Dissociation Between Postprandial Lipemia and High Density Lipoprotein Cholesterol Concentrations in Endurance-Trained Men

Jonathan C. Cohen, James Stray-Gundersen, and Scott M. Grundy

Previous studies have indicated an inverse relation between circulating high density lipoprotein (HDL) concentrations and the rate of chylomicron clearance. Because chronic exercise has been shown to augment chylomicron clearance, we measured HDL cholesterol concentrations and plasma triglyceride and retinyl palmitate responses to high- (140 g) and low- (50 g) fat meals in endurance-trained men. Plasma HDL cholesterol concentrations in these men ranged from 36 to 105 mg/dl. Intraindividual variation in the cholesterol concentration of the HDLs occurred primarily in HDL2. The magnitude of postprandial lipemia induced by both the high- and the low-fat meals was uniformly low compared with values reported previously for sedentary men and was not correlated with HDL cholesterol concentrations. Postprandial retinyl palmitate concentrations, which reflect chylomicron remnant metabolism, also showed no correlation with HDL cholesterol concentrations. These data indicate that the degree of postprandial lipemia is not the primary determinant of HDL cholesterol concentrations in endurance-trained men. Accordingly, the wide range of HDL cholesterol concentrations measured in these men must be attributable to other factors. (Arteriosclerosis and Thrombosis 1991;11:838–843)

Several epidemiological studies have indicated that plasma high density lipoprotein (HDL) cholesterol concentrations are negatively correlated with coronary heart disease (see Reference 1 for review). Despite extensive investigation, however, the mechanism(s) underlying this link have not been elucidated. One possibility is that HDL attenuates the development of atherosclerosis directly, perhaps by facilitating reverse cholesterol transport. Alternatively, some authors have suggested that high concentrations of HDL are not intrinsically antiatherogenic but instead reflect a metabolic process that independently confers protection from atherosclerosis. Patsch et al proposed that HDL concentrations are largely determined by postprandial plasma triglyceride concentrations. Consequently, individuals with low HDL concentrations may be susceptible to coronary atherosclerosis because of their diminished capacity to remove triglyceride-rich lipoproteins from the circulation and not because of a deficiency of HDL per se.

If interindividual variations in plasma HDL concentrations are determined primarily by variations in postprandial plasma triglyceride concentrations, then interventions that augment the clearance of triglycerides would be expected to cause concomitant increases in plasma HDL concentrations. Evidence to support this concept comes from the observation that triglyceride-lowering drugs often raise HDL cholesterol levels and from the finding that exercise training tends to lower triglyceride concentrations and to raise HDL.

Endurance exercise adaptation provides a useful paradigm for examining the interaction between HDL and chylomicron metabolism because chronic exercise diminishes postprandial lipemia and increases the rate of clearance of chylomicrons. Accordingly, the present study was undertaken to investigate the relation between total HDL (HDL2) cholesterol and HDL2 cholesterol concentrations and postprandial lipemia in endurance-trained men. In this report, we present the results of fat tolerance tests performed in 52 healthy men who were in regular training at the time of the study and whose
HDL₃ cholesterol concentrations spanned a 20-fold range. Two of these men were also studied during periods of abstinence from vigorous exercise.

Methods
The procedures used in this study were approved by the Institutional Review Board of the University of Texas Southwestern Medical Center at Dallas and the Ethics and Research Committee of the University of Cape Town, South Africa. All procedures were performed on an outpatient basis on men who were following self-selected diets. The subjects were requested to abstain from alcohol (for 72 hours) and from vigorous exercise, food, and beverages except water (for 12 hours) before each test.

