I: A-II).

The present study investigated the relations between HDL, postprandial TGRLs, and coronary atherosclerosis. Plasma levels of HDL cholesterol and triglycerides, HDL subfractions that were separated with respect to particle size and apolipoprotein composition, and TGRLs were determined before and after intake of an oral fat load. Two groups of middle-aged men with angiographically ascertained coronary atherosclerosis and either normal plasma triglyceride levels (NTG) or mild to moderate hypertriglyceridemia (HTG) were investigated along with matched control subjects.

### Methods

**Patients and Control Subjects**

A total of 32 male patients who had survived a first myocardial infarction before the age of 45 years and who had subsequently participated in an angiographic study of the mechanisms associated with the progression of coronary atherosclerosis were enrolled in the study. Patients with polygenic or monogenic hypercholesterolemia (based on repeated lipoprotein classifications showing a type IIa pattern and on studies of first-degree relatives) were excluded. Obese subjects (≥130% ideal weight), subjects with manifest diabetes mellitus (repeated fasting blood glucose values ≥7.0 mmol/l), and subjects who were taking lipid-lowering medication or who were abusing alcohol were also excluded. Because of these exclusion criteria, the coronary patient group consisted of 17 NTG subjects (14 normolipidemic subjects and three subjects with sporadic hypercholesterolemia) and 15 HTG subjects. Lipoprotein phenotyping was based on the 90th percentiles of VLDL triglyceride (1.65 mmol/l) and low density lipoprotein (LDL) cholesterol (5.35 mmol/l) values in an age- and sex-matched control population. None of the patients studied had suffered reinfarction within the preceding 6 months. Coronary artery bypass grafting had been performed on nine patients with severe exertional angina, all of whom had recovered well after the operation. More than 1 year had elapsed between bypass surgery and the present study of postprandial lipoprotein metabolism. A coronary disease–free control group comprising 10 normolipidemic men of similar age (mean age±SD, 49.2±3.2 years; range, 41–52 years) was recruited from participants in a previous population survey. They had initially been drawn from a register containing all permanent residents of Stockholm County. There was no clinical or laboratory evidence of thyroid dysfunction or other conditions that might lead to secondary hyperlipoproteinemias in any of the patients or control subjects studied.

At the time of the infarction, all coronary patients had been instructed by a dietitian regarding a diet low in fat, rich in complex carbohydrates, and with a limited intake of alcohol. The percent composition of the different sources of energy in the recommended diet was 10–15% protein, 30% fat, and the remaining energy from carbohydrates. The ratio of saturated to mono-unsaturated and polyunsaturated fat was 1:1:1. Control subjects, on the other hand, had not received any dietary advice before this study.

At the time of the second coronary angiography, a majority (69%) of the patients had developed multiple-vessel disease. Progression of coronary atherosclerosis between the angiographies had occurred in 26 patients (81%). There was no difference in lesion progression or in the severity of lesions on either angiographic investigation between NTG and HTG patients. Three quarters of the patients were treated with cardioselective β-blockers (metoprolol or atenolol) at the time of the study.

**Oral Fat Tolerance Test**

All participants were admitted early in the morning to the Clinical Research Unit for a mixed-meal type of oral fat tolerance test, which was performed within 6
months after the second coronary angiography. Participants had been fasting for 12 hours and had been asked to refrain from smoking during the fasting period and from alcohol during the preceding 3 days. The protocol for the fat tolerance test was a slight modification of that of Cohn et al. An emulsion consisting of soybean oil (Karlshamns Oils & Fats AB, Karlshamn, Sweden; 50 g/m² body surface area), glucose (50 g/m²), egg white protein (Sigma 500, 25 g/m²), dried egg yolk (Sigma 0625, 6.3 g/m²), and 200 ml water prepared with some lemon flavor (60.2% fat, 13.3% protein, and 26.5% carbohydrate by energy) was ingested within 10 minutes between 7 and 7:30 AM. The test meal was well tolerated by all subjects. Blood samples were obtained through an indwelling catheter. A fasting blood sample was taken before intake of the test meal. Subsequent blood samples were drawn hourly for the first 9 hours, and the last sample was taken 12 hours after ingestion of the emulsion. Participants were allowed to be ambulant throughout the test. Smoking was prohibited. Water but no food was allowed during the test.

**Blood Sampling**

Venous blood samples were drawn into precooled sterile tubes (Vacutainer, Becton Dickinson, Meylan Cedex, France) containing Na₂EDTA (1.4 mg/ml), which were instantly put into an ice water bath. Plasma was then recovered within 30 minutes by low-speed centrifugation (1,750g, 20 minutes, 1°C) and kept at this temperature throughout the preparation procedures. Sodium azide (1.0 mol/l), phenylmethylsulfonyl fluoride (10 mmol/l, dissolved in isopropanol), and aprotinin (10,000 KIE/ml) (Trasylol, Bayer, Leverkusen, FRG) were immediately added to the isolated plasma before fractionation of TGRLs to final concentrations of 1.0 mmol/l, 10 μmol/l, and 50 KIE/ml, respectively.

**Fractionation of Major Plasma Lipoproteins**

The major plasma lipoproteins (TGRLs [density 1.006 kg/l], LDL, and HDL) were determined by a combination of preparative ultracentrifugation and precipitation of apolipoprotein B-containing lipoproteins, followed by lipid analyses as reported on samples obtained before the test meal and at 3, 6, and 12 hours after the meal. The "LDL fraction" determined by this method is actually composed of the intermediate density plus LDL density fractions (1.006<d<1.063 kg/l).

