Postprandial Lipoprotein Metabolism in Normolipidemic Men With and Without Coronary Artery Disease


A delayed clearance of postprandial lipoproteins from the plasma may play a role in the etiology of premature coronary atherosclerosis. To address this hypothesis, we studied chylomicron (remnant) metabolism in two groups of 20 selected normolipidemic men aged 35–65 years, a group of coronary artery disease (CAD) patients, and a matched control group with documented minimal coronary atherosclerosis. Subjects received an oral fat load supplemented with cholesterol and retinyl palmitate. Plasma samples obtained during the next 24-hour period were analyzed for total as well as \(d<1.019\) g/ml and \(d>1.019\) g/ml triacylglycerol, cholesterol, and retinyl ester concentrations. Although both groups of patients responded identically in terms of the appearance of gut-derived lipids in the plasma, CAD patients showed a marked delay in the clearance of retinyl esters as well as in the normalization of plasma triacylglycerol concentrations. Postheparin plasma hepatic lipase activity was significantly lower in the CAD group. Apolipoprotein E phenotype measurements did not reveal marked differences in frequency between both groups. The frequency distribution was not unusual in comparison with the normal Dutch population. The magnitude of the postprandial responses of triacylglycerol and retinyl esters was correlated positively with the fasting levels of plasma triacylglycerol and negatively with high density lipoprotein subtraction 2 cholesterol concentrations. These data indicate that the clearance of postprandial lipoproteins in normolipidemic CAD patients as selected in the present study is delayed as compared with that of controls without coronary atherosclerosis and suggest that postprandial lipoproteins may play a role in the etiology of their disease. (Arteriosclerosis and Thrombosis 1991;11:653–662)

Studies of experimental animals have revealed that cholesteryl ester–rich remnants of triacylglycerol-rich plasma lipoproteins may play a role in the deposition of lipids in the arterial wall. Most of these studies were performed with cholesterol-fed dogs or rabbits, and large concentrations of abnormal lipoproteins, derived from the catabolism of chylomicrons as well as hepatic very low density lipoprotein (VLDL), accumulated in these animals. High concentrations of gut- and liver-derived remnants of triacylglycerol-rich lipoprotein metabolism are also found in humans with a rare disorder of lipoprotein metabolism, familial dysbetalipoproteinemia, a disease that is associated with premature coronary atherosclerosis. It has been demonstrated that the clearance of chylomicron remnants from the plasma in these patients is seriously hampered.

In normal humans, the presence of chylomicron remnants in the plasma is associated with the postprandial phase, and plasma residence times as low as 15–30 minutes have been measured. However, there is evidence that the removal of chylomicron remnants from the plasma is a saturable process, and plasma residence times of these lipoproteins can increase substantially if larger loads of lipids are consumed. Nevertheless, marked delays in the clearance of chylomicron remnants could remain undetected when the fasting plasma of individuals is screened for lipoprotein and lipid abnormalities. Zilversmit has advocated the concept that the
postprandial phase is critical in atherogenesis. Disturbances in the removal of chylomicron remnants from plasma would expose the vascular bed more intensively to these atherogenic lipoproteins and fate these subjects for premature coronary atherosclerosis. In this process, macrophage macrophages in the arterial wall are thought to play a central role, and uptake of remnants of triacylglycerol-rich lipoproteins by these cells may lead to an intracellular accumulation of cholesteryl esters and conversion of these macrophages into foam cells. Consequently, the importance of studies on the metabolism of postprandial lipoproteins in humans has been stressed, and recently, several studies have appeared on this subject. In the present article, we report on studies of postprandial lipoprotein metabolism in two groups of selected normolipidemic patients, a group with severe coronary atherosclerosis (coronary bypass patients) and a matched control group with normal coronary arteries at angiography.

Methods

Materials

Water-miscible emulsions of retinyl palmitate (Arovit, 150,000 IU/ml) were from Roche, Basel, Switzerland. Retinol, retinyl ester standards, and other chemicals were from Sigma Chemical Co., St. Louis, Mo. Organic solvents of high-performance liquid chromatography (HPLC) grade and all other chemicals of analytical grade were obtained from Merck (Darmstadt, F.R.G.).