Subjects
Fifty-two normolipidemic men aged between 21 and 66 years volunteered for this study. Twenty-two of the men (group A) were studied in Cape Town, and the other 30 men (group B) were studied in Dallas. None of the men were obese, none smoked or used any medication known to affect lipoprotein metabolism, and all were in regular exercise training, running at least 32 km per week, for the 3 months preceding the study. Two of the men were restudied after 2 months of abstinence from exercise training. The height and weight of each subject were determined by standard methods. The percentage body fat of subjects in group B was determined from three skinfold measurements as described by Jackson and Pollock. The dietary intakes of men in group B were assessed from 3-day diet records analyzed by use of a commercial computer program (COMPUTRITION, Computrition Inc., Chatsworth, Calif.).

Procedures
Fat tolerance tests were performed after an overnight fast. Men in group A were tested by the procedure described by Patsch et al. Each consumed a meal comprising 175 ml heavy whipping cream (70 g fat) and 2.5 g chocolate syrup per square meter of body surface area. Blood samples were drawn before and at 2-hour intervals for 8 hours after the test.

To test a more physiological fat load, the men in group B were given a meal containing 120 ml heavy whipping cream (50 g fat), 5 g chocolate powder, and 120 ml water. Vitamin A (50,000 units; Aquasol, Armour Pharmaceutical, Kankakee, Ill.) was added to the meal to label chylomicron remnants. We have shown previously that fat tolerance tests with fat loads of this magnitude are sensitive enough to distinguish between runners and healthy sedentary men. Because the magnitude of postprandial lipemia in normal adult men is largely independent of body weight, lean body mass, or body surface area, each man was given the same dose of fat and vitamin A. Blood samples were drawn into vacuum tubes containing EDTA before and at 2, 3, 4, 6, and 8 hours after the meal.

Analytical Methods
Plasma and HDL cholesterol and plasma triglyceride concentrations were determined by enzyme assay with commercial kits (cholesterol reagent No. 236691, Boehringer Mannheim, Indianapolis, Ind., and triglyceride reagent No. 338-50, Sigma Chemical Co., St. Louis, Mo.). HDL₁ cholesterol and HDL₃ cholesterol were measured by the precipitation methods of Gidez et al. HDL₂ cholesterol was calculated from the difference between HDL₁ cholesterol and HDL₃ cholesterol.

Chylomicron remnant clearance was assessed by measuring the postprandial excursion of plasma retinyl palmitate concentrations. Because the demarcation between chylomicrons and their remnants has not been elucidated, we did not attempt to isolate a chylomicron remnant fraction. Berr et al have shown that the rate of disappearance of retinyl palmitate from the plasma is not limited by the rate of intravascular lipolysis in normolipidemic individuals; therefore, measurements of retinyl palmitate concentrations in postprandial plasma presumably reflect the endocytic step of chylomicron remnant removal.

For the retinyl palmitate assay, neutral lipids were extracted from plasma with CH₂OH and hexane. All tubes used in the extraction were covered with aluminum foil to shield retinyl esters from light. Five hundred microliters of plasma and 100 μl ethanol containing 300 ng retinyl undecanoate (internal standard) was added dropwise to 5 ml CH₂OH. The CH₂OH was vortexed during addition of the plasma. Hexane (5 ml) was then added, and the mixture was vortexed for 30 seconds. The samples were allowed to stand to effect phase separation, the upper phase was removed, and the lower phase was washed with 5 ml hexane. The hexane phase was dried under N₂, and the residue was resuspended in 50 μl benzene.

Retinyl palmitate concentrations in the lipid extracts were measured by reversed-phase high-performance liquid chromatography (HPLC) with a Waters Model 600A chromatograph (Waters Associates, Milford, Mass.) and a 3-μm Ultrasphere ODS column, 4.6×75 mm (Alttech Associates, Deerfield, Ill.). A guard column was also used. The mobile phase was HPLC-grade CH₂OH. Column effluent was monitored at 326 nm, and chromatograms were recorded on a Beckman Model 3390A integrator (Beckman Instruments, Palo Alto, Calif.) with an attenuation setting of 3. Peaks were quantified by the area-ratio method.