**Determination of HDL GGE Subclasses, LpA-I, and LpA-I:A-II**

HDL GGE subclasses were determined by a modification of the nondenaturing polyacrylamide GGE method described by Blanche et al. In principle, HDL of density 1.070–1.21 kg/l was first separated from plasma by sequential ultracentrifugation. The protein content of the essentially albumin-free solution of HDL was measured according to the method of Lowry et al. GGE of isolated HDL from fasting plasma and from plasma obtained from blood samples drawn at 3, 6, and 12 hours after intake of the oral fat load was performed with polyacrylamide gels (PAA 4/30, Pharmacia, Uppsala, Sweden). All four samples from one individual were always applied to the same gel. Staining was performed with amido black, and wet gels were scanned at 570 nm in a Shimadzu CS 930 scanner (Shimadzu, Kyoto, Japan). The location of the limits of each HDL subclass on the scanning curves was derived from the relative migration of protein standards (thyroglobulin, lactate dehydrogenase, catalase, ferritin, and bovine serum albumin) of known Stokes' diameters (d) applied to each gel: HDL₁, 9.71<d<12.9; HDL₂, 8.77<d<9.71; HDL₃, 8.17<d<8.77; HDL₄, 7.76<d<8.17; and HDL₅, 7.21<d<7.76 nm. The relative distribution of HDL between the major subclasses was obtained by calculation of the area under the curve (AUC) for each subclass. To obtain measurements of HDL GGE subclass protein concentrations, the percentage of the total AUC for the HDL scanning curve accounted for by each subclass was multiplied by the total HDL protein concentration.

Shifts of the major peaks of the HDL GGE pattern after intake of the oral fat load were measured by superimposing the transparent scanning curves for each individual HDL GGE pattern at 3, 6, and 12 hours onto the fasting HDL GGE pattern (0 hours) on a light table. Alterations of the particle size of the major HDL₁, HDL₃, and HDL₅ peaks were measured in millimeters and converted to nanometers with the use of conversion factors. These factors were derived from the known sizes of the standard protein markers for each location on the gels. Since the relative size of the polyacrylamide gel was nonlinear, a 1-mm shift of the HDL₁, HDL₃, and HDL₅ peaks on the scanning curve corresponded to particle size differences amounting to 0.027, 0.020, and 0.014 nm, respectively. The HDL₅ peak was frequently located on the boundary between HDL₃ and HDL₅.

The plasma concentration of LpA-I was determined by a rocket immunoelectrophoresis technique. Fresh plasma samples at 4°C were shipped overnight from Stockholm to Lille, France, the day after the oral fat tolerance test. Briefly, the plasma was run in an agarose gel that contained antibodies against both apolipoprotein A-I and apolipoprotein A-II, with the latter in excess. Lipoprotein particles containing apolipoprotein A-II were then precipitated close to the application point, whereas LpA-I was precipitated as a rocket within the gel. The plasma concentrations of LpA-I:A-II were measured by a differential antibody immunoassay with double antibodies. In the first step, a separation of lipoprotein particles was performed on a microtiter plate covered with antibodies against apolipoprotein A-II. Plasma material that was unbound was washed away, and the remaining material was quantified after addition of peroxidase-labeled antibodies to apolipoprotein A-I. Measurement of LpA-I and LpA-I:A-II was made for 10 control subjects and 22 patients, 12 NTG and 10 HTG cases.

**Subfractionation of Sf 12–400 Lipoproteins**

TGRLs were subfractionated by cumulative rate ultracentrifugation from plasmas drawn before ingestion of the test meal and at 3, 6, and 12 hours thereafter. In principle, the plasma was adjusted to a density of 1.00 kg/l with sodium chloride. A density gradient consisting of 4 ml of 1.10 kg/l plasma and 3 ml each of 1.065, 1.020, and 1.006 kg/l saline solutions was then formed in Beckman Ultracentrifuge tubes (volume, 13.4 ml) that had been coated with polyvinyl alcohol (BDH...
Chemicals Ltd., Poole, England). Ultracentrifugation was performed in a Beckman SW40 Ti swinging-bucket rotor at 40,000 rpm and 15°C (Beckman L8-55 ultracentrifuge). Consecutive runs were made that were calculated to float particles of the following Svedberg flotation rates (Sf) to the top of the tube: Sf>400, Sf 60–400, and Sf 20–60. After each centrifugation, the top 0.5 ml of the gradient containing the respective lipoprotein subclasses was aspirated, and a 1.006 kg/l density saline solution was used to refill the tube before the next run. In addition, the Sf 12–20 fraction was finally recovered by slicing the tube 29 mm from the top after the Sf 20–60 lipoproteins had been aspirated after the last ultracentrifuge run. Isolated samples of TGRLs were immediately frozen at —80°C. The frozen samples were shipped on dry ice from Stockholm to Toronto no later than 2 weeks after they had been isolated.

All salt solutions used to prepare the density gradients were adjusted to pH 7.4 and contained 0.02% NaN₃ and 0.01% EDTA. Densities were verified to the fourth decimal place in a Paar densitometer (Graz, Austria).