Patients

Coronary artery disease (CAD) patients and lesion-free controls were selected from the data base of the Cardiology Department of the Zuiderziekenhuis Rotterdam by selection criteria as described below. All patients were men between the ages of 35 and 65 years who underwent exploratory coronary angiography in the 12-month period before entry into the study. Controls underwent coronary angiography for complaints of chest pain, but the angiograms showed no or only minimal signs of coronary atherosclerosis (i.e., stenosis in major vessels <20%). The CAD group consisted of subjects suffering from severe coronary atherosclerosis (at least three major coronary vessels occluded by 70% or more) who underwent coronary artery bypass surgery in the preceding 3–9 months. All had recovered well and were fit enough to allow the cessation of medication that could affect plasma lipoprotein metabolism (e.g., β-adrenergic antagonists, mainly metoprolol [standard dose, 50 mg b.i.d., regular preparation], used by 11 CAD and three control patients, and calcium channel blockers, mainly nifedipine [standard dose, 10 mg t.i.d., regular preparation], used by one CAD and six control patients) starting 3 days before the study.

The frequency of physical activity of all subjects was recorded in a questionnaire and found to be rather low and not different between the groups (no sport activity or less than once a week, 12 CAD and 11 control patients; once a week, five CAD and five control patients; twice a week or more, three CAD and four control patients). If anything, the intensity of physical activity was higher in the CAD group, as several of these patients still participated in rehabilitation programs.

CAD and control patients were selected according to the following criteria: 1) plasma cholesterol less than 260 mg/dl*, 2) low density lipoprotein (LDL) cholesterol less than 200 mg/dl, 3) plasma triacylglycerol concentration less than 200 mg/dl, 4) normal blood pressure without medication, 5) absence of obesity, liver disease, diabetes mellitus, thyroid dysfunction, kidney disease, or gastrointestinal disorders, and 6) not smoking or only limited use of cigarettes. (Two thirds were nonsmokers, the others used only 1–15 cigarettes per day.) However, nearly all subjects had been smokers in the past but had stopped 9 months to 30 years earlier. The numbers of current smokers in the CAD and control groups were seven and four, respectively. By excluding patients with strong established risk factors for coronary atherosclerosis or disturbed plasma lipoprotein metabolism, and subsequent patient matching (see below), we aimed to exclude the confounding influence of these factors in the interpretation of our data.

CAD and control patients were matched for age (difference <5 years), LDL cholesterol concentration (difference <50 mg/100 ml), and Quetelet index (difference <3). Only if a matched pair could be formed were they entered into the study.

Patients were asked to participate after being informed in a letter about the protocol and the objectives of the study. More than 80% reacted positively. The protocol for the study was approved by the ethical committee of the Zuiderziekenhuis Rotterdam.

Protocol

Patients were hospitalized at the end of the afternoon of the day preceding the study. In each session, two subjects, a CAD patient and his matched control, were studied. After a 6 PM evening meal, patients were fasted overnight. At 8 AM the next morning, blood samples were taken for various measurements (see below), and patients received a liquid fat load consisting of dairy cream (40% fat) supplemented with egg yolk and retinyl palmitate. This mixture was made into a milk shake after addition of a small amount of milk powder. Each patient received a fat load adjusted to his body composition (77.5 g fat, 0.5 g cholesterol, and 27,000 IU retinyl palmitate per square meter of body surface area). The fat load was consumed during a period of 15 minutes, and patients were deprived of any source of energy for the

*The present study started in 1984. According to current concepts, the cutoff for "normal" (desired) plasma concentration of cholesterol for men aged 40–65 years is considered to be 200 mg/dl.
next 24 hours. Blood samples were taken during this period at 2, 4, 6, 8, 10, 12, 14, and 24 hours after consumption of the fat load. The fat load was well tolerated in all subjects. When asked, no one complained about gastrointestinal problems or steatorrhea. After 24 hours, patients received an intravenous injection of heparin (100 IU/kg body wt). Twenty minutes later, a blood sample was collected for postheparin plasma isolation, and patients received an intravenous injection of protamine sulfate (1 mg/kg body wt) to restore hemostasis.