Retinyl undecanoate was synthesized by the method of Huang and Goodman and purified by preparative HPLC on a Waters C18 μBondapak column (Waters Associates).

Statistical Methods
Postprandial lipemia was defined as the area under the curve described by plasma triglyceride concentrations (normalized to the 0-hour value by subtracting...
the fasting value from each subsequent value) plotted against time. This area was calculated by use of the trapezoidal rule. The relations between the parameters measured in this study were assessed in two ways. First, Pearson's product-moment correlations between parameters were calculated for the 22 men in group A and for the 30 men in group B. Second, the data for each group were stratified according to HDL\textsubscript{2} cholesterol concentration and divided into a “low” HDL\textsubscript{2} cholesterol subgroup and a “high” HDL\textsubscript{2} cholesterol subgroup. Unpaired t tests were used to compare the mean value of each parameter in the low HDL\textsubscript{2} cholesterol subgroup with the corresponding value in the high HDL\textsubscript{2} cholesterol group.

Results

Plasma Lipids and High Density Lipoprotein

Fasting plasma triglyceride concentrations were well within the normal range (<200 mg/dl) in all of the men, and all but one had plasma total cholesterol concentrations below 250 mg/dl. One man had an elevated plasma total cholesterol value (270 mg/dl). HDL\textsubscript{T} cholesterol concentrations ranged from 37 to 76 mg/dl in group A and from 36 to 105 mg/dl in group B (see Tables 1 and 2 for mean values). HDL\textsubscript{T} cholesterol and HDL\textsubscript{2} cholesterol concentrations were poorly correlated with postprandial lipemia (see Figures 1 and 2) and with postprandial plasma retinyl palmitate concentrations (see Figure 3). Correlation coefficients between HDL cholesterol concentrations and plasma lipid concentrations, anthropometry, and running mileage are shown in Tables 3 and 4.

Oral Fat Tolerance

The fat tolerance test procedure was well tolerated by all of the subjects, and none had diarrhea or any gastrointestinal symptoms of fat malabsorption during the tests. The mean values for postprandial lipemia (see Tables 1 and 2) were similar to those reported previously for endurance-trained men given comparable fat loads\textsuperscript{9} and were substantially lower than those reported previously for normolipidemic sedentary men.\textsuperscript{9} When the subjects were divided into subgroups according to HDL\textsubscript{2} cholesterol concentration, postprandial lipemia was similar in the high HDL\textsubscript{2} cholesterol subgroup and in the low HDL\textsubscript{2} cholesterol subgroup (see Table 1). Dietary fat and cholesterol intakes were also similar in the low and high HDL\textsubscript{2} subgroups in group B. Fat constituted 30±11\% (range, 14−44\%) of the caloric intake of the low HDL\textsubscript{2} subgroup and 30±7\% (range, 21−43\%) of the caloric intake of the high HDL\textsubscript{2} subgroup. Mean

### Table 1. Age, Anthropometry, and Running Mileage of 52 Endurance-Trained Men

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (yr)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>Body fat (%)</th>
<th>Weekly mileage (km)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group A (n=22)</strong></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Low HDL\textsubscript{2} (n=11)</td>
<td>27±1</td>
<td>176±1</td>
<td>72±1</td>
<td>...</td>
<td>&gt;50</td>
</tr>
<tr>
<td>High HDL\textsubscript{2} (n=11)</td>
<td>28±1</td>
<td>176±1</td>
<td>72±2</td>
<td>...</td>
<td>&gt;50</td>
</tr>
<tr>
<td><strong>Group B (n=30)</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Low HDL\textsubscript{2} (n=15)</td>
<td>40±2</td>
<td>178±1</td>
<td>74±1</td>
<td>11±1</td>
<td>61±6</td>
</tr>
<tr>
<td>High HDL\textsubscript{2} (n=15)</td>
<td>36±2</td>
<td>179±1</td>
<td>75±2</td>
<td>11±1</td>
<td>58±10</td>
</tr>
</tbody>
</table>

Data are mean±SD.