Determination of Apolipoproteins B-48 and B-100

An assay that was based on the relative chromogenic activities (milliunits/ml) of apolipoproteins B-100 and B-48 of freshly isolated and processed lipoprotein samples was used for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as described by Kane et al. In principle, lipoprotein fractions containing 200–500 μg total protein were delipidated. The delipidated precipitate was dissolved overnight in sample buffer and applied to a 3.3% SDS-PAGE. Four or five rod gels were run for each sample, one for each of four or five dilutions of the delipidated lipoprotein–sample buffer preparation. A total apolipoprotein B mass of approximately 20–60 μg was applied to each gel. Gels were stained with Coomassie blue R-250 for 24 hours and then destained for 3 days in 10% acetic acid before scanning. Scanning was performed on an LKB Ultrascan laser densitometer (Pharmacia LKB Biotechnology AB) linked to an Apple IIe personal computer (Apple, Cupertino, Calif.), and the AUC for each peak was integrated automatically by the instrument with use of the LKB gelscan software package. Graphs were constructed that related the intensity of staining (AUC) for the apolipoprotein B-100 and B-48 peaks to the lipoprotein sample dilution, and the relative amounts of the two molecular variants of apolipoprotein B in the sample could then be calculated. The absolute mass of apolipoproteins B-100 and B-48 was derived from their relative quantities and the total apolipoprotein B value. The latter was quantified in all fractions of TGRLs and in intermediate density lipoproteins by electroimmunoassay after pretreatment of the samples with lipase.

Table 1. Characteristics of Patients and Control Subjects at the Time of the Study

<table>
<thead>
<tr>
<th></th>
<th>NTG patients (n=17)</th>
<th>HTG patients (n=15)</th>
<th>Control subjects (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>48.8±3.5</td>
<td>48.7±3.6</td>
<td>49.2±3.6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.8±3.8</td>
<td>27.7±3.0</td>
<td>24.5±2.8</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total plasma</td>
<td>5.80±0.86</td>
<td>6.11±0.98</td>
<td>5.58±0.55</td>
</tr>
<tr>
<td>VLDL</td>
<td>0.36±0.16</td>
<td>1.20±0.07*</td>
<td>0.27±0.18</td>
</tr>
<tr>
<td>LDL</td>
<td>4.56±0.84</td>
<td>4.22±0.71</td>
<td>4.06±0.61</td>
</tr>
<tr>
<td>HDL</td>
<td>1.08±0.35†</td>
<td>0.86±0.14‡</td>
<td>1.38±0.36</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total plasma</td>
<td>1.36±0.37</td>
<td>3.11±1.44*</td>
<td>1.09±0.40</td>
</tr>
<tr>
<td>VLDL</td>
<td>0.74±0.30</td>
<td>2.41±1.36*</td>
<td>0.56±0.25</td>
</tr>
<tr>
<td>LDL</td>
<td>0.46±0.13</td>
<td>0.42±0.07</td>
<td>0.37±0.08</td>
</tr>
<tr>
<td>HDL</td>
<td>0.12±0.05</td>
<td>0.14±0.04</td>
<td>0.11±0.05</td>
</tr>
<tr>
<td>Postheparin plasma lipase activities (milliunits/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPL</td>
<td>431±146</td>
<td>303±80§</td>
<td>483±180</td>
</tr>
<tr>
<td>HL</td>
<td>280±113</td>
<td>416±175∥</td>
<td>338±128</td>
</tr>
<tr>
<td>Oral glucose tolerance¶</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>9</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Borderline</td>
<td>6</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Decreased</td>
<td>2</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

NTG, normotriglyceridemic; HTG, hypertriglyceridemic; BMI, body mass index; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; LPL, lipoprotein lipase; HL, hepatic lipase. Values are number of subjects in groups or mean±SD.

Differences were tested by analysis of variance or χ² test. The Scheffé F test was employed to identify differences among the groups when the overall F statistic was significant.

* p<0.001 compared with NTG patients and control subjects.
† p<0.05 compared with control subjects.
‡ p<0.001 compared with control subjects.
§ p<0.05 compared with NTG patients and control subjects.
|| p<0.05 compared with NTG patients.
¶ The oral glucose tolerance test was performed and evaluated according to Reaven et al. 36
TABLE 2. HDL Subclass Concentrations

<table>
<thead>
<tr>
<th></th>
<th>NTG patients (n=17)</th>
<th>HTG patients (n=15)</th>
<th>Control subjects (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL GGE subclasses*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2b (g/l)</td>
<td>0.19±0.14</td>
<td>0.06±0.06†</td>
<td>0.25±0.17</td>
</tr>
<tr>
<td>2a (g/l)</td>
<td>0.38±0.15</td>
<td>0.22±0.08†</td>
<td>0.41±0.18</td>
</tr>
<tr>
<td>3a (g/l)</td>
<td>0.46±0.11</td>
<td>0.38±0.09‡</td>
<td>0.50±0.13</td>
</tr>
<tr>
<td>3b (g/l)</td>
<td>0.34±0.09</td>
<td>0.36±0.08</td>
<td>0.31±0.08</td>
</tr>
<tr>
<td>3c (g/l)</td>
<td>0.18±0.06</td>
<td>0.18±0.06</td>
<td>0.16±0.09</td>
</tr>
<tr>
<td>LpA-I (g/l)</td>
<td>0.38±0.11</td>
<td>0.36±0.06</td>
<td>0.50±0.14</td>
</tr>
<tr>
<td>LpA-I:A-II (g/l)</td>
<td>0.73±0.15</td>
<td>0.74±0.11</td>
<td>0.84±0.14</td>
</tr>
</tbody>
</table>

NTG, normotriglyceridemic; HTG, hypertriglyceridemic; HDL GGE subclasses, high density lipoprotein subclass determined by gradient gel electrophoresis; LpA-I, lipoproteins with apolipoprotein A-I but no apolipoprotein A-II; LpA-I:A-II, lipoproteins with both apolipoproteins A-I and A-II. Values are mean±SD.

*Nomencature according to Blanche et al.15

Group differences were identified by analysis of variance and the Scheffé F test.

†p<0.01 compared with control subjects and p<0.05 compared with NTG patients.

