Heparin-induced lipolysis in hypertriglyceridemic subjects results in the formation of atypical HDL particles

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Abstract This study reports on the characterization of high density lipoprotein (HDL) in normotriglyceridemic and hypertriglyceridemic (HTG) subjects, after a fat meal and heparin-induced release of lipases. Samples for detailed analysis of HDL by density gradient ultracentrifugation and nondenaturing gradient gel electrophoresis were collected at 0 h and 5 h after the meal and 15 min after the administration of heparin. The normotriglyceridemic subjects were subdivided into two groups: those who remained normotriglyceridemic 5 h after the meal (NTG) or those who were hypertriglyceridemic at this time point (NTG-HTG). At the outset of the study, mean triglyceride levels were significantly higher ($P < 0.001$) and HDL cholesterol levels lower ($P < 0.02$) in the HTG group. The HDL particles in this group were enriched with triglyceride ($P < 0.001$). Serum triglyceride levels rose in all three groups after the fat meal and this was associated with further triglyceride enrichment of the HDL particles. In all groups, rapid lipolysis induced by heparin caused a significant decrease in plasma triglycerides and increase in free fatty acid levels, these changes being greatest in the HTG group. HDL density profiles of the study groups prior to the administration of heparin demonstrated two distinct peaks at density 1.09 g/ml (HDL_2) and 1.13 g/ml (HDL_3). However, after the administration of heparin to the HTG group, only a single peak in the HDL profile was evident that was located at the density region corresponding to HDL_2 (1.09 g/ml). Upon gradient gel electrophoresis of this peak, there was an increased number ($P < 0.005$ vs. NTG) of small particles (<4.37 nm) whose size was similar to the size range normally associated with HDL_{3b} and HDL_{3c}. Similar changes in HDL density and size after the administration of heparin were observed in the NTG-HTG group who were also hypertriglyceridemic postprandially. By contrast, the density gradient profiles and sizes of the HDL particles did not change after the administration of heparin to NTG subjects. Thus, the activation of lipolysis in HTG subjects leads to the generation of atypical HDL particles that are small but of reduced density. Rapid clearance of such particles could account for the inverse relationship between triglyceride and HDL cholesterol in this population subgroup. **—O'Meara, N. M., V. G. Cabana, J. R. Lukens, B. Loharikar, T. M. Forte, K. S. Polonsky, and G. S. Getz.** Heparin-induced lipolysis in hypertriglyceridemic subjects results in the formation of atypical HDL particles. *J. Lipid Res.* 1994. **35:** 2178–2190.

Supplementary key words free fatty acid • postheparin lipase • small but light HDL particles

Prospective epidemiological studies have demonstrated an inverse association between high density lipoprotein (HDL) cholesterol levels and ischemic heart disease (1–3). Although the anti-atherogenic properties of HDL may, in part, be due to its role in the reverse transport of cholesterol from peripheral tissues to the liver (4, 5), the interaction of the metabolism of HDL and triglyceride-rich particles may also be important. Many patients with low HDL cholesterol levels are hypertriglyceridemic (HTG) (6, 7). There is an active and dynamic interaction between HDL and triglyceride-rich particles in the circulation characterized by an exchange of core lipid (cholesteryl ester and triglyceride) between these lipoprotein particles, this exchange being facilitated by cholesteryl ester transfer protein (CETP) (5). HDL also has an important role in the lipolytic process. During lipolysis of very low density lipoproteins (VLDL) and chylomicrons, surface lipids (phospholipid and cholesterol) and proteins (apoA-I, A-II and Cs) are transferred to HDL (8, 9). It is believed that this exchange results in the formation of large HDL_2 particles from small HDL_3 particles. Con-

Abbreviations: FFA, free fatty acid; HDL, high density lipoprotein; HEP, postheparin; HL, hepatic lipase; HTG, hypertriglyceridemic; LCAT, lecithin:cholesterol acyltransferase; LDL, low density lipoprotein; LPL, lipoprotein lipase; NIDDM, non-insulin dependent diabetes mellitus; NTG, normotriglyceridemic; PP, postprandial; VLDL, very low density lipoprotein.

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versely, HDL acts as a reservoir for E and C, apolipoproteins which transfer to triglyceride-rich particles postprandially, apoC-II being a prerequisite for the activation of lipoprotein lipase (LPL) and initiation of lipolysis (10). Thus HDL may modulate or in turn be modified by the lipolytic process.

The mechanistic relationship between HDL levels and metabolism on the one hand and plasma triglyceride levels on the other remains to be clarified.

The link between LPL and HDL has been demonstrated in NTG subjects (11, 12), diabetics (13), and in patients with familial hyperalphalipoproteinemia (14). The role of LPL in mediating the transformations between HDL_2 and HDL_3 has also been demonstrated after ingestion of fat (15, 16) and in response to heparin (17). In the latter study, the administration of heparin to three HTG subjects also resulted in the appearance of small (5.8 nm) particles, of unknown origin. In particular, it was unclear whether these responses in HDL to the in vivo lipolysis were unique to such markedly HTG subjects or whether a similar response would be observed in more modestly HTG and NTG subjects. In this current study, we made a further attempt to characterize the changes in HDL and its subfractions during lipolysis after a high fat meal by evaluating the changes in density, size, and composition of these particles in groups of NTG and HTG subjects after the administration of heparin.

The characterization of these changes in HDL during the metabolism of plasma triglyceride may provide the basis for the exploration of the mechanism that accounts for the low HDL levels in hypertriglyceridemic subjects. These studies have indicated that the rapid metabolism of triglyceride is associated with a reduction in the size of HDL. It has been suggested that smaller HDL particles may be more rapidly removed from the circulation (18, 19). However, further mechanistic studies are required to validate this hypothesis.

The primary aim of the study was to investigate the inverse relationship between triglyceride metabolism and HDL and to investigate the importance of the lipolytic process in this relationship. The interaction of triglyceride concentration, lipolysis, and HDL characteristics were examined. These studies provide some insight into mechanisms that might account for the low HDL levels observed in hypertriglyceridemic subjects. Two strategies were used. We have compared the basal or fasting state with the peak postprandial state when triglyceride metabolism is accentuated markedly. We have also induced a state of very rapid triglyceride lipolysis by the injection of heparin, known to activate lipoprotein and hepatic lipases, at the postprandial triglyceride peak. We compared postprandial responses in subjects (NTG) who maintained a normal triglyceride level throughout the postprandial excursion, subjects (NTG-HTG) whose postprandial triglycerides were substantially elevated even though their fasting triglyceride levels were within the normal range, and subjects (HTG) who were frankly hypertriglyceridemic under basal conditions. A small group of diabetic subjects were also included because of the association between diabetes and high triglyceride and low HDL cholesterol levels.

