Production of Small High-Density Lipoprotein Particles After Stimulation of In Vivo Lipolysis in Hypertriglyceridemic Individuals: Studies Before and After Triglyceride-Lowering Therapy

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In hypertriglyceridemic states, triglyceride enrichment of high-density lipoprotein (HDL) may play an important role in decreasing the HDL cholesterol and apolipoprotein (apo) A-1 plasma concentration. We have shown previously that HDL particles are transformed into small HDLs when lipolysis is stimulated in vivo or in vitro, and this process is more marked if the HDL is triglyceride-rich. The present study was conducted to determine whether the susceptibility of HDL to transformation can be altered by triglyceride-lowering therapy in humans. Seventeen moderately hypertriglyceridemic individuals (nine with type II diabetes mellitus and eight moderately hypertriglyceridemic nondiabetic subjects) were studied before and after 3 months of triglyceride-lowering therapy with gemfibrozil. Since no significant differences in postprandial and postheparin HDL metabolism were detected between type II diabetic and nondiabetic subjects, results are reported for the two groups combined (N = 17). Fasting HDL was triglyceride-rich with a preponderance of HDL3, and became more enriched with triglycerides postprandially. Heparin administration resulted in a rapid decrease in plasma and HDL triglycerides and an increase in plasma and HDL free fatty acids (FFAs). Postheparin, there was a reduction in HDL size and an increase in the proportion of small (HDL3c) HDL particles (HDL3c constituted 7.1% ± 1.8% of total HDL preheparin and 26.6% ± 3.8% postheparin, $P < .001$). Triglyceride-lowering treatment resulted in a decrease in fasting triglycerides (−54%, $P < .001$) and HDL triglyceride content (−36%, $P = .002$), an increase in fasting HDL cholesterol (19%, $P = .004$), and proportionately fewer (13.2% ± 2.1%, $P < .001$) HDL3c particles formed postheparin. Postheparin HDL size correlated inversely with the fasting triglyceride level ($r = -.55$, $P < .001$) and HDL triglyceride concentration ($r = -.34$, $P = .02$). These results show that the postprandial increase in triglyceride levels in hypertriglyceridemic subjects is associated with increased production of small HDL particles when lipolysis is stimulated, and that lipid-lowering therapy can contribute to favorably reduce this postprandial production of small HDL particles. Further studies are needed to clarify how these abnormalities ultimately lead to a decrease of plasma HDL cholesterol and apo A-1 in hypertriglyceridemic states.

THERE HAVE BEEN MANY STUDIES in recent years examining the relationship between hypertriglyceridemia and high-density lipoprotein (HDL) metabolism, but the precise mechanism whereby hypertriglyceridemia leads to a decrease of HDL cholesterol remains to be clarified. A dynamic interaction between triglyceride-rich lipoprotein (TRL) and HDL in the circulation results in constant remodeling of HDL particles.[1] The transfer of triglyceride from TRL to HDL, accompanied by the reciprocal transfer of cholesteryl ester from HDL, is mediated by cholesteryl ester transfer protein (CETP).[2] Hypertriglyceridemia due to a variety of causes also results in increased mass transfer of triglyceride from TRL to HDL,[1] and levels of HDL cholesterol and apolipoprotein (apo) A-1, the major apolipoprotein of HDL, are inversely associated with hypertriglyceridemia.[3-9] The relation of triglyceridemia to HDL cholesterol levels does not appear to be linear, and most of the variation in plasma HDL cholesterol is observed at triglyceride concentrations less than 2.5 mmol/L.[10]

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Reciprocal transfer of triglycerides and cholesteryl esters is increased in the postprandial state, when chylomicrons and very–low-density lipoproteins increase in the circulation, resulting in HDL enriched with triglycerides.[11-15] Consequently, HDL isolated from hypertriglyceridemic individuals has been found to be triglyceride-rich.[11,16,17] The degree of fasting and postprandial triglyceride enrichment of HDL correlates inversely with the fasting HDL cholesterol concentration,[14,15,18] suggesting that triglyceride enrichment of HDL may be implicated in the process of HDL cholesterol lowering.