‡p<0.05 compared with control subjects.

In this work the apolipoprotein B-48 concentration in the Sf 20–60 and Sf 60–400 fractions is equated with the plasma level of small and large chylomicron remnants, respectively. Intestinal secretion of apolipoprotein B-48-containing lipoproteins directly into the Sf 60–400 fraction, however, cannot be entirely ruled out.

Lipid Analyses

Total cholesterol and triglyceride contents were determined in triplicate in plasma and in the major plasma lipoproteins. Lipids were first extracted with chloroform/methanol.30 Cholesterol31 and triglyceride32 contents were then determined on an Ultrolab (LKB).

Postheparin Plasma Lipase Activities

An intravenous injection of heparin (100 units/kg body wt) was given at the time of a separate visit to the clinic 1 week after the oral fat tolerance test. Venous blood for determination of postheparin plasma LPL and HL activities was drawn from the opposite arm before and 15 minutes after the heparin injection. Assay conditions for LPL (sonicated emulsion of [1H]oleic acid–labeled triolein in 20% Intralipid [Kabi Pharmacia Parenterals, Stockholm]) and HL (sonicated emulsion of [3H]-labeled triolein in gum arabic) were the same as described by Bengtsson-Olivecrona and Olivecrona.33 In the LPL assay, goat antiserum to HL (to suppress the HL activity) was added. For assay of HL, LPL was inhibited by a high salt concentration in the assay medium. Lipase activities were expressed in milliunits per milliliter, which corresponds to 1 nanomole of fatty acid released per minute per volume of postheparin plasma.

Coronary Angiography

The first and second coronary angiographies were done by the percutaneous transfemoral technique according to a standard protocol34 and recorded on 35-mm cine film with the aid of cesium-iodide–activated image intensifiers. Cine films were processed in the usual manner and were assessed by an angiographer who was blinded to the clinical characteristics and lipoprotein profiles of the patient. A semiquantitative scoring system was used to determine the presence and severity of coronary atherosclerosis in 15 proximal coronary arterial segments.34 Atherosclerotic lesions were defined as sharp edged, plaquelike, or irregularly indented (often multiple) into the vessel lumen but without features that were suggestive of fibromuscular hyperplasia. Accordingly, a single stenosis with smooth contours or a single occlusion in the absence of additional changes in the same or any other coronary artery was not classified as atherosclerosis, whereas multiple lesions always were classified as such. Segments that were distal to a significant stenosis in the absence of sufficient poststenotic contrast filling or that were distal to a total occlusion were not evaluated, nor were segments of a hypoplastic coronary artery. A global coronary atherosclerosis score was obtained by dividing the sum of all segmental atherosclerosis scores by the number of segments accessible to evaluation. Both films were simultaneously projected for purpose of comparison. Progression of coronary atherosclerosis was defined as an increase in the coronary atherosclerosis score. The detailed methodology of the coronary atherosclerosis score determination and the statistical analysis of the reliability of this system have been described elsewhere.34

Statistical Analysis

Conventional methods were used for calculation of means and standard deviations. Coefficients of skewness and kurtosis were calculated to test deviations from a normal distribution. Statistical significance for differences in continuous variables between more than two groups was tested by a one-way analysis of variance. The Scheffé F test was employed to identify differences among the groups when the overall F statistic was significant. Within-group comparisons at various time points during the test were made by Student's paired t test. The Bonferroni method was used to correct for the

TABLE 3. Relations of Fasting Plasma Concentrations of LpA-I and LpA-I:A-II to Plasma Levels of HDL Cholesterol, HDL Triglycerides, and HDL Gradient Gel Electrophoresis Subclasses

<table>
<thead>
<tr>
<th></th>
<th>HDL Cholesterol</th>
<th>HDL Triglycerides</th>
<th>HDL GGE subclasses</th>
</tr>
</thead>
<tbody>
<tr>
<td>LpA-I</td>
<td>0.62†</td>
<td>0.10</td>
<td>2b  0.60†</td>
</tr>
<tr>
<td>LpA-I:A-II</td>
<td>0.37*</td>
<td>0.24</td>
<td>2a  0.60†</td>
</tr>
</tbody>
</table>

LpA-I, lipoprotein particles containing apolipoprotein A-I and no apolipoprotein A-II; LpA-I:A-II, lipoprotein particles containing apolipoprotein A-I and apolipoprotein A-II; HDL, high density lipoprotein; GGE, gradient gel electrophoresis subclasses. Values are Spearman rank correlation coefficients.

†p<0.05, ‡p<0.001.
effect of multiple comparisons. To estimate the total amounts of lipid and lipoprotein components present in the plasma during the entire 12-hour postprandial period, areas between the curves for plasma measurements (plotted against time after the test meal) and the x axis were calculated. Associations between lipoprotein parameters were determined by calculation of Spearman rank correlation coefficients. Partial correlation coefficients were calculated between lipoprotein variables and estimates of progression of coronary atherosclerosis. Because of the varying time period (4–6.8 years) between the two coronary angiographies in individual patients and their differing ages at the time of reangiography, the time between angiograms and age, respectively, were always entered as forced variables in the regression equations.

Ethical Considerations

The experimental protocol was approved by the ethics committee of the Karolinska Hospital, Stockholm. All subjects gave their informed consent to participate in the study.

Results

Plasma Lipoproteins and Activity of Lipolytic Enzymes

Fasting cholesterol and triglyceride concentrations in the major plasma lipoprotein fractions in patients and control subjects are shown in Table 1. The VLDL lipid levels were elevated among the HTG patients, as a consequence of the criteria for patient selection. The HDL cholesterol concentration was significantly decreased in both patient groups, particularly in the HTG patients. The postheparin plasma LPL activity was significantly lower in the HTG patient group compared with both the NTG patients and the control subjects, whereas the HL activity was elevated in the HTG patient group compared with the NTG patient group.