MATERIALS AND METHODS

Subjects

Studies were performed in groups of NTG (n = 10) and HTG (n = 7) subjects. The NTG subjects were divided into two groups on the basis of whether they were still normotriglyceridemic 5 h postprandially (NTG, n = 6) or whether they became hypertriglyceridemic at this time (NTG-HTG, n = 4). Subjects were deemed to be hypertriglyceridemic basally or postprandially when their fasting and/or 5-h triglyceride concentration exceeded 2.25 mmol/l (200 mg/dl). The mean ages, weights, and BMIs of all three groups are recorded in **Table 1.** Three members of the HTG group had non-insulin-dependent diabetes mellitus (NIDDM). The diabetic subjects were included as diabetes is often associated with hypertriglyceridemia and a tendency to a low HDL cholesterol level. The inclusion of these subjects allowed us to explore whether a qualitatively distinct mechanism might account for the hypertriglyceridemia and low HDL in diabetes. As there is no discernible difference between the diabetic and non-

TABLE 1. Age, weight, BMI, total cholesterol, and HDL cholesterol

	NTG (n = 6)	NTG-HTG (n = 4)	HTG (n = 7)
Age (yr)	34.3 ± 4.6	36.0 ± 3.0	43.9 ± 3.5
Wt (kg)	87.5 ± 11.7	116.3 ± 33.2	108.2 ± 9.8
BMI (kg/m^2)	31.3 ± 3.2	35.5 ± 7.8	36.7 ± 2.7
Cholesterol (mmol/l)	3.92 ± 0.2	4.41 ± 0.5	4.93 ± 0.4
HDL cholesterol (mmol/l)	1.08 ± 0.07	1.08 ± 0.16	0.73 ± 0.06[a]

Results expressed as mean ± SEM.
[a] $P < 0.02$ vs. NTG.

diabetic subjects for the parameters examined in this study, data for the two groups of subjects were combined. In an attempt to match the groups, one diabetic patient was included in each of the other two groups. All diabetic patients were controlled on diet or oral agents, the latter being discontinued 2 weeks before the study. Both groups were matched for age and weight. All studies were performed in the Clinical Research Center at the University of Chicago. Informed consent was obtained from all participants and the protocol was approved by the Institutional Review Board.

Experimental protocol

Subjects were admitted to the Clinical Research Center after a 14-h overnight fast, and an intravenous sampling catheter was inserted into the forearm. Normal saline was infused into the catheter to maintain patency. After fasting blood samples were drawn, all subjects received a high fat test meal containing 60 g fat/m^2 body surface area. The detailed composition of this test meal, which was consumed in 20 min, has previously been described (20, 21). Having consumed the meal, all subjects did not eat again for the remainder of the study. Blood samples were again drawn at 5 h. This time point was chosen as it was considered to be representative of the average time at which peak triglyceride concentrations were observed in earlier studies (20-22) where NTG and HTG subjects consumed an identical test meal. After the 5-h blood sample was drawn, each subject received, by intravenous injection, 60 U Heparin sodium/kg body weight and, after 15 min, a blood sample was obtained for lipoprotein analysis and determination of postheparin lipolytic activity.

Lipid and lipoprotein determination

Blood was collected in tubes containing a final concentration of 1.2 g/l sodium EDTA, 1 mM PMSF, 0.1 g/l sodium azide, 1 mM BHT, 80 mg/l chloramphenicol, 80 μg/l gentamicin sulfate, and 10,000 μ/l kallikrein inhibitor. Plasma was immediately separated by centrifugation (3000 rpm) for 10 min at 4°C. Enzymatic kits were used to analyze the concentration of triglycerides (Boehringer Mannheim, Indianapolis, IN), cholesterol, and cholesteryl esters (Lancer, St. Louis, MO), and free fatty acids (FFA) (NEFA Kit, Wako Pure Chemicals, Dallas, TX) in plasma and in the HDL subfractions. The phospholipid content of HDL was determined by the Bartlett inorganic phosphorus method (23) while the protein content of the respective lipoprotein fractions was measured by a modified Lowry procedure (24) with SDS to disrupt the lipid micelles (25). All measurements were performed in the chemistry core laboratory for the SCOR in Atherosclerosis at the University of Chicago according to the standardization criteria established by the Center for Disease Control, National Heart, Lung, and Blood Institute Standardization Program.

Isolation of HDL

HDL and its subfractions were isolated from samples at all time points by density gradient ultracentrifugal flotation. Ultracentrifugation was performed at 15°C in a Beckman SW 41Ti rotor at 38,000 rpm for 66 h using a simplified gradient procedure adapted in this laboratory (26). In this procedure, 4.6 ml of 20% NaBr are initially layered at the bottom of a 14 × 89 mm Beckman centrifuge tube. Two ml of plasma are then added after which the tube is filled to within 2 mm of the top with 3% NaBr. With this gradient, the lipoproteins banded between $1.006 < d < 1.25$ g/ml. Fractions of 0.4 ml were collected using an ISCO gradient collector with UV monitor at 280 nm (Instrument Specialities Co., Lincoln, NE). The density of the fractions was determined from the refractive index of the solution based on the density of a reference solution of sodium bromide. Fractions corresponding to the relevant lipoprotein peaks were pooled, dialyzed in Tris-buffered saline (10 mM Tris, 150 mM NaCl, 0.01% EDTA, 20 mM NaN_3, pH 7.4) and used for lipid and lipoprotein analysis. Fractions designated as total HDL were taken as the pooled fractions in the density range 1.063 to 1.21 g/ml while fractions designated as HDL_2 and HDL_3 represented single tubes taken at the respective peaks of the density gradient distribution.

Polyacrylamide gradient gel electrophoresis

The size of the HDL particles was determined in a nondenaturing gel system using commercially prepared 4-30% polyacrylamide gels (Pharmacia, Pistcataway, NJ) using procedures recommended by the manufacturer. Fifteen micrograms of protein as a mixture by volume with three parts sample and one part solution of 40% sucrose with 0.01% bromphenol blue was applied to each of twelve lanes in a gel set. A mixture of standard molecular weight proteins (HMW Calibration Kit, Pharmacia) consisting of thyroglobulin (radius 8.50 nm), ferritin (6.10 nm), catalase (5.20 nm), lactate dehydrogenase (4.08 nm), and bovine serum albumin (3.55 nm) was included on a separate lane in each gel. The gels were stained with Coomassie G250 in perchloric acid (0.1% stain, 5% perchloric acid), destained, and stored in 7% acetic acid. The radius of the particles was assessed after densitometric scanning (Instruments Specialities Co., Lincoln, NE) based on the relative distance of migration of the standard. The proportion of particles of different sizes was estimated from the output of the scanner using a computerized curve-fitting digitizer program (Sigma Scan, Jandel Scientific, Corte Madera, CA).

Electron microscopy

HDL were transported to Berkeley in their salt background and were immediately dialyzed against ammonium acetate buffer (126 mM ammonium acetate, 0.26 mM

EDTA, pH 7.4). Aliquots were examined in the JEOL 100C electron microscope after staining with sodium phosphotungstate as previously described (27).

Statistical methods

All results are expressed as mean ± SEM. Baseline differences between groups were tested for significance using a General Linear Modeling procedure with a Tukey allowance for multiple comparisons ($P < 0.05$ was regarded as statistically significant). Within-group changes after the meal and after administration of heparin were tested for significance using the paired *t*-test. Data analysis was performed using the Statistical Analysis System (SAS version 6 for personal computers, SAS Institute, Cary, NC).