**Analytical Methods**

**Lipoprotein fractionation.** Lipoproteins were fractionated from fasting serum samples (0.5 ml) by isopycnic density gradient ultracentrifugation essentially as described by Redgrave et al. Lipoprotein cholesterol profiles were obtained during elution of the gradients with an ISCO gradient fractionator (model 184, ISCO, Lincoln, Neb.) coupled to a Technicon autoanalyzer II, using specifications essentially as described by Boehringer for high density lipoprotein (HDL) cholesterol measurements on this instrument (Technicon autoanalyzer II, Boehringer-Mannheim, Mannheim, F.R.G., HDL cholesterol, cholesterol oxidase/phenol/4-amino-phenazone/per-oxidase [CHOD-PAP] method, July 1983 edition, No. 783-6718-1217.1). This procedure resulted in total separation of VLDL, LDL, and HDL cholesterol peaks. Serum lipoprotein cholesterol concentrations were calculated by planimetry using sets of standard sera run before and after each gradient. Recoveries of cholesterol in this procedure were 100±8.6% (mean±SD).

HDL cholesterol subclass distributions were determined in a similar setup after fractionation of serum by rate zonal density gradient ultracentrifugation in an SW 40 rotor (Beckman Instruments, Palo Alto, Calif.) essentially as described previously. Cholesterol profiles in these gradients normally showed two HDL peaks and a large VLDL plus LDL peak “floating” on top of the gradient. A “shoulder” was often present on the “heavy” edge of the HDL3 peak and was designated HDL3b. Contributions of HDL2, HDL3 (“light” HDL3, the main HDL peak), and HDL3b were calculated by a newly developed deconvolution program run on an Olivetti M242 computer. Reproducible HDL subclass distributions could be calculated this way, and the resulting three-component profiles matched perfectly with measured cholesterol profiles after superimposition. Copies of the software can be obtained from the authors (P.H.E.G.).

Postprandial plasmas were fractionated in a VLDL plus intermediate density lipoprotein (IDL) fraction and an LDL plus HDL fraction by ultracentrifugation at d=1.019 g/ml and 4°C with a 40.3 rotor (Beckman), essentially as described earlier. Plasma was kept at 0–4°C to inhibit lipid transfer activity during the ultracentrifugal procedure that could otherwise affect cholesteryl ester, retinyl ester, and triacylglycerol distributions.

**Apolipoprotein quantification and phenotyping.** Apolipoproteins A-I, A-II, and B were measured in samples of fasting serum by radial immunodiffusion as described earlier. Methods were standardized to give absolute protein values using pure apolipoprotein and LDL standards. Apolipoprotein E was also quantified by radial immunodiffusion using a pool of human sera as a standard. Consequently, for apolipoprotein E, data are expressed in relative units. Apolipoprotein E phenotyping was performed by isoelectric focusing of delipidated plasma, followed by immunoblotting, using apolipoprotein E antisem as first antibodies, exactly as described by Havekes et al.

**Retinyl ester quantification.** Retinyl esters were determined in plasma and in the d<1.019 g/ml (VLDL plus IDL) and d>1.019 g/ml (LDL plus HDL) plasma fractions. Plasma samples for analyses were prepared at temperatures between 0°C and 4°C, shielded from light, and kept under N2 at −20°C until analysis (usually within 1 week). Lipid extractions were performed at temperatures below 4°C and were shielded from light. Plasma (0.8 ml), VLDL plus IDL fractions (0.8 ml), or LDL plus HDL fractions (1.6 ml) were extracted with a chloroform/methanol mixture containing 0.05% butylated hydroxytoluene according to Bligh and Dyer after addition of an internal retinyl acetate standard (16 μg). The chloroform phase of the extraction was removed, and 1.5 ml was dried under a stream of N2. Lipid residues were solubilized in 0.75 ml of a mixture of methanol and chloroform (3:1, vol/vol). Retinyl esters were separated and quantified by reverse-phase HPLC using a Radial-PAK C18 analytical column (Waters, Taunton, Mass.) equipped with a guard column (Guard-Pak precolumn RCSS C18) under isocratic conditions with a mixture of ethyl acetate and methanol (3:7, vol/vol) as the mobile phase (flow rate, 2 ml/min), essentially as described by Knook and de Leeuw. Retinol and the acetyl, lauryl, myristyl, palmityl, stearyl, oleyl, and linoleyl esters of retinol could be separated this way. The retinyl palmitate peak was by far the most prominent (∼70% of the long-chain retinyl ester mass), and its relative contribution did not vary between patients and over time after fat ingestion. Therefore, we routinely measured this peak and calculated plasma concentrations using the internal retinyl acetate standard. Interassay variation was less than 2%, and plasma concentrations as low as 50 ng/ml could be measured accurately.