No subject with decreased oral glucose tolerance was found in the control group, whereas two NTG and six HTG patients had decreased oral glucose tolerance. The body mass index was not significantly higher in the patient groups compared with the control group.

HDL Subclasses

HDL GGE subclass protein, LpA-I, and LpA-I:A-II concentrations in fasting plasma are given in Table 2. The most pronounced differences in the particle size distribution of HDL measured on GGE were seen in the HDL<sub>2b</sub> range in the HTG patients. In this group, the HDL<sub>2b</sub> protein concentration was only one fourth of that measured in the control group. The corresponding level of HDL<sub>2b</sub> was reduced to one half of that in the control group. In addition, the HDL<sub>3a</sub> level was lower in HTG patients compared with control subjects. The plasma concentrations of HDL<sub>3a</sub> and HDL<sub>2b</sub> were also significantly lower in HTG than in NTG patients. Fasting levels of LpA-I were reduced to the same level in the NTG and HTG patient groups. However, because of the smaller variance the reduction of LpA-I reached statistical significance only in the latter group. No major differences in fasting plasma concentrations of LpA-I:A-II were found between the groups.

Interrelations Between HDL Subclasses

Fairly strong positive correlations were noted between the plasma concentration of LpA-I and the plasma level of HDL cholesterol and the larger HDL GGE subclasses HDL<sub>2b</sub>, HDL<sub>3a</sub>, and HDL<sub>2b</sub> (Table 3). The corresponding relations for LpA-I:A-II appeared to be considerably weaker.

Postprandial Responses of HDL Subclasses

Plasma levels of HDL cholesterol and triglycerides during the fat tolerance test are shown in Figure 1. The control group did not exhibit any change in plasma HDL cholesterol concentration in the postprandial state. By contrast, in the patient group the levels were reduced.
TABLE 4. Absolute Change in Particle Diameter of Three Dominant HDL Peaks on Gradient Gel Electrophoresis at 6 Hours After Intake of the Oral Fat Load

<table>
<thead>
<tr>
<th>Study group</th>
<th>HDL&lt;sub&gt;2b&lt;/sub&gt; peak shift (nm)</th>
<th>HDL&lt;sub&gt;3b&lt;/sub&gt; peak shift (nm)</th>
<th>HDL&lt;sub&gt;3b&lt;/sub&gt; peak shift (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control subjects</td>
<td>+0.13±0.17 (+0.01 to +0.26)</td>
<td>+0.13±0.09 (+0.01 to +0.11)</td>
<td>+0.06±0.07 (+0.01 to +0.26)</td>
</tr>
<tr>
<td>NTG patients</td>
<td>+0.12±0.15 (+0.05 to +0.20)*</td>
<td>+0.11±0.09 (+0.03 to +0.09)*</td>
<td>+0.06±0.06 (+0.03 to +0.10)</td>
</tr>
<tr>
<td>HTG patients</td>
<td>+0.04±0.11 (+0.04 to +0.13)*</td>
<td>+0.09±0.08 (+0.02 to +0.09)*</td>
<td>+0.06±0.06 (+0.02 to +0.10)</td>
</tr>
</tbody>
</table>

HDL, high density lipoprotein; NTG, normotriglyceridemic; HTG, hypertriglyceridemic. Values are mean±SD. The 95th percentile confidence intervals are given within parentheses. Values had a Gaussian distribution for all HDL subclasses in all groups, with the exception of HDL<sub>2b</sub> in HTG patients. Furthermore, only five of 15 HTG patients had a distinct peak within the HDL<sub>2b</sub> particle size range. The mean diameter size of HDL peaks on the gradient gel chromatograms was 10.4 nm for HDL<sub>2b</sub>, 8.4 nm for HDL<sub>3b</sub>, and 8.0 nm for HDL<sub>3b</sub>.

*When the lower limit of the 95th percentile confidence interval of the change in peak HDL particle diameter size between the 0-hour and 6-hour values was positive, the particle size was considered to be increased at the <i>p</i> < 0.05 level.

compared with fasting concentrations at 3 and 6 hours in the HTG group and at 6 hours in NTG subjects. Plasma levels in both patient groups had returned to the fasting level by 12 hours, and the significant group differences in HDL cholesterol concentration found in fasting plasma remained in the 3-, 6-, and 12-hour postprandial samples. The HDL triglyceride concentration rose by about 30% in all three groups and peaked at 6 hours. The HDL triglycerides had returned to fasting levels at 12 hours. There were no major differences in HDL triglyceride concentration between the groups at any time point throughout the test.

No major changes in the HDL GGE subclass protein concentration occurred during alimentary lipemia (data not shown). Subtle shifts in particle size for the major HDL subclass peaks were seen when scanning curves of the 3-, 6-, and 12-hour samples were superimposed onto the scanning curve of the fasting HDL sample. The changes were discrete and not accompanied by significant alterations in HDL GGE subclass protein concentrations. The most pronounced increase in particle size for the major peaks encountered in the HDL<sub>2b</sub>, HDL<sub>3b</sub>, and HDL<sub>3b</sub> ranges was found at 6 hours (Table 4). Increases in diameter in the range of 1–2% were seen in all three study groups. There were no significant differences between the groups. Superimposed scanning curves of fasting and 6-hour samples from one representative subject from each group are shown in Figure 2.