O'Meara et al[12] characterized HDL in normotriglyceridemic and hypertriglyceridemic subjects after a high-fat meal and heparin-induced release of lipase. In hypertriglyceridemic but not in normotriglyceridemic subjects, rapid lipolysis induced by heparin resulted in the formation of atypical HDL particles that were small but of reduced density. The quantity of small particles formed after lipolysis was directly related to the preheparin HDL triglyceride content. An unresolved question arising from that study was whether the transformation of HDL particles with heparin is an intrinsic property of the individual's HDL, or whether this process can be modified with lipid-lowering therapy, presumably by altering the composition of the HDL particles.

In the present study, we examined whether the generation of lipolysis-induced small HDL particles in hypertriglyceridemic individuals can be reduced after 3 months of triglyceride-lowering treatment with gemfibrozil. HDL density, size, and composition were characterized before and after a high-fat meal and then after administration of heparin to stimulate in vivo lipolysis in moderately hypertriglyceridemic subjects. Studies were repeated following 3 months of triglyceride-lowering therapy.

SUBJECTS AND METHODS

Subjects

Nine moderately hypertriglyceridemic subjects with type II diabetes and eight nondiabetic hypertriglyceridemic subjects participated in the study. The fasting lipid parameters, body mass index, and waist to hip ratio (WHR) of subjects with and without type II diabetes were similar. All subjects with type II diabetes had moderate glycemic control (hemoglobin A_{1C} [HbA_{1C}], 7% to 10%). No subject was treated with insulin. Seven type II diabetic subjects were treated with oral hypoglycemic agents (three with glyburide alone and four with glyburide plus metformin), and two individuals were diet-controlled. Oral hypoglycemic agents were continued throughout the study (but were withheld on the morning of the high-fat test meal), and no changes in dose were made. No subjects were taking any other medication known to affect lipid metabolism for at least 6 months before entrance into the study. Subjects with systemic illness, malignancy, or renal or hepatic disease were excluded from the study. Informed written consent was obtained from all participants in accordance with the guidelines of The Toronto Hospital Human Subjects Review Committee. All studies were conducted in The Toronto Hospital Clinical Investigation Unit.

Experimental Protocol

Subjects were instructed to consume their regular diet until 6 PM the evening before the fat-feeding study, and then to fast. At approximately 8 AM, an intravenous sampling catheter was inserted into a forearm vein and 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 meal contained 67.9% of calories from fat (polyunsaturated to saturated ratio, 0.526), 23.7% from carbohydrate, and 10.4% from protein. It consisted of a high-fat milkshake containing ice cream, cream, peanut butter, and corn oil and a cheese sandwich. After consuming the meal, the subjects did not eat again for the duration of the test meal study (5.25 hours). Blood samples were drawn hourly for measurement of glucose, free fatty acid (FFA), total cholesterol, HDL cholesterol, and triglyceride levels. Samples for lipid and lipoprotein analysis were collected into chilled tubes on ice containing 0.4 μmol/L/mL blood of the lipase inhibitor APBA (*m*-Aminophenylboronic acid; Sigma Pharmaceuticals, St Louis, MO). At 5 hours, additional blood samples were drawn for the detailed analysis of HDL. After the 5-hour blood sample was drawn, each subject received heparin sodium 60 U/kg body weight (Organon Tieknika, Toronto, Ontario, Canada) by intravenous injection. Fifteen minutes after heparin administration (5.25 hours after the test meal), a final blood sample was obtained for HDL analysis and plasma lipases.

After completing the initial fat-feeding study, all subjects received gemfibrozil 600 mg orally twice per day (Parke-Davis, Toronto, Ontario, Canada) for 3 months, following which an identical fat-feeding study was performed. Throughout this report, the initial fat-feeding study will be referred to as the pretreatment study, and the 3-month fat-feeding study as the posttreatment study.