RESULTS

Basal and postprandial lipid levels

The age, weight, BMI, and total and HDL cholesterol concentrations of the three study groups are presented in Table 1 while the triglyceride concentrations are presented in **Table 2.** Basal triglyceride concentrations were significantly higher in the HTG group ($P < 0.001$). In these subjects, comparable increases in triglyceride concentration were observed after the high fat meal and the mean plasma triglyceride concentration 5 h after the meal was significantly higher ($P < 0.05$) than the basal value. Although fasting plasma triglyceride levels in the NTG and NTG-HTG groups were within the normal range, the levels in the NTG-HTG group were still significantly higher than those in the NTG group ($P < 0.01$). The differences in the triglyceride levels at baseline between these two groups were magnified postprandially where the NTG-HTG group became notably hypertriglyceridemic. No significant differences in total cholesterol levels were observed between the three groups. By contrast, basal HDL cholesterol levels were significantly lower ($P < 0.02$) in the HTG group (Table 1).

TABLE 2. Basal, postprandial, and post heparin triglyceride levels (mM/l)

	NTG (n = 6)	NTG-HTG (n = 4)	HTG (n = 7)
Basal	0.62 ± 0.11	1.09 ± 0.07	3.1 ± 0.5[a]
Postprandial	1.809 ± 0.13[d]	3.18 ± 0.12[c]	5.2 ± 1.3[b]
Postheparin	0.48 ± 0.05[e]	1.13 ± 0.36[e]	2.3 ± 0.9[f]

Results expressed as mean ± SEM.
[a] $P < 0.001$ vs. NTG-HTG and NTG groups.
[b] $P < 0.05$ vs. basal.
[c] $P < 0.005$ vs. basal.
[d] $P < 0.0005$ vs. basal.
[e] $P < 0.005$ vs. postprandial.
[f] $P < 0.0005$ vs. postprandial.

TABLE 3. Basal, postprandial, and postheparin plasma FFA (mM)

	NTG	NTG-HTG	HTG
Basal	0.57 ± 0.10	0.66 ± 0.14	0.92 ± 0.15
Postprandial	0.59 ± 0.06	1.29 ± 0.23	0.94 ± 0.24
Postheparin	2.58 ± 0.20	3.81 ± 0.57	6.31 ± 1.11[a]

[a] $P < 0.02$ vs. NTG.

Plasma FFA concentrations were measured in the three study groups (**Table 3**). Basal FFA values were higher in the HTG group but only reached statistical significance after the injection of heparin.

Effect of heparin on lipid concentrations

The administration of heparin 5 h after the meal caused an immediate and dramatic decrease in plasma triglyceride levels in all study subjects (Table 2). This reduction in triglyceride concentration was most evident in subjects who were HTG postprandially. Plasma triglycerides decreased by 0.61 ± 0.09 mmol/l ($P < 0.005$) in the NTG subjects, by 2.05 ± 0.25 mmol/l ($P < 0.005$) in the NTG-HTG subjects, and by 2.96 ± 0.42 mmol/l ($P < 0.005$) in the HTG group. Within all groups, no obvious differences in the response to heparin were observed between the diabetic and non-diabetic subjects. Total plasma cholesterol and HDL cholesterol concentrations did not change appreciably in the 15 min immediately after the administration of heparin. FFA levels were notably increased after heparin injection in all three study groups, with the most marked increment occurring in the HTG group (Table 3).

Effect of fat feeding and heparin on density and size of HDL particles

Figure 1 shows the changes in density and size of postprandial HDL from a representative HTG subject after the administration of heparin. In this figure, the HDL density profiles at baseline are resolved into two distinct peaks at density 1.09 g/ml (HDL_2) and at 1.13 g/ml (HDL_3). After the high fat meal, a slight shift in the density of 5-h postprandial HDL_2 and HDL_3 was observed. Fifteen minutes after the administration of heparin, the density profiles of the HDL particles were significantly altered. At this time point, only a single peak in the HDL profile was evident and this was located at the density region corresponding to HDL_2 (1.09 g/ml). HDL_3 was not evident. However, when the material in this peak was submitted to nondenaturing gradient gel electrophoresis, an increased number of small particles was observed with size similar to the size range normally associated with HDL_3. Similar changes in density and size of HDL after

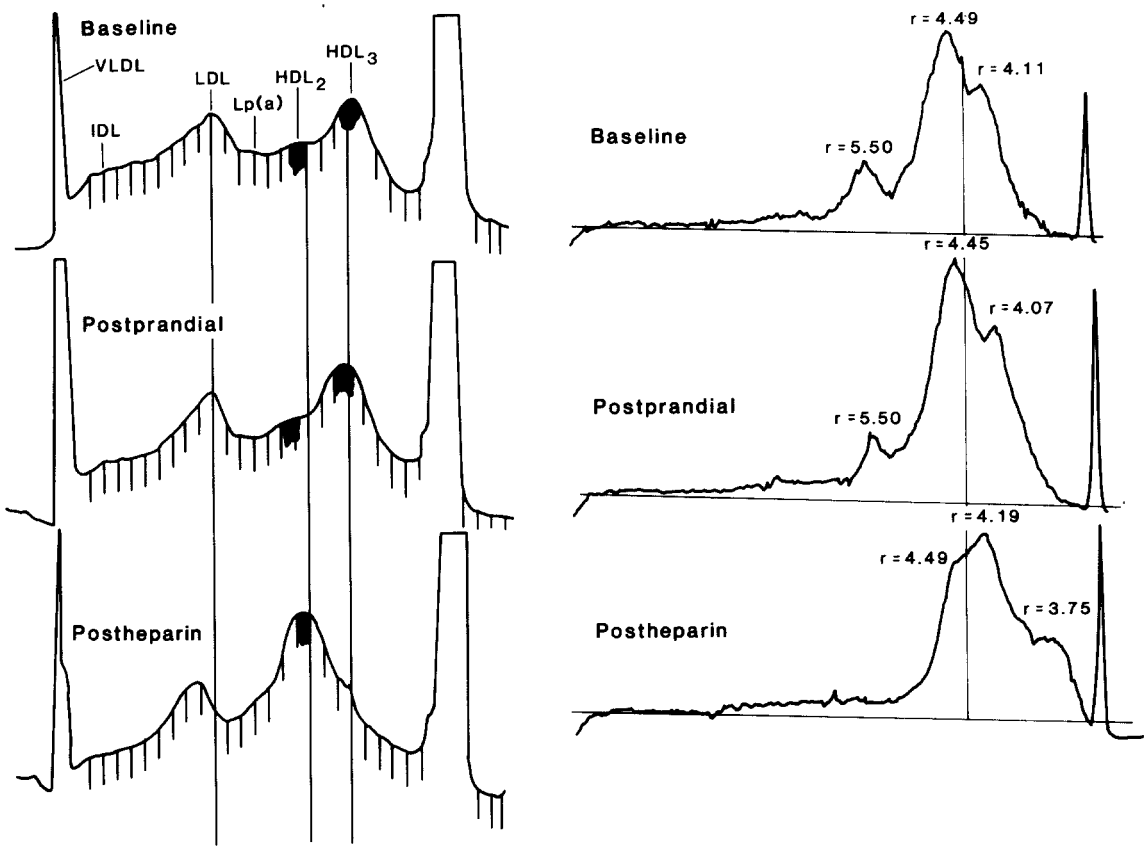

Fig. 1. Changes in the density and size of HDL. Plasma samples obtained at baseline, 5 h postprandial, and 15 min after the infusion of heparin were subjected to density gradient ultracentrifugal flotation (left panel). The corresponding HDL isolated from the samples on the left were subjected to nondenaturing gradient gel electrophoreses and scanned (right panel). The numbers above the peaks of the scans correspond to the radius (nm) of the peak. The shift in density (left) and appearance of small HDL particles (right) are evident in the plasma obtained after the injection of heparin (bottom).

the administration of heparin were observed in the NTG-HTG subjects (**Fig. 2**). By contrast, no change in HDL density gradient profiles or HDL particle size was observed after the administration of heparin to the NTG subjects (**Fig. 3**).