No major alterations in the plasma levels of LpA-I and LpA-I:A-II were recorded in the postprandial state in either patients or control subjects. The lower fasting LpA-I concentration in the patients was maintained during the entire postprandial period (Figure 3).

**Relations Between HDLs and Postprandial Responses of TGRLs**

The concentrations of cholesterol and triglycerides in TGRLs during the fat tolerance test are shown in Figure 4. The TGRL triglyceride concentration in the control group had increased fourfold at 3 hours to 2.05±0.84 mmol/l and was still well above the fasting concentration at 6 hours (Figure 4, upper panel). The 3-hour postprandial triglyceride level in TGRLs in the NTG patient group reached the same level as in the control group, but the peak level was attained between 3 and 6 hours. In the HTG patient group, TGRL triglycerides rose from 2.41±1.36 mmol/l to a peak of 6.51±2.99 mmol/l at 6 hours and returned to baseline by 12 hours. Similar patterns of response were found in the three groups for TGRL cholesterol (Figure 4, lower panel).

To determine the relations between the fasting plasma concentrations of HDL cholesterol and HDL

**FIGURE 2.** Scanning curves of high density lipoprotein (HDL) gradient gel electrophoresis (GGE) in one control subject, one normotriglyceridemic patient (NTG), and one hypertriglyceridemic patient (HTG). Solid line shows the HDL particle distribution in the fasting state, whereas the broken line shows the HDL particle distribution 6 hours after intake of the oral fat load. Short, thick, vertical lines show the boundaries between the HDL GGE subclasses (2b, 2a, 3a, 3b, and 3c), and long, broken, vertical lines show the particle sizes in nanometers (nm) of the three main peaks in the fasting state.
Figure 3. Line plots showing responses of lipoproteins containing only apolipoprotein A-I (Lp A-I) (upper panel) and lipoproteins containing both apolipoproteins A-I and A-II (Lp A-I:A-II) (lower panel) to the oral fat load in the control group and in normotriglyceridemic (NTG) and hypertriglyceridemic (HTG) patient groups. Control subjects (n=10) are shown by open circles, NTG patients (n=12) by filled circles, and HTG patients (n=10) by filled squares. Levels are given as mean±SEM. No statistically significant changes in concentration were found after intake of the oral fat load. The significantly lower LpA-I concentration in fasting plasma of NTG and HTG patient groups persisted throughout the test.

Figure 4. Line plots showing responses of triglyceride-rich lipoprotein (TGRL) (Sf>20) triglycerides (upper panel) and TGRL cholesterol (lower panel) to the oral fat load in the control group and in normotriglyceridemic (NTG) and hypertriglyceridemic (HTG) patient groups. Control subjects (n=10) are shown by open circles, NTG patients (n=17) by filled circles, and HTG patients (n=15) by filled squares. Levels are given as mean±SEM. A significant increase of both TGRL triglycerides and cholesterol was seen at both 3 and 6 hours after intake of the oral fat load in all groups: *p<0.001 compared with fasting levels. Group mean differences found at baseline (elevated very low density lipoprotein triglycerides and cholesterol in the HTG patient group, p<0.001) were maintained throughout the test.
Correlations to Angiographic Scores

Inverse associations were also found between the HDL GGE subclasses HDL2b and HDL3a, and the postprandial lipoproteins of larger particle size (AUCs for both apolipoproteins B-48 and B-100 in Sf 60–400 lipoproteins), whereas the corresponding relations of LpA-I and LpA-I:A-II to postprandial lipoproteins were weaker and mostly statistically insignificant (Table 5).

It is notable that associations of similar statistical power to the ones obtained for postprandial lipoproteins of larger particle size were found between the fasting plasma VLDL triglyceride concentration and the HDL parameters. Postheparin plasma LPL activity correlated positively with fasting plasma HDL cholesterol and LpA-I concentrations, whereas postheparin HL activity was negatively correlated with HDL cholesterol, more specifically with the large HDL GGE subspecies HDL2b, and HDL2b.

Correlations to Angiographic Scores

Relations of HDL cholesterol, HDL subclasses, and postprandial concentrations of apolipoproteins B-48 and B-100 in the various lipoprotein subfractions to coronary atherosclerosis were derived from correlations with the global severity of atherosclerotic lesions, as determined from the second coronary angiogram (global atherosclerosis severity score) and the rate of lesion progression between the first and the second coronary angiograms (atherosclerosis progression score). Neither fasting plasma HDL cholesterol nor HDL GGE subclasses, LpA-I, or LpA-I:A-II correlated significantly with either of the two coronary scores. In contrast, as reported previously, a strong positive association was found between the AUC for Sf 20–60 apolipoprotein B-48 (small chylomicron remnants) and progression of coronary atherosclerosis (*r=0.51, p=0.01). Introduction of the various HDL parameters as a forced variable in the regression equation did not substantially alter the strength of the correlation between postprandial levels of small chylomicron remnants and the atherosclerosis progression score. No significant correlations with the two coronary atherosclerosis scores were found for fasting plasma VLDL or LDL cholesterol concentrations, postprandial levels of large and small VLDL (AUCs for apolipoprotein B-100 in Sf 60–400 and Sf 20–60 lipoproteins), or postprandial levels of large chylomicron remnants (AUC for apolipoprotein B-48 in Sf 60–400 lipoproteins).