**Postheparin lipases and cholesteryl ester transfer activity measurements.** The activities of postheparin plasma lipoprotein lipase and hepatic lipase were determined as described previously. The amount of active cholesteryl ester transfer protein (CETP) was determined in samples of plasma delipidated by lipoprotein precipitation with dextran sulfate/MnCl2, by use of carbon-14–labeled cholesteryl oleate HDL as the donor and human LDL as the acceptor. The
assay contained human HDL (3 μg cholesteryl ester, 18,900 cpm), human LDL (150 μg cholesteryl ester), 25 mM sodium phosphate buffer, pH=7.4, 2 mM 5,5'-dithiobis-(2-nitrobenzoic acid), 150 mM NaCl, and 25 μl of delipidated plasma samples in a total volume of 250 μl. The assay mixture was incubated at 37°C for 4 hours, and radioactivity transferred to LDL was determined after LDL precipitation by dextran sulfate/MgCl₂. Blanks in the assay contained 25 μl delipidated porcine plasma, a species lacking CETP activity. Samples were run in triplicate. Under the conditions employed, the transfer of radioactivity was linear over time. An evaluation of the assay has been published earlier.²⁹

Data analysis. The mean and SEM of plasma parameters were calculated. As some of these parameters showed a non-Gaussian distribution, comparisons between CAD and control patients were performed by the Mann–Whitney U test. To evaluate changes in the distribution of lipids between the d<1.019 g/ml and d>1.019 g/ml plasma density fractions before and after an oral lipid load, a Wilcoxon signed-rank test was used. Possible associations between several parameters were determined using linear regression as well as by Spearman’s rank correlation analysis. To quantify the magnitude of the postprandial responses in triacylglycerol and retinyl esters, the surface areas of those parts underneath the response peaks, defined by the connecting line between data points and a line originating at the t=0-hour value parallel to the * axis, were calculated for each person for several time intervals after ingestion of the lipid load. The resulting data sets were analyzed by the Mann–Whitney U test. Analyses were performed on a Digital VAX computer using BMDP or RS/E statistical packages.

Results

Characterization of Lipoprotein Parameters

Lipid and lipoprotein concentrations, determined in the plasmas of CAD patients and controls after an overnight fast, are given in Table 1. As a consequence of the selection protocol, plasma concentrations of total and LDL cholesterol are nearly identical in both groups of patients, as were age and Quetelet index. Plasma triacylglycerol concentrations were slightly higher in the CAD group, but this difference was not statistically significant. Larger differences between groups were found in HDL cholesterol concentrations, being statistically significantly lower in CAD patients for total HDL cholesterol and HDL₃ cholesterol. The lower levels of HDL in this group are also reflected by the lower concentrations in plasma of apolipoproteins A-I and A-II. Apolipoprotein B and apolipoprotein E levels in plasma were similar in both groups. As chylomicron remnant uptake by the liver is known to be delayed in individuals with the apolipoprotein E2/E2 phenotype,¹⁰ ¹⁹ all patients were phenotyped for apolipoprotein E. The data for these

![Table 1. Lipid and Lipoprotein Parameters in Plasma of Coronary Artery Disease Patients and Controls After 14 Hours' Fasting](http://atvb.ahajournals.org/)