The changes in HDL size and density depicted in Figs. 1–3 were representative of the two different responses to heparin observed, depending on whether subjects were hypertriglyceridemic or normotriglyceridemic at the time of heparin administration. **Table 4** highlights the changes in particle size after the administration of heparin. In the HTG group, significant increases were observed in the proportion of small particles of the HDL_{3b} and HDL_{3c} subclass (i.e., <4.37 nm). Similarly in the NTG-HTG group, an increased proportion of small particles was observed after the administration of heparin. By contrast, heparin did not cause any significant increase in the proportion of small particles in the NTG subjects. To further evaluate the influence of the 5-h triglyceride concentration on HDL particle size post heparin, the correlation between the 5-h triglyceride concentration and the proportion of small particles (<4.37 nm) present post heparin was calculated. This correlation was highly significant ($r = 0.92$, $P < 0.0001$). These data are illustrated in **Fig. 4.** When the data were re-analyzed after the exclusion of the most hypertriglyceridemic subject, this correlation remained highly significant ($r = 0.78$, $P < 0.001$).

The changes in the size and density of the HDL particles after the administration of heparin appear to be a function of the level of plasma triglyceride rather than the postprandial state as the administration of heparin to a HTG subject under fasting conditions produced changes in HDL very similar to those described above.

Significant differences in particle size were also observed between groups. Under fasting conditions the HTG group had increased proportions of small HDL particles (<4.37 nm) in comparison to the NTG group ($P < 0.05$) while the proportion of small particles in the NTG-HTG group was intermediate between those observed in the NTG and HTG groups. These differences persisted postprandially and after heparin administration. Across all study subjects, a highly significant correlation

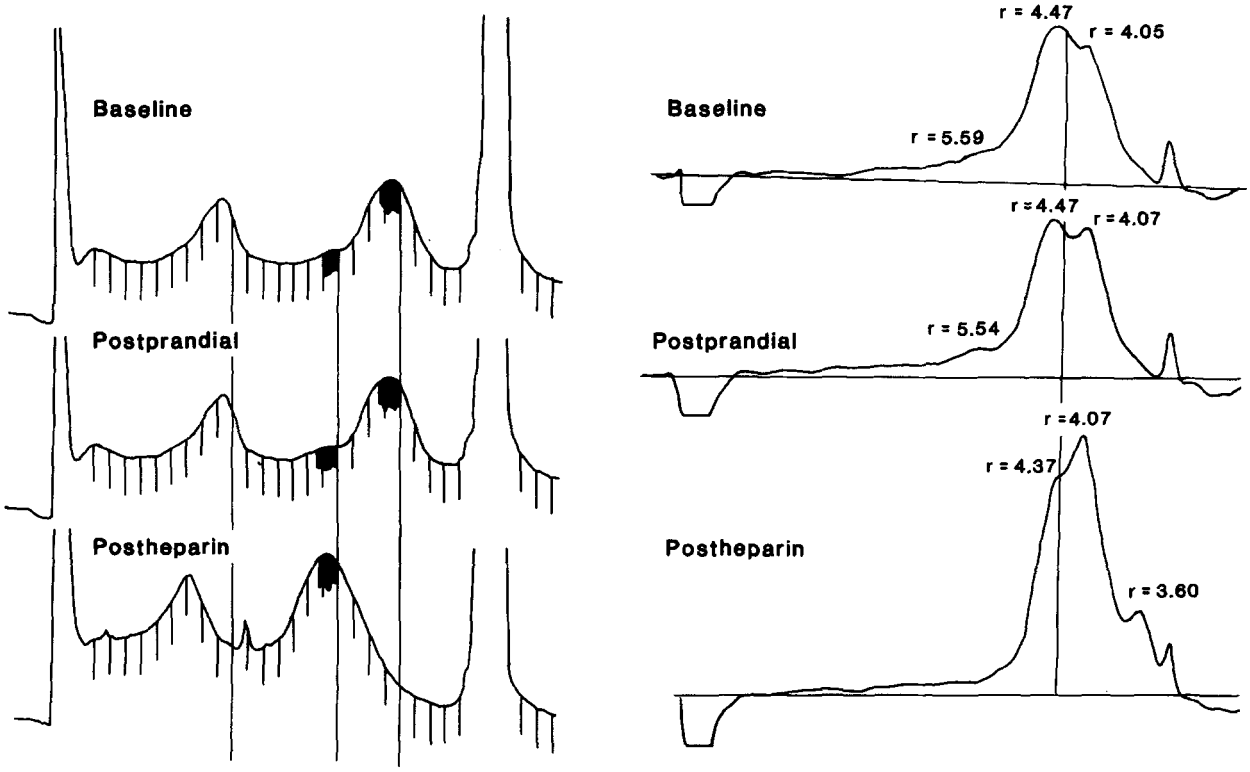

Fig. 2. Changes in the density and size of HDL in the NTG-HTG subjects. Legend is as for Fig. 1. Similar changes occurred as in the HTG subjects depicted in Fig. 1.