Discussion

The present study dealt with alterations of HDL during alimentary lipemia and the relations between HDL composition and subclass distribution and the metabolism of postprandial TGRls. Specific determinations of apolipoproteins B-48 and B-100 in subfractions of TGRls allowed for quantification of intestinal and hepatic TGRls. Strong inverse associations were found between postprandial accumulations of large chylomicron remnants and large VLDL on the one hand and the fasting plasma concentrations of HDL cholesterol and the larger HDL particle species defined by GGE on the other. Interestingly, the corresponding relations for the smaller TGRls were weaker. Furthermore, our results indicate that the strong inverse relation between the plasma concentrations of either large chylomicron remnants or large VLDL and HDL cholesterol was confined to the lower range of HDL cholesterol concentrations, whereas no association was apparent at higher HDL cholesterol levels. Accordingly, the efficiency of the metabolism of TGRls was not the only determinant of the plasma level of HDL cholesterol or the larger HDL subspecies.

### Table 5. Relations of Fasting Plasma Concentrations of HDL Cholesterol and HDL Subclasses to Fasting Plasma VLDL Triglycerides and Postprandial Plasma Triglyceride, Chylomicron, and VLDL Remnant Levels During the Oral Fat Tolerance Test and Postheparin Plasma Lipase Activity Levels

<table>
<thead>
<tr>
<th>HDL GGE subclasses</th>
<th>HDL chol</th>
<th>2b</th>
<th>2a</th>
<th>3a</th>
<th>3b</th>
<th>3c</th>
<th>LpA-I</th>
<th>LpA-I:A-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL TG</td>
<td>-0.59†</td>
<td>-0.56‡</td>
<td>-0.58‡</td>
<td>-0.45‡</td>
<td>0.17</td>
<td>0.19</td>
<td>-0.26</td>
<td>-0.13</td>
</tr>
<tr>
<td>AUC TG</td>
<td>-0.46†</td>
<td>-0.36*</td>
<td>-0.29</td>
<td>-0.28</td>
<td>0.30</td>
<td>0.32*</td>
<td>-0.24</td>
<td>-0.28</td>
</tr>
<tr>
<td>AUC apo B-48</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sf 60–400</td>
<td>-0.60‡</td>
<td>-0.40*</td>
<td>-0.40*</td>
<td>-0.37*</td>
<td>0.25</td>
<td>0.24</td>
<td>-0.29</td>
<td>-0.27</td>
</tr>
<tr>
<td>Sf 20–60</td>
<td>-0.38*</td>
<td>0.10</td>
<td>0.01</td>
<td>0.06</td>
<td>0.16</td>
<td>0.11</td>
<td>-0.02</td>
<td>-0.18</td>
</tr>
<tr>
<td>AUC apo B-100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sf 60–400</td>
<td>-0.58‡</td>
<td>-0.54‡</td>
<td>-0.50‡</td>
<td>-0.42*</td>
<td>0.19</td>
<td>0.22</td>
<td>-0.37*</td>
<td>-0.19</td>
</tr>
<tr>
<td>Sf 20–60</td>
<td>-0.50†</td>
<td>-0.38*</td>
<td>-0.39*</td>
<td>-0.26</td>
<td>0.04</td>
<td>0.15</td>
<td>-0.20</td>
<td>-0.30</td>
</tr>
<tr>
<td>LPL</td>
<td>0.43†</td>
<td>0.18</td>
<td>0.27</td>
<td>0.19</td>
<td>-0.21</td>
<td>-0.27</td>
<td>0.43*</td>
<td>0.06</td>
</tr>
<tr>
<td>HL</td>
<td>-0.32*</td>
<td>-0.48‡</td>
<td>-0.56‡</td>
<td>-0.27</td>
<td>0.07</td>
<td>0.21</td>
<td>-0.18</td>
<td>-0.04</td>
</tr>
</tbody>
</table>

HDL, high density lipoprotein; VLDL, very low density lipoprotein; chol, cholesterol; GGE, gradient gel electrophoresis; LpA-I, lipoprotein particles containing apolipoprotein A-I and no apolipoprotein A-II; LpA-I:A-II, lipoprotein particles containing both apolipoprotein A-I and apolipoprotein A-II; TG, triglycerides; AUC, area under the curve; apo B-48, apolipoprotein B-48; Sf, Svedberg flotation unit; apo B-100, apolipoprotein B-100; LPL, postheparin plasma lipoprotein lipase activity; HL, postheparin plasma hepatic lipase activity. Values are Spearman rank correlation coefficients.

*p<0.05, †p<0.01, ‡p<0.001.
The recent studies of postprandial lipoprotein metabolism of Patsch and colleagues suggested a link between the chylomicron remnant hypothesis and the known inverse relation between HDL and CHD. According to their concept, individuals with normal fasting plasma lipids and high levels of HDL$_2$ catabolize chylomicrons and potentially atherogenic chylomicron remnants at a faster rate than do individuals with normal fasting lipid and low HDL$_2$ levels. Subsequent studies by the same group demonstrated the roles of lipases in determining HDL$_2$ levels. The hypothesis advanced by these authors now states that LPL activity may limit the magnitude of postprandial lipemia, which determines the triglyceride enrichment of HDL$_2$ that in turn influences the levels of HDL$_2$ through the action of HL. High plasma concentrations of HDL$_2$ could therefore be a result rather than a cause of an efficient plasma triglyceride transport system. The lipase activity associations with HDL cholesterol and HDL subfractions demonstrated in the present study would be consonant with this hypothesis.