<table>
<thead>
<tr>
<th>Parameter measured (mg/dl)*</th>
<th>CAD patients</th>
<th>Control patients</th>
<th>p t</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>220±8</td>
<td>215±6</td>
<td>NS</td>
</tr>
<tr>
<td>Triacylglycerol</td>
<td>145±8</td>
<td>126±8</td>
<td>NS</td>
</tr>
<tr>
<td>VLDL cholesterol</td>
<td>26±3</td>
<td>23±4</td>
<td>NS</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>158±8</td>
<td>152±6</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>33±2</td>
<td>43±2</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>HDL₃ cholesterol</td>
<td>4.0±0.5</td>
<td>5.4±0.7</td>
<td>NS</td>
</tr>
<tr>
<td>HDL₄₉ cholesterol</td>
<td>24±1</td>
<td>31±2</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Apolipoprotein A-I</td>
<td>129±3</td>
<td>154±5</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Apolipoprotein A-II</td>
<td>51±2</td>
<td>59±2</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Apolipoprotein B</td>
<td>115±6</td>
<td>107±4</td>
<td>NS</td>
</tr>
<tr>
<td>Apolipoprotein E₂</td>
<td>124±9</td>
<td>126±5</td>
<td>NS</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>52±1</td>
<td>51±1</td>
<td>NS</td>
</tr>
<tr>
<td>Quetelet index (kg/m²)</td>
<td>25±0.6</td>
<td>25±0.4</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean±SEM. CAD, coronary artery disease; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; NS, not significant.

*All units are in mg/dl except for age and Quetelet index.
†Probability value of CAD versus control patients by Mann–Whitney U test.
‡Concentration in percent of standard serum.

analyses are given in Table 2. Apolipoprotein E2 alleles were present in two CAD and four control patients, but only one of these CAD patients was found to be of the E2/E2 phenotype. Although groups were small, the observed frequencies are not very much different from those found in a normal Dutch population (see Reference 30).

Postprandial Triacylglycerol and Retinyl Ester Responses

CAD and control patients received the oral fat load at 8:30 AM. None of them complained about gastrointestinal problems or steatorrhea. During the day, patients moved freely through the ward and spent only limited time in bed. Complaints about hunger usually started in the beginning of the evening, but none of the patients had problems finishing the study. Results of the retinyl ester, cholesterol, and triacylglycerol analyses in the d<1.019 g/ml and d>1.019 g/ml fractions obtained by ultracentrifugation are shown in Figures 1–3. In response to the fat load, plasma triacylglycerol and retinyl ester concentrations rose sharply due to in-

![Table 2. Apolipoprotein E Phenotype of Coronary Artery Disease Patients and Controls](http://atvb.ahajournals.org/)

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>CAD patients (n)</th>
<th>Control patients (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E3/E3</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>E3/E4</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>E3/E2</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>E2/E2</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

CAD, coronary artery disease.
Peak levels were reached after about 6 hours (Figures 1 and 2), and no differences were found between the CAD and control patients in this ascending phase after ingestion of the fat load. In the time period between 6 and 12 hours after fat ingestion, plasma triacylglycerol concentrations returned to baseline, while retinyl esters showed a somewhat slower clearance from plasma. The magnitude of the triacylglycerol and retinyl ester responses were calculated for different time intervals as indicated in “Methods,” and data are given in Tables 3 and 4. For all time intervals calculated, CAD patients showed larger responses to the oral fat load than did controls, the difference being particularly evident for the retinyl ester responses.

Plasma cholesterol concentrations were only minimally affected by consumption of the (cholesterol-rich) fat load (data not shown). Supposedly, as a consequence of increased concentration of acceptor lipoproteins (chylomicrons) and cholesteryl ester transfer activity, the cholesterol content of the $d>1.019 \text{ g/ml}$ fraction decreases (Figure 3) while its triacylglycerol content increases (Figure 1). It is interesting to note that the triacylglycerol associated with the $d>1.019 \text{ g/ml}$ fraction was higher in the CAD patient group than in controls, both before and after ingestion of the fat load (Figure 1). Cholesterol associated with the $d<1.019 \text{ g/ml}$ fraction increased during the first 6 hours after ingestion of the fat load (Figure 3). No differences were evident between CAD and control patients in this phase. Eight hours after fat ingestion, cholesterol distributions started to return to prefeeding values. The profiles in Figure 3 indicate that this process takes more time in the CAD than in the control patient group. Cholesterol ester transfer activities in delipidated samples of plasmas of all CAD and control patients were determined in the fasted plasmas using an assay with $[^{14}C]$cholesteryl ester labeled donor HDL and unlabeled acceptor LDL. In this assay, which measures the amount of active CETP protein, no difference was observed between the two groups (145+2 versus 143+2 ng cholesteryl ester/hour/mg delipidated plasma protein, mean±SEM).

**Measurement of Postheparin Plasma Lipase Activities**

To investigate whether lipoprotein lipase and hepatic lipase activities are related to the observed difference in handling of plasma lipoproteins in the postprandial phase, patients received an intravenous...
Statistically significant positive associations were found between the level of fasting triacylglycerol and the magnitude of the triacylglycerol and retinyl ester responses in plasma (linear regression correlation coefficients as well as Spearman rank correlation coefficients between 0.34 and 0.40) but not with HDL₃₅ or HDL₃₇ cholesterol. No statistically significant associations were found between the magnitude of the triacylglycerol or retinyl ester responses over any of the time periods and activities of lipoprotein lipase or hepatic lipase, neither after linear regression nor after rank correlation analysis.

Discussion

It has been repeatedly suggested that a delayed clearance of postprandial lipoproteins from plasma may stimulate atherogenesis, but whether an abnormally high postprandial hyperlipemia is a risk factor is not well established. The present study was aimed at investigating the validity of these suggestions.

To overcome complications in interpretation of results due to the presence of multiple risk factors, stringent selection and entry criteria were used for patients, thereby allowing us to investigate postprandial plasma lipoprotein metabolism in groups of normolipidemic CAD and control patients with similar plasma total and LDL cholesterol concentration, Quetelet index, and age distributions. Bias toward history of medication was difficult to avoid. β-Adrenergic blockers (mainly metoprolol) were used by 11 CAD and three control patients, while calcium channel blockers (mainly nifedipine) were used by one CAD and six control patients. Metoprolol administration in humans has, in some studies, been associated with a mild hypertriglyceridemia and HDL-lowering effects, and although the use of this medication was stopped 3 days before the study, some effects of history of medication on the outcome of our study is at present difficult to exclude.

Challenging CAD and control patients with an oral fat load resulted in a marked and nearly identical increase of plasma triacylglycerol and retinyl ester concentrations in both groups due to the appearance of chylomicrons in plasma. The most significant finding in the present study is that the triacylglycerol and especially the retinyl ester concentrations in the plasma of CAD patients remained elevated for a prolonged period as compared with that of control patients, suggesting a delay in the clearance of postprandial lipoproteins. As postheparin lipoprotein lipase activities were not different between the two groups, a slower rate of chylomicron remnant removal could explain these findings. Differences in gastric emptying after the lipid load or efficiency of lipid absorption could also affect postprandial responses, but because we excluded patients with gastrointestinal disorders and had no indications for lipid malabsorption during our studies, an explanation of our findings at that level seems unlikely.

During the postprandial phase, transfer of cholesterol esters from HDL to triacylglycerol-rich lipoproteins and transport of triacylglycerol in the reverse direction has been found to be increased. It is of interest to note that in CAD patients, the triacylglycerol concentration associated with the d>1.019 g/ml fraction is increased compared with controls, both before as well as during the oral lipid load test.
(Figure 1). Triacylglycerol concentrations associated with the \(d<1.019\) g/ml fractions before ingestion of the fat load were not different between the two groups (Figure 1). Speculating on the mechanism of these findings, it seems possible that the prolonged circulation of postprandial (triacylglycerol donor) lipoproteins favors transfer of triacylglycerol to the \(d>1.019\) g/ml lipoproteins, thereby explaining these observations. Cholesterol concentrations associated with the \(d>1.019\) g/ml fractions decreased during the postprandial phase (Figure 3), supposedly due to transfer of cholesterol ester from \(d>1.019\) g/ml to postprandial lipoproteins as described earlier by others.\(^3\)

The decrease in the \(d>1.019\) g/ml cholesterol is slightly larger in the CAD patient group between 10 and 24 hours after the lipid load, in line with the previously mentioned concept of prolonged acceptor availability in CAD patients. CETP activity in delipidated, fasted plasma samples was found not to differ between CAD and control patients.

In the present study, postprandial lipoprotein metabolism was studied in normolipidemic CAD patients. A delayed removal of postprandial lipoproteins from plasma was described earlier by several investigators in patients with dyslipoproteinemia (type III) and endogenous hypertriglyceridemia (type IV).\(^9\),\(^10\),\(^17\),\(^18\) and was suggested to play a role in their premature atherosclerosis. Chylomicrons were found to accumulate in type IV and chylomicron remnants in type III hyperlipoproteinemic plasmas.\(^18\) Cortner et al\(^10\) reported recently that the delayed clearance of postprandial lipoproteins in type IV patients is secondary to overproduction of VLDL particles, whose remnants compete with chylomicron remnants for removal by the hepatic chylomicron remnant receptors. In this study and a second one by Brenninkmee-

### Table 3. Plasma and \(d<1.019\) g/ml Triacylglycerol Responses After Ingestion of Oral Fat Load

<table>
<thead>
<tr>
<th>Response interval (hr)</th>
<th>CAD patients (g/[l×hr])</th>
<th>Control patients (g/[l×hr])</th>
<th>(p^*)</th>
<th>CAD patients (g/[l×hr])</th>
<th>Control patients (g/[l×hr])</th>
<th>(p^*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–24</td>
<td>17.1±2.1</td>
<td>14.5±2.5</td>
<td>(p&gt;0.05)</td>
<td>15.1±1.9</td>
<td>12.1±1.9</td>
<td>(p&gt;0.05)</td>
</tr>
<tr>
<td>6–24</td>
<td>13.8</td>
<td>12.1</td>
<td>(p&gt;0.05)</td>
<td>11.6</td>
<td>10.0</td>
<td>(p&gt;0.05)</td>
</tr>
<tr>
<td>8–24</td>
<td>7.6±1.1</td>
<td>4.9±1.3</td>
<td>(p&lt;0.05)</td>
<td>6.4±1.0</td>
<td>3.9±1.0</td>
<td>(p&lt;0.05)</td>
</tr>
<tr>
<td>10–24</td>
<td>6.7</td>
<td>2.6</td>
<td>(p&lt;0.01)</td>
<td>4.6</td>
<td>2.0</td>
<td>(p&lt;0.01)</td>
</tr>
<tr>
<td>12–24</td>
<td>2.8</td>
<td>0.7±0.2</td>
<td>(p&lt;0.05)</td>
<td>2.1</td>
<td>0.2</td>
<td>(p&lt;0.01)</td>
</tr>
<tr>
<td>14–24</td>
<td>2.8</td>
<td>0.2</td>
<td>(p&lt;0.01)</td>
<td>1.7</td>
<td>0.1</td>
<td>(p&lt;0.01)</td>
</tr>
</tbody>
</table>

*Plus-minus values are mean±SEM; single values are median. Magnitudes of the triacylglycerol responses were calculated as described in "Methods." CAD, coronary artery disease.

### Table 4. Plasma and \(d<1.019\) g/ml Retinyl Palmitate Responses After Ingestion of Oral Fat Load

<table>
<thead>
<tr>
<th>Response interval (hr)</th>
<th>CAD patients (mg/[l×hr])</th>
<th>Control patients (mg/[l×hr])</th>
<th>(p^*)</th>
<th>CAD patients (mg/[l×hr])</th>
<th>Control patients (mg/[l×hr])</th>
<th>(p^*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–24</td>
<td>18.7±2</td>
<td>14.0±1.6</td>
<td>(p&gt;0.05)</td>
<td>14.7±1.9</td>
<td>10.7±1.5</td>
<td>(p&gt;0.05)</td>
</tr>
<tr>
<td>6–24</td>
<td>17</td>
<td>11.5</td>
<td>(p&gt;0.05)</td>
<td>12.4</td>
<td>9.3</td>
<td>(p&gt;0.05)</td>
</tr>
<tr>
<td>8–24</td>
<td>13.3±1.6</td>
<td>8.7±1.1</td>
<td>(p&lt;0.05)</td>
<td>10.0±1.5</td>
<td>6.2±1.1</td>
<td>(p&lt;0.05)</td>
</tr>
<tr>
<td>10–24</td>
<td>12.4</td>
<td>7.7</td>
<td>(p&lt;0.05)</td>
<td>8.6</td>
<td>4.8</td>
<td>(p&lt;0.05)</td>
</tr>
<tr>
<td>12–24</td>
<td>9.2±1.3</td>
<td>5.4±0.7</td>
<td>(p&lt;0.01)</td>
<td>5.5±0.7</td>
<td>2.5±0.7</td>
<td>(p&lt;0.01)</td>
</tr>
<tr>
<td>14–24</td>
<td>8.2</td>
<td>4.8</td>
<td>(p&lt;0.01)</td>
<td>5.4</td>
<td>2.5</td>
<td>(p&lt;0.01)</td>
</tr>
<tr>
<td>16–24</td>
<td>6.1±0.9</td>
<td>3.5±0.4</td>
<td>(p&lt;0.01)</td>
<td>3.8±0.8</td>
<td>1.8±0.3</td>
<td>(p&lt;0.01)</td>
</tr>
<tr>
<td>18–24</td>
<td>5.1</td>
<td>3</td>
<td>(p&lt;0.01)</td>
<td>2.9</td>
<td>1.5</td>
<td>(p&lt;0.01)</td>
</tr>
<tr>
<td>20–24</td>
<td>4.3±0.6</td>
<td>2.5±0.2</td>
<td>(p&lt;0.01)</td>
<td>2.4±0.5</td>
<td>1.2±0.2</td>
<td>(p&lt;0.01)</td>
</tr>
<tr>
<td>22–24</td>
<td>3.7</td>
<td>2.2</td>
<td>(p&lt;0.01)</td>
<td>1.7</td>
<td>1.0</td>
<td>(p&lt;0.01)</td>
</tr>
<tr>
<td>24–24</td>
<td>3.2±0.4</td>
<td>1.9±0.2</td>
<td>(p&lt;0.01)</td>
<td>1.7±0.4</td>
<td>0.9±0.2</td>
<td>(p&lt;0.01)</td>
</tr>
</tbody>
</table>

*Plus-minus values are mean±SEM; single values are median. Magnitude of retinyl palmitate responses was calculated as described in "Methods." CAD, coronary artery disease.

*Probability value of CAD versus control patients by Mann–Whitney \(U\) test.
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with low levels of plasma HDL and that subjects with low levels of plasma HDL or HDL show a marked postprandial hypertriglyceridemia. HDL levels of HDL may reflect an efficient handling of reverse cholesterol transport, or alternatively, high HDL may be directly implicated in counteracting atherosclerosis. In the present study, it was found that plasma HDL parameters were lower in CAD patients as compared with controls. Low levels of plasma HDL have been associated with HDL, postprandial responses, and CAD is not still very well defined. Therefore, our data and those of others on postprandial plasma lipoprotein metabolism indicate that chylomicron remnants could be implicated in atherogenesis but do not prove a direct causal relation.

Of interest is the present finding that activities of hepatic lipase are lower in CAD patients than in controls. Low hepatic lipase activities have been found earlier in groups of normolipidemic coronary angiography patients. Furthermore, it was found recently in the dietary Leiden Intervention Trial that hepatic lipase activities were significantly lower in the patient group that showed progression of atherosclerosis as compared with the group that showed no progression. Hepatic lipase activity has been associated with HDL and HDL cholesterol uptake by the liver, as well as chylomicron remnant and LDL catabolism. No statistically significant correlation was found in our present data between HDL and hepatic lipase activities. However, HDL cholesterol concentrations in our patients were low compared with those of normal healthy men analyzed by the same method (compare References 21 and 46), and the clustering of data points at the lower end of the normal HDL concentration range may explain our inability to find such an inverse relation.

Although postprandial lipoprotein clearance in the CAD group was delayed and hepatic lipase activities were low, no statistically significant association between retinyl ester responses (total or between 8 and 24 hours) and hepatic lipase activities could be demonstrated. This finding makes it difficult to speculate that low levels of hepatic lipase in CAD patients are implicated in the delayed clearance of postprandial lipoproteins in these patients. Other factors, that is, the activity of hepatic chylomicron remnant receptors and/or the apolipoprotein E and C content of chylomicron remnants, may play a more prominent role in this process. The identification of these factors will be critical in understanding disturbed postprandial lipoprotein metabolism and its implications for atherogenesis.

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**KEY WORDS** • chylomicrons • chylomicron remnants • postprandial lipoproteins • oral fat load • coronary artery disease
Postprandial lipoprotein metabolism in normolipidemic men with and without coronary artery disease.

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