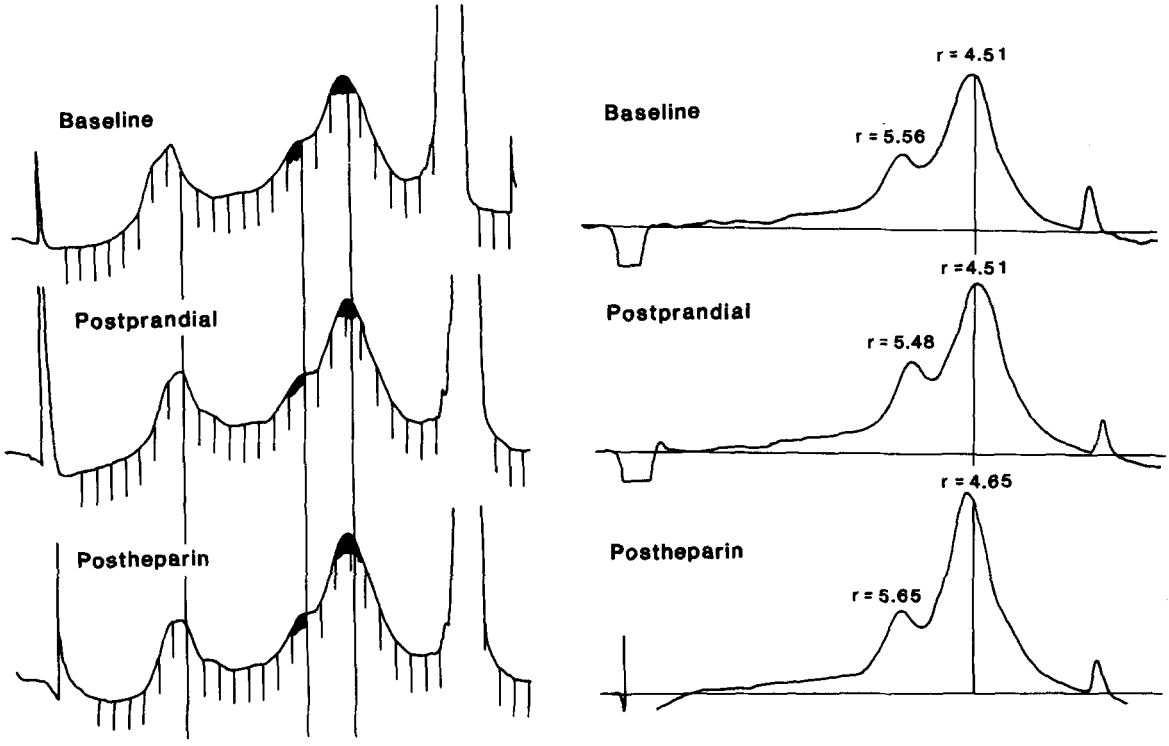

Fig. 3. Density and size of HDL in NTG subjects. Legend is as for Fig. 1. No changes in density (left) or size (right) of HDL were evident in the postheparin plasma (bottom) from the NTG subjects.

TABLE 4. Proportion of small HDL particles (<4.37 nm) in the study groups (%)

	NTG	NTG-HTG	HTG
Basal	10.1 ± 4.7	23.2 ± 4.5	43.7 ± 10.1[b]
Postprandial	10.8 ± 5.0	26.0 ± 6.2	43.2 ± 9.7[b]
Postheparin	13.0 ± 6.0	44.6 ± 9.6	71.0 ± 7.4[a,c]

Sizes (radii) reported in this table are determined from nondenaturing gradient gel electrophoresis, performed as described in the Methods section. Percentage refer to the proportion of small HDL particles among total HDL particles.

[a] $P < 0.05$ vs. HTG group at 5 h.

[b] $P < 0.05$ vs. NTG group.

[c] $P < 0.005$ vs. NTG group.

was observed between the basal triglyceride concentration and the proportion of small HDL particles present at time 0 ($r = 0.89$, $P < 0.0001$).

Effect of feeding and heparin on electron microscopic structure of HDL from hypertriglyceridemic subjects

To elucidate the anomalous behavior of HTG and NTG-HTG HDL in separations by ultracentrifugation contrasted with those by nondenaturing gel electrophoresis (Figs. 1 and 2), ultracentrifugal fractions between the density range of 1.06 to 1.21 g/ml from three HTG subjects were examined by electron microscopy. As substantial ultracentrifugal differences were noted between postprandial and postheparin fractions, these two conditions were evaluated. Particle size distribution of the major subfractions from a representative experiment is shown in **Table 5.** In the postprandial samples, round HDL particles predominate (**Fig. 5,a and b**) and their mean particle diameter decreases with increasing density (Table 5). In the postheparin samples, mean particle diameters for spherical HDL are consistently smaller than their counterparts of similar density in the postprandial sample (Table 5). Electron microscopy revealed that postheparin HDL are uniquely different from postprandial HDL as seen in Fig. 5c and d. Large irregular surface remnants are present in the least dense subfraction and discoidal particles are present in the denser fraction.

HDL composition

HDL composition for all groups is represented in **Fig. 6** and **Table 6.** At baseline, the HDL particles of HTG subjects were enriched with triglyceride ($P < 0.001$) while the proportion of cholesteryl ester in the particles was reduced ($P < 0.005$). In all three groups, after the meal, the triglyceride content of HDL increased, this increase being statistically significant in the NTG and NTG-HTG groups. Although the administration of heparin caused a decrease in the triglyceride content of the particles in each group, these differences were not statistically significant. In the case of HDL_2 particles (**Table 7**), there was a decline in triglyceride content in response to heparin, but only in the HTG particles was this statistically significant ($P < 0.001$).

Analysis of HDL_2 (Table 7) samples yielded results that were qualitatively similar to those of total HDL. Major differences were that, in contrast to total HDL, HDL_2 in all samples had proportionately lower relative protein content and higher lipid content, especially cholesteryl ester, free cholesterol, and phospholipid. Changes after heparin administration were similar for both total HDL and HDL_2.

DISCUSSION

In these experiments, groups of NTG and HTG subjects received identical lipid-rich meals and similar doses of heparin 5 h after the meal. Changes in HDL associated with these manipulations have been described (22). All three groups of subjects contained diabetic (NIDDM) and non-diabetic subjects. As we have no evidence that diabetes per se influences the behavior of the HDL particles in the postprandial and the postheparin states, the diabetic and non-diabetic subjects have been considered together in the analysis of the results.

Postprandial and postheparin changes in HDL

The changes in HDL observed in these studies of postprandial and acute postheparin responses fall into two categories: those relating to the physical properties of

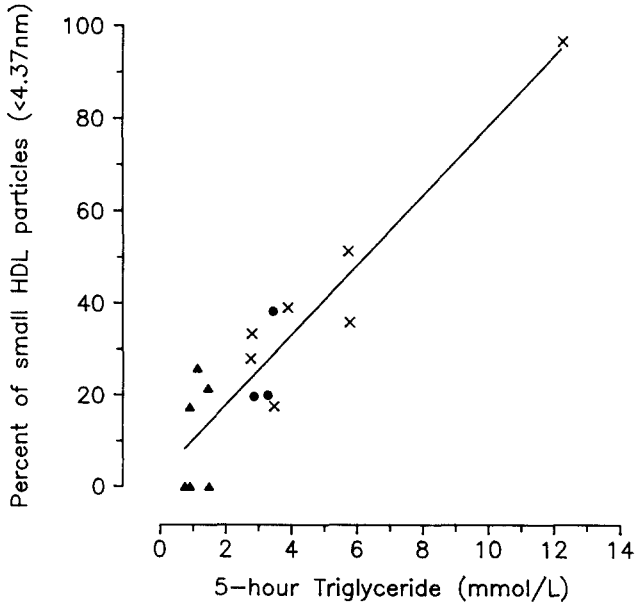

Fig. 4. Correlation between the postprandial triglyceride response (x-axis) and the percentage of small ($r = <4.37$ nm) HDL particles present after the injection of heparin (y-axis); (▲) NTG; (●) NTG-HTG; (x) HTG.

TABLE 5. Size and morphology of HDL particles from ultracentrifuge subfractions isolated from an HTG subject after feeding and heparin administration

Sample	Fraction Number	Density	Spherical Particle Diameter	Disc/Remnant
		g/ml	*nm*	*nm*
PP	18	1.0780	10.2 ± 1.8	
PP	20	1.0959	8.7 ± 1.7	
PP	22	1.1168	8.1 ± 1.4	
PP	24	1.1337	7.3 ± 1.5	
HEP	18	1.0780	8.0 ± 1.7	50–120, remnant
HEP	20	1.0959	7.5 ± 1.3	
HEP	22	1.1168	7.2 ± 1.3	
HEP	24	1.1337	6.7 ± 1.5	18.5 ± 4.4, disc

Particle diameters are determined from calibrated electron microscopic images as described in the Methods section. PP, postprandial sample; HEP, postheparin sample.

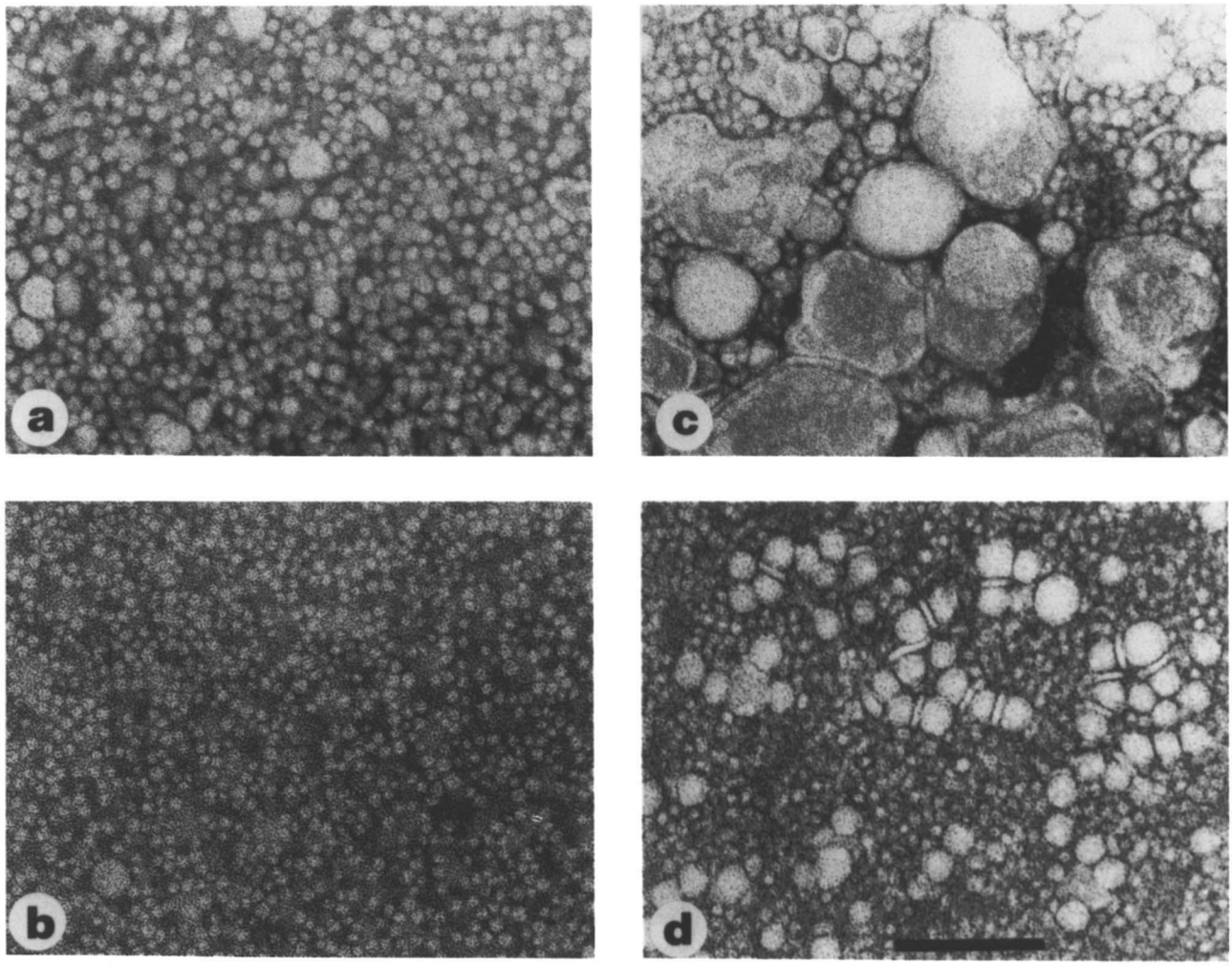

Fig. 5. Electron micrographs of negatively stained HDL fractions from an HTG subject. (a) Postprandial fraction of density 1.0780 g/ml. The majority of particles are spherical with a mean diameter of 10.2 ± 1.8 nm. (b) Postprandial fraction of density 1.1337 g/ml. Small spherical particles (7.3 ± 1.5 nm) predominate. (c) Postheparin HDL fraction of density 1.0780 g/ml. Two types of particles are present: small round ones (8.0 ± 1.4 nm) and irregular shaped large remnants ranging from 50 to 120 nm in size. (d) Postheparin fraction of density 1.1337 g/ml. Small round particles (6.7 ± 1.5 nm) predominate but discoidal particles (18.5 ± 4.4 nm) seen "on edge" are also present; the large round profiles are 18.5 ± 2.8 nm diameter and are presumed to be disc en face. The bar marker represents 100 nm and is representative for all micrographs.

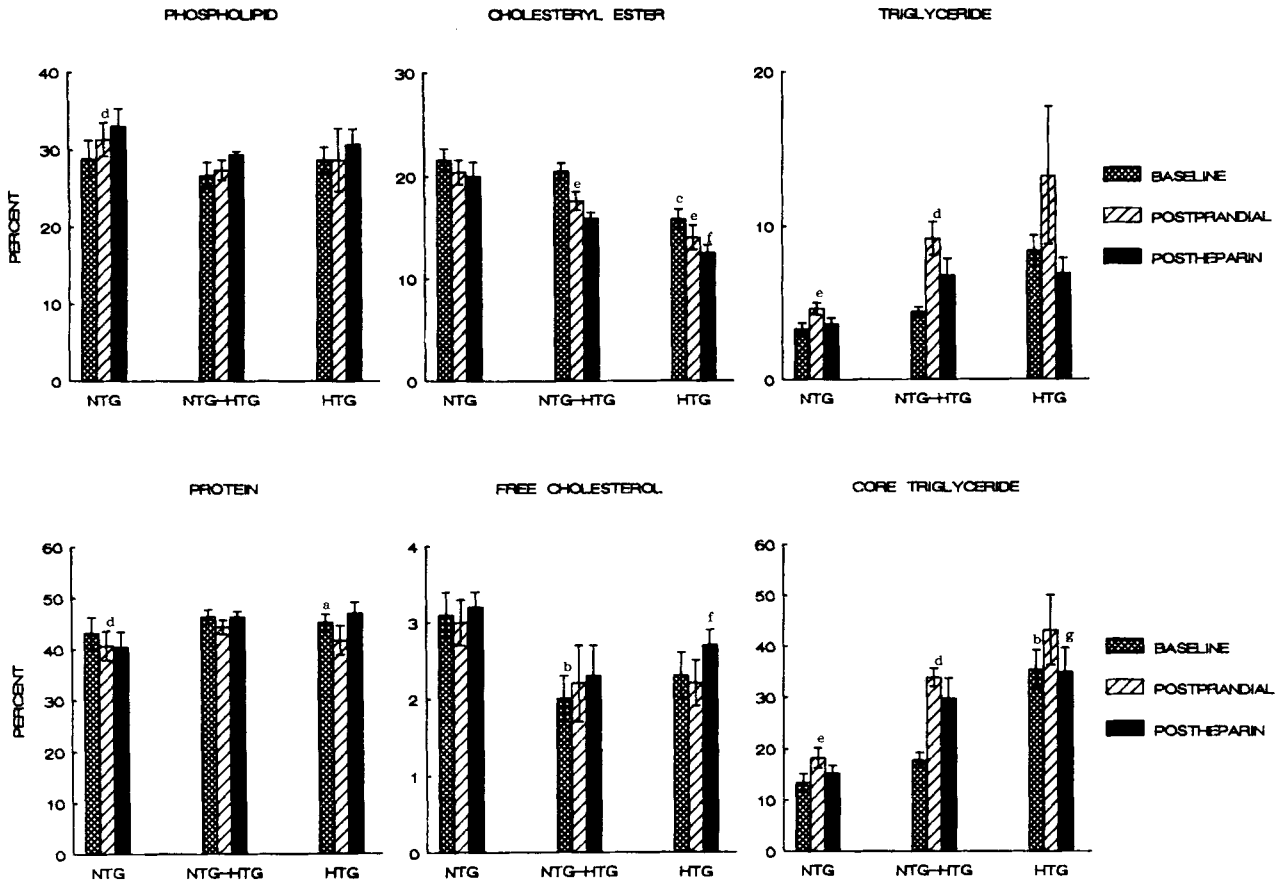

Fig. 6. Composition of HDL (weight percent) in the different study groups. Refer to Table 6 for the significance (P) values.

TABLE 6. Composition of HDL by weight (%)

Time (h)	Protein	TG	FC	CE	PL	TG Core
			NTG			
0	43.2 ± 3.1	3.3 ± 0.4	3.1 ± 0.3	21.6 ± 1.1	28.9 ± 2.4	13.4 ± 1.8
5	40.7 ± 2.9[d]	4.6 ± 0.4[c]	3.0 ± 0.3	20.4 ± 1.2	31.4 ± 2.2[d]	18.6 ± 2.0[c]
5.25	40.4 ± 3.0	3.6 ± 0.4	3.2 ± 0.2	20.0 ± 1.4	33.1 ± 2.3	15.2 ± 1.6
			NTG-HTG			
0	46.3 ± 1.4	4.4 ± 0.3	2.0 ± 0.3[b]	20.5 ± 0.8	26.8 ± 1.7	17.8 ± 1.5
5	44.3 ± 1.4	9.2 ± 1.1[d]	2.2 ± 0.5	17.6 ± 0.9[c]	27.5 ± 1.3	33.9 ± 1.8[d]
5.25	46.3 ± 1.1	6.8 ± 1.1	2.3 ± 0.4	15.9 ± 0.6	29.5 ± 0.4	29.8 ± 4.0
			HTG			
0	45.2 ± 1.6[a]	8.4 ± 1.0	2.3 ± 0.3	15.8 ± 1.0[c]	28.8 ± 1.7	34.5 ± 3.8[b]
5	41.7 ± 2.9	13.3 ± 4.5	2.2 ± 0.3	14.0 ± 1.2[c]	28.8 ± 4.1	43.2 ± 6.9
5.25	47.0 ± 2.2	6.9 ± 1.0	2.7 ± 0.2[f]	12.5 ± 0.8[f]	30.8 ± 2.0	35.0 ± 4.7

The time 0 refers to the basal fasting sample, the 5-h sample refers to samples taken 5 h after consumption of a standard fat meal, and the 5.25-h sample refers to the sample taken 15 min after the administration of heparin intravenously to the 5-h postprandial subject.

[a] $P < 0.001$ vs. NTG and NTG-HTG.
[b] $P < 0.05$ vs. NTG.
[c] $P < 0.005$ vs. NTG and NTG-HTG.
[d] $P < 0.05$ vs. 0 hr.
[e] $P < 0.01$ vs. 0 hr.
[f] $P < 0.05$ vs. 5 hr.

TABLE 7. Composition of HDL_2 by weight (%)

Time (h)[a]	Protein	TG	FC	CE	PL	TG Core
	NTG					
0	32.9 ± 3.0	3.9 ± 0.6	4.4 ± 0.5	25.2 ± 1.7	30.8 ± 1.7	13.3 ± 1.7
5	30.6 ± 2.8	5.4 ± 0.8	4.8 ± 0.6	25.0 ± 1.2	34.4 ± 2.8	17.7 ± 2.5
5.25	31.2 ± 2.7	4.2 ± 0.5	5.0 ± 0.3	24.4 ± 1.3	35.1 ± 2.4	14.6 ± 1.9
	NTG-HTG					
0	35.2 ± 3.0	7.0 ± 0.9	3.7 ± 1.3	24.2 ± 1.3	30.0 ± 2.8	22.7 ± 3.4
5	35.1 ± 2.5	11.7 ± 1.3	2.9 ± 0.4	21.7 ± 0.7	30.6 ± 2.7	34.8 ± 2.7
5.25	43.8 ± 2.0	5.7 ± 1.1	1.7 ± 0.2	17.5 ± 0.4	31.2 ± 0.4	24.1 ± 3.7
	HTG					
0	29.2 ± 2.9	12.0 ± 2.4	4.7 ± 0.7	21.7 ± 2.5	32.5 ± 2.3	34.9 ± 5.0
5	34.4 ± 4.3	14.7 ± 3.1	4.0 ± 0.9	19.6 ± 2.6	29.2 ± 1.2	42.3 ± 7.3
5.25	40.3 ± 3.5	6.1 ± 1.1	2.5 ± 0.3	17.4 ± 1.2	33.8 ± 1.7	25.5 ± 3.6

[a]See legend to Table 6 for the description of the 0-, 5-, 5.25-h samples.

HDL and to their lipid composition. In some subjects, there are changes in the physical characteristics of HDL as reflected in their behavior in the ultracentrifuge, upon non-denaturing gradient gel electrophoresis, and when fractions of these lipoproteins are observed by negative staining electron microscopy. No significant changes are observed in those subjects who maintained their plasma triglyceride concentration below 2.26 mmol/l (200 mg/dl) throughout the course of the experiment. On the other hand, in both the NTG-HTG and the HTG groups of subjects, the changes in HDL properties after the heparin injection and mobilization and activation of lipases are quite dramatic especially in the ultracentrifugal profile of HDL, which increasingly comes to resemble HDL_2 in the observed profile. A striking and surprising observation is that when these same samples are examined by non-denaturing gradient gel electrophoresis, contrary to expectation, the particles appeared to be smaller in size than prior to heparin injection. This paradox remains to be explained, and will be discussed in more detail below.

Independently of the postprandial and heparin challenges, there are quite substantial differences in the proportion of small-sized HDL particles in the three groups of subjects: the more hypertriglyceridemic is the plasma, the higher is the proportion of small-sized particles. This is well represented in the data as plotted in Fig. 4. These differences are noted even in the basal samples (Table 4). These differences are not only correlated with the total plasma triglyceride concentration, but also with the mean HDL triglyceride content.

The second category of changes relates to the lipid composition of HDL particles, with special emphasis on both the postprandial and postheparin responses affecting mainly the HDL triglyceride composition. HDL becomes enriched with triglyceride in relation to the increment in the size of the total plasma triglyceride pool. The HDL-triglyceride is thus increased during the postprandial hypertriglyceridemia, and this is decreased rapidly as lipolysis is activated by the injection of heparin. The increase in HDL triglyceride is presumably the result of an exchange of VLDL triglyceride for HDL cholesteryl ester, facilitated by the cholesteryl ester transfer protein. Thus, at the height of triglyceride elevation postprandially in hypertriglyceridemic subjects, over 40% of the core lipid of HDL is triglyceride. This is two and a half times the core lipid proportion made up of triglyceride in the postprandial HDL of normotriglyceridemic subjects. Even under basal conditions, hypertriglyceridemic HDL contains two and a half times as much core triglyceride as normotriglyceridemic HDL (Tables 6 and 7). Under basal conditions, there is a progressive increase in the HDL triglyceride from normotriglyceridemic, NTG-HTG, and hypertriglyceridemic (HTG) subjects.

There is a similar trend in the relative proportion of small HDL particles (nondenaturing gel electrophoresis) across these three subject groups. As might be expected, the triglyceride core proportion in HDL drops 15 min after an intravenous injection of heparin, the largest proportional drop being seen in the hypertriglyceridemic subjects. The total plasma triglyceride concentration also drops after heparin injection, being most profound in the hypertriglyceridemic group (Table 2). Not only does the core triglyceride of HDL drop after heparin, but so does the cholesteryl ester content. It is likely that the postheparin changes in HDL triglyceride are attributable to the direct lipolysis of this core lipid by either lipoprotein or hepatic lipase. The postheparin HDL has a lower proportion of core to surface mass (including protein) (calculated from data in Tables 6 and 7) than HDL isolated from subjects under basal or postprandial condi-

tions. This is most profound in the case of the frankly hypertriglyceridemic subjects. The fact that both triglyceride and cholesteryl ester decline from the core of HDL particles isolated after heparin injection suggests that there has probably been a more profound reorganization of HDL components (28). Compatible with these changes in HDL lipid core is the reduction in size of HDL particles after heparin injection, as reflected either by nondenaturing gel electrophoresis (Table 4) or electron microscopy (Table 5). In the latter case, in all ultracentrifugal samples ranging in isolation density from 1.078 g/ml to 1.1337 g/ml, the mean particle diameters are smaller in the postheparin samples than in the postprandial samples.

The electron microscopic data show the presence of discs and surface remnants in some subfractions that may, in part, be responsible for the skewing of particles in the direction of larger, less dense particles during ultracentrifugation. On nondenaturing gradient gel electrophoresis such particles are probably diffused over the large-pore region of the gel, or perhaps stuck at the top of the gel. The appearance of large surface remnants is not surprising as in vitro incubation studies with triglyceride-rich particles and lipase have demonstrated the formation of redundant surface material (8); similar structures were also previously reported after in vivo heparin-induced lipolysis in hypertriglyceridemic subjects (17). The appearance of discoidal particles after acute administration of heparin is consistent with the early observation of Chajek and Eisenberg (9) who noted the formation of discoidal particles after perfusion of VLDL through the isolated rat heart. The mechanism for the formation of the discoidal HDL after lipolysis is not well understood.

HDL size in subject groups

The size distribution of HDL particles in the three subject groups is of interest. Despite almost identical HDL cholesterol concentrations in the plasma at baseline in the NTG and NTG-HTG subjects (Table 1), there was a difference in the size profile of HDL in these two groups. The NTG-HTG subjects at baseline had at least twice the proportion of small particles ($<$ 4.4 nm radius) as did the NTG subjects. They also had lower free cholesterol content in their HDL than in that of NTG subjects. These subjects (NTG-HTG) also had slightly higher basal triglyceride levels and a more profound increase in small particles postprandially and postheparin, an alteration that was associated with postprandial hypertriglyceridemia. While the causes and effects are not easily distinguished, it is clear that there are subtle changes in HDL metabolism and triglyceride metabolism that accompany the postprandial state in individuals with high normal triglyceride levels under basal conditions. This serves to emphasize the close interaction of triglyceride and HDL metabolism. How these subtle differences relate to risk of atherosclerosis remains to be explored.

Paradoxical behavior of HDL examined by centrifugation and gel electrophoresis

The behavior of HDL obtained from postheparin samples is paradoxical when examined by ultracentrifugation on the one hand and by nondenaturing gel electrophoresis on the other. In the first case, HDL appears to be less dense, leading one to expect a high lipid core and large size. Quite the contrary is suggested by three approaches: lipid analysis, nondenaturing gel electrophoresis, and electron microscopy, all of which suggest smaller particles with less lipid core. Also, HDL particles seem to be more heterogeneous when examined by these latter techniques than when examined by centrifugation. It is not clear how one can explain this paradox, but in any event it seems that the ultracentrifugation yields the aberrant result. Thus, under these special circumstances, ultracentrifugation of HDL does not appear to provide a reliable characterization of these lipoprotein particles.

The activation of lipolysis that follows the release of lipoprotein lipase and hepatic lipase induced by heparin leads to a substantial increase in free fatty acids particularly in the hypertriglyceridemic subjects. We are currently exploring the hypothesis and have preliminary supportive data that suggest that the free fatty acids liberated by activated lipases are at least partially responsible, by virtue of their surface activity, for refashioning HDL particles, particularly when their concentration exceeds the capacity of albumin to bind them tightly.

Other compositional changes in HDL during lipolysis could play a role in the alterations in HDL physical features discussed above. These include the lipolytic generation of lysolecithin, the acquisition of redundant surface constituents, C apolipoproteins, phospholipid, and cholesterol from triglyceride-rich lipoproteins. The contribution of the triglyceride enrichment of HDL to the observed changes in size remains to be established. In other words, are the HDL transformations related largely to the lipolytic products generated or does the HDL triglyceride enrichment per se make an important contribution? This question remains to be further investigated.

The plasma fatty acid concentration seldom, if ever, reaches the levels observed acutely in hypertriglyceridemic subjects after heparin administration in this study. The fatty acid concentration is held at low levels by their very rapid uptake from the plasma into the tissues. Our results show that these systems can be overwhelmed at least acutely. While this is seldom the case physiologically and systemically, it is possible in local microenvironments, as for example where triglyceride-rich lipoproteins are being rapidly lipolyzed in the vicinity of capillary endothelial lipoprotein lipase. HDL particles that enter

these microenvironments may be modified as described in this study. HDL, modified in this fashion, may be rapidly cleared from the circulation, either as such or after interaction with hepatic lipase. Such a chain of events could account for the low HDL observed in hypertriglyceridemic subjects.

Conclusion

In conclusion, many previous reports have emphasized the inverse relationship between plasma triglyceride and HDL cholesterol levels. The data in this study highlight the structural differences in HDL between NTG and HTG subjects, differences that are enhanced during the metabolism of triglyceride-rich lipoproteins. It remains to be seen whether the altered size and density of the HDL particles in this setting are associated with an increased clearance of these particles from the circulation and perhaps with an increased susceptibility to atherosclerosis.

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