An alternative interpretation that would also agree with our data is that low levels of HDL cholesterol or of the larger HDL particle species can limit the rate of lipolytic degradation of postprandial TGRLs, whereas at high levels of HDL, TGRL lipolytic degradation depends on factors other than HDL. This impact of a low HDL level might be mediated by decreased availability of C apolipoproteins in TGRLs. All three C apolipoproteins appear to modulate the apolipoprotein E-mediated hepatic uptake of TGRLs, so long as they are part of these lipoprotein particles. A relative deficiency of apolipoprotein C-II in the HDL fraction might be a limiting mechanism for lipolytic removal of postprandial TGRL triglycerides. This hypothesis is in good agreement with the results from a study by Kasyap et al. They found that the response and reciprocal changes of apolipoprotein C-II in HDL and TGRLs in healthy individuals who are given an oral fat load substantiated the notion that apolipoprotein C-II might be of regulatory importance in the removal of postprandial TGRLs. It is also noteworthy that a recent study of endurance-trained men, whose HDL cholesterol levels were well above the distribution in the general population, failed to demonstrate a relation between the fasting HDL cholesterol or its larger subspecies and the response of TGRLs after an oral fat load.
During alimentary lipemia, HDL is enriched in triglycerides and phospholipids and depleted of apolipoproteins CI–III and E.\(^4\) This causes a shift in the HDL density profile.\(^4\) In the present study a rise in HDL triglycerides amounting to about 30\% was found at the time of peak postprandial triglyceridemia in both patients and control subjects. Concomitantly, the HDL cholesterol concentration was markedly reduced in the HTG patients. By contrast, HDL was essentially uninfluenced by the oral fat load in NTG patients and control subjects. However, discrete shifts were found in the mean diameter (size) of the major HDL peaks in the postprandial state. These alterations in HDL lipid composition are in accordance with those published in previous reports.\(^4\) During alimentary lipemia, cholesteryl ester transfer is stimulated because of an increased mass of triglyceride-rich acceptor lipoproteins, a redistribution of cholesteryl ester transfer protein (CETP) to larger HDL species, an increase in total CETP activity (possibly also due to a limited increased CETP mass), and favorable compositional changes of acceptor lipoproteins.\(^4\) Accordingly, HTG subjects with low HDL cholesterol concentrations are likely to have an accelerated transfer of cholesteryl esters into TGLRs during alimentary lipemia. This chain of events, although potentially a component of “reverse cholesterol transport,” may also promote the formation of presumably atherogenic cholesteryl ester–rich remnants.

The HDL GGE subclass protein concentrations as well as the LpA-I and LpA-I:A-II levels were not substantially influenced by the oral fat load in any study group. Plasma concentrations of LpA-I and LpA-I:A-II were less closely linked to the metabolism of TGLRs than were the plasma levels of HDL cholesterol or HDL GGE subclasses. LpA-I promotes efflux of cholesterol from adipose cells,\(^4\) and the plasma LpA-I concentration has been indicated to be lower in subjects with coronary atherosclerosis.\(^3\) However, no statistically significant inverse relation between the plasma level of LpA-I and progression of coronary atherosclerosis was found in the present study.

Overall, no associations were found between either HDL cholesterol, HDL GGE subclasses, or LpA families and coronary atherosclerosis in the present study. In contrast, a strong positive relation was present between progression of coronary atherosclerosis and plasma levels of small chylomicron remnants during the fat tolerance test.\(^37\) Importantly, accumulation of large chylomicron remnants and large VLDL after the oral fat load was strongly linked to lower plasma concentrations of several of the HDL parameters that were measured in the present study, whereas the corresponding associations for the small chylomicron remnants were considerably weaker or absent. In a larger group of young postinfarction patients we have recently demonstrated an inverse relation between the plasma concentration of the largest HDL particles, the HDL\(_{2b}\) subclass, and the severity and progression of coronary lesions over time.\(^37\) Importantly, this association was exclusively accounted for by NTG patients. A negative correlation, albeit statistically insignificant, between the HDL\(_{2b}\) protein level and disease progression was also found in the group of NTG patients in the present study.

A limitation of the present study with respect to a demonstration of angiographic associations is the fairly small patient group and the retrospective design, which confers an inherent selection bias. A major proportion of the patients were on medication with cardioselective \(\beta\)-blockers. This may influence fasting and postprandial lipoprotein levels. Furthermore, we selected male patients in whom genetic hypercholesterolemias were not present. Therefore, we cannot say whether our results also extend to subjects with hypercholesterolemia and to women. These restrictions notwithstanding, the implication of our findings is that the link between postprandial plasma levels of small chylomicron remnants and progression of coronary artery disease, as described in greater detail elsewhere,\(^37\) is not accounted for by close metabolic interrelations with the HDL system, by a concomitant low HDL cholesterol concentration, or by an inefficient reverse cholesterol transport. Rather, the association with coronary artery disease is probably explained by a direct atherogenic action of the small chylomicron remnants per se.

Acknowledgment
We are indebted to Christian Landou, MD, at the Department of Thoracic Radiology, Karolinska Hospital, Stockholm, for his help with the scoring of coronary angiograms.

References
4. Patsch JR, Prasad S, Gotto AM, Bengtssoon-Olivecrona G: Postprandial lipemia, a key for the conversion of high density lipoprotein to high density lipoprotein, by hepatic lipase. J Clin Invest \(1984;74:2017-2023\)
12. Patsch JR, Prasad S, Gotto AM, Patsch W: High density lipoprotein–, relationship of the plasma levels of this lipoprotein species to its composition, to the magnitude of postprandial lipemia, and to the activities of lipoprotein lipase and hepatic lipase. J Clin Invest \(1987;80:341-347\)
22 Arteriosclerosis and Thrombosis Vol 13, No 1 January 1993


HDLs and alimentary lipemia. Studies in men with previous myocardial infarction at a young age.

F Karpe, J M Bard, G Steiner, L A Carlson, J C Fruchart and A Hamsten

doi: 10.1161/01.ATV.13.1.11

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1993 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/13/1/11

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/