HDL, high density lipoprotein.

*p<0.02, low HDL\textsubscript{2} vs. high HDL\textsubscript{2}.

### Table 2. Plasma Lipids, High Density Lipoproteins, and Postprandial Lipemia in 52 Endurance-Trained Men

<table>
<thead>
<tr>
<th>Group</th>
<th>Total Cholesterol (TC)</th>
<th>Fasting Triglycerides (FTG)</th>
<th>HDL\textsubscript{T}-C</th>
<th>HDL\textsubscript{2}-C</th>
<th>HDL\textsubscript{3}-C</th>
<th>PPL (mg/dl·8 hr)</th>
<th>PP-RP (ng/ml·8 hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group A (n=22)</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Low HDL\textsubscript{2} (n=11)</td>
<td>172±7</td>
<td>77±8</td>
<td>60±3</td>
<td>21±2</td>
<td>39±2</td>
<td>263±44</td>
<td>...</td>
</tr>
<tr>
<td>High HDL\textsubscript{2} (n=11)</td>
<td>161±4</td>
<td>94±12</td>
<td>52±3</td>
<td>14±2</td>
<td>39±2</td>
<td>270±39</td>
<td>...</td>
</tr>
<tr>
<td><strong>Group B (n=30)</strong></td>
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</tr>
<tr>
<td>Low HDL\textsubscript{2} (n=15)</td>
<td>183±13</td>
<td>59±7*</td>
<td>68±3†</td>
<td>29±3†</td>
<td>39±3</td>
<td>257±32</td>
<td>...</td>
</tr>
<tr>
<td>High HDL\textsubscript{2} (n=15)</td>
<td>182±5</td>
<td>75±4</td>
<td>60±3</td>
<td>25±2</td>
<td>35±1</td>
<td>103±14</td>
<td>4,743±277</td>
</tr>
<tr>
<td><strong>Group A (n=22)</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low HDL\textsubscript{2} (n=11)</td>
<td>172±8</td>
<td>77±5</td>
<td>49±2</td>
<td>15±1</td>
<td>34±1</td>
<td>105±19</td>
<td>4,670±274</td>
</tr>
<tr>
<td>High HDL\textsubscript{2} (n=15)</td>
<td>192±6</td>
<td>73±6</td>
<td>71±4†</td>
<td>34±3†</td>
<td>37±1</td>
<td>102±23</td>
<td>4,817±492</td>
</tr>
</tbody>
</table>

Values are mean±SD.

TC, total plasma cholesterol; FTG, fasting plasma triglyceride concentration; HDL\textsubscript{T}-C, high density lipoprotein total cholesterol concentration; PPL, postprandial lipemia; PP-RP, postprandial retinyl palmitate. PPL is defined as the area under the curve described by plasma TG concentrations (normalized to the 0-hour value) plotted against time.

*p<0.05, †p<0.01, low HDL\textsubscript{2} vs. high HDL\textsubscript{2}.

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Cohen et al
Postprandial Lipemia and HDL

Daily cholesterol intake was 375 ± 212 mg (range, 67–732 mg) in the low HDL₂ subgroup and 301 ± 144 mg (range, 72–528 mg) in the high HDL₂ subgroup.

Cessation of training in one man led to a fourfold increase in postprandial lipemia (122 mg/dl/8 hr to 400 mg/dl/8 hr), with no concomitant change in HDL₃ cholesterol (36 mg/dl) or HDL₄ cholesterol (6 mg/dl) concentration. In another subject, abstinence from exercise training led to a fall in HDL₂ cholesterol, from 16 to 8 mg/dl, with little change in postprandial lipemia (220–272 mg/dl/8 hr).