Laboratory Measurements

Glucose was analyzed enzymatically using a Glucose Analyzer II (Beckman Instruments, Fullerton, CA). Cholesterol levels were measured using the CHOD-PAP enzymatic colorimetric kit (Boehringer Mannheim Diagnostica, Montreal, Quebec, Canada; catalog #236691) (intraassay coefficient of variation [CV], 1.4%; interassay CV, 2.9%). Triglycerides were measured as esterified glycerol using an enzymatic colorimetric kit (Boehringer Mannheim Diagnostica; catalog #450032). Free glycerol was eliminated from the sample in a preliminary reaction followed by enzymatic hydrolysis of triglyceride, with subsequent determination of the liberated glycerol by colorimetry (intraassay CV, 3.0%; interassay CV, 4.9%). FFA levels were measured by an enzymatic colorimetric method relying on the acylation of coenzyme A by the fatty acids (kit supplied by Wako Chemical Industries, Osaka, Japan; intraassay CV, 4.7%; interassay CV, 8.2%). The phospholipid content was measured using a kit (Boehringer Mannheim Diagnostica; catalog #691844; intraassay CV, 1.7%; interassay CV, 3.3%). The cholesteryl ester content was measured using the cholesterol kit after degrading free cholesterol in the samples with a mixture of cholesterol oxidase, peroxidase, phenol, Triton-x, and phosphate buffer (intraassay CV, 2.1%; interassay CV, 4.7%).[19] Free cholesterol was calculated as the difference between total cholesterol and cholesterylester. Protein levels were measured by the technique described by Lowry et al.[20] HDL cholesterol was also quantified on the supernatant remaining after precipitation of apo B–containing lipoproteins in the plasma with manganese heparin.[21] The apo A-1 level was measured using the Sebia Hydragel ApoA1B kit (Gelman Sciences, Toronto, Ontario, Canada, catalog #4050) according to the method of Laurell.[22]

Plasma Lipase Activity

Blood for lipase determination was collected into chilled tubes containing EDTA (1 mg/mL) 15 minutes after the 5-hour injection of heparin (60 U/kg). The plasma was separated immediately and then frozen at −70°C until the assay for lipoprotein lipase (LPL) and hepatic lipase (HL) activity. Both activities were determined by the method of Krauss et al,[23] after inhibition of LPL with protamine. The assay in this laboratory has previously been validated and cross-checked against assays using two preparations of anti–hepatic lipase antibodies, obtaining correlation coefficients (*r*) for the procedures of greater than .9 and greater than 85% inhibition of measurable LPL activity with protamine.[24]

Isolation of HDL

Blood samples for HDL determination were adjusted to a final concentration of 1.2 g/L sodium EDTA, 1 mmol/L PMSF, 0.1 g/L sodium azide, 1 mmol/L BHT, 80 mg/L chloramphenicol, 80 ng/L gentamicin sulfate, and 10,000 U/L kallikrein inhibitor. HDL was isolated by gradient-density ultracentrifugal flotation in a discontinuous 3% to 20% NaBr gradient for 66 hours at 38,000 rpm and 15°C in a Beckman SW40 Ti rotor (Beckman Instruments, Mississauga, Ontario, Canada). Using this simplified gradient procedure adapted by Cabana et al,[25] plasma from normolipidemic individuals produces well-defined peaks of HDL2 and HDL3 clearly separated from the bottom plasma fraction, thereby minimizing albumin contamination. Fractions of 0.4 mL were collected through a gradient fractionator with UV monitor at 280 nm. Fraction density was estimated by the refractive index compared with the refractive index of a standard salt solution of known density. The peak density of the HDL fraction was taken as the density of the fraction corresponding to the peak of the HDL refractance curve, which in these hypertriglyceridemic subjects corresponds to the major HDL3 fraction. By pooling tubes corresponding to the HDL peak subfraction density (1.063 to 1.210 g/mL), clean samples of HDL with minimal contamination from other fractions are obtained. Fractions were dialyzed in Tris-buffered saline (10 mmol/L Tris, 150 mmol/L NaCl, 0.01% EDTA, and 20 mmol/L NaN_3, pH 7.4) and used for lipoprotein analysis. HDL components were expressed as the percentage composition by weight. Since the FFA content of HDL is significantly increased postheparin, the mass of HDL FFA was also considered in the calculation of HDL composition of the postheparin samples. Using this high-salt equilibrium centrifugation, we generally find less than 10% apo A-1 in the bottom fraction.

Polyacrylamide Gradient Gel Electrophoresis

Particle size was analyzed by electrophoresis in a nondenaturing