Discussion

Plasma concentrations of HDL₃ cholesterol often are elevated in men who perform chronic endurance exercise, and several studies have indicated that the increase in HDL cholesterol occurs primarily in the HDL₂ subfraction (see Reference 7 for a review). Results of the present study are consistent with these observations. The mean plasma HDL₃ cholesterol concentration in our subjects was substantially higher than corresponding values reported for normal men sampled at random from the general population,¹⁵ the elevation occurring primarily in the HDL₂ subfraction. Although most of the men in our study had relatively high HDL₃ cholesterol concentrations, considerable interindividual variation was evident: HDL₃ cholesterol concentrations ranged from 36 to 105 mg/dl, whereas HDL₂ cholesterol levels ranged from 3 to 60 mg/dl.

Neither HDL₃ cholesterol nor HDL₂ cholesterol concentrations were correlated with the magnitude of postprandial lipemia elicited by the high-fat meals (group A). The administration of smaller, more physiological fat loads did not improve these correlations. Equally poor correlations were observed between HDL₂ cholesterol concentrations and postprandial plasma retinyl palmitate concentrations, an index of chylomicron remnant removal. Dissociation between these parameters is also strongly indicated by the observation that when the men were grouped according to HDL₂ cholesterol concentrations, mean postprandial lipemia was essentially identical in the high and the low HDL₂ cholesterol subgroups. These data, taken as a whole, indicate that postprandial lipemia is not the primary determinant of HDL₂ cholesterol concentrations in endurance-trained men.
Although the relation between HDL$_T$ cholesterol and postprandial lipemia in endurance-trained men has not been studied in detail, possible mechanisms responsible for interindividual heterogeneity in HDL$_T$ cholesterol concentrations of exercising men have been examined by Sady et al. In a cross-sectional study in which runners with high HDL$_T$ cholesterol concentrations and runners with low concentrations were compared, the activity of lipoprotein lipase was similar in the two groups. Because lipoprotein lipase catalyzes the principal step in the chylomicron–triglyceride clearance pathway, these data suggest that interindividual differences in HDL$_T$ cholesterol concentrations among endurance-trained men cannot be ascribed to differences in the rate of lipolysis of chylomicron–triglycerides, a prediction entirely consistent with the results of the present study.

Weintraub et al reported that an exercise training program decreased postprandial lipemia in all participants but that no systematic changes in HDL$_T$ cholesterol concentrations occurred. Although their sample size (n=7) precluded definitive statistical analysis, these data are also consistent with a dissociation between HDL$_T$ cholesterol concentrations and chylomicron metabolism in exercise.

Because the present study was conducted in a highly select group of subjects, our findings should not be interpreted as evidence against a relation between HDL concentrations and postprandial lipemia in the general population. To assess the implications of our results for this relation, our data should be considered in the light of a previous study by Patsch et al, who...
found a strong negative correlation between HDL$_2$ cholesterol and postprandial lipemia in a heterogeneous group of men and women whose exercise status ranged from sedentary to marathon running. The range of HDL$_T$ cholesterol concentrations in the two studies was remarkably similar. In contrast, the interindividual variation in postprandial lipemia in the present study was far smaller than that reported previously. Taken together, these results suggest that high postprandial lipemia is incompatible with high HDL concentrations but that low postprandial lipemia does not automatically confer high HDL$_T$ cholesterol concentrations. Accordingly, impaired triglyceride transport is not the only cause of low HDL$_T$ cholesterol concentrations. If plasma HDL$_T$ cholesterol concentrations are influenced by factors unrelated to chylomicron clearance, then it is premature to ascribe the epidemiological correlation between HDL$_T$ cholesterol and coronary artery disease to latent defects in the catabolism of chylomicrons or their remnants. Further studies are now required to determine whether men with low HDL$_T$ cholesterol concentrations due to poor chylomicron–triglyceride clearance are at greater risk of premature coronary heart disease than are men with low HDL$_T$ cholesterol concentrations due to other factors.

References

3. Patsch JR, Prasad S, Gotto AM, Patsch W: High density lipoprotein 2: 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 1984;80:341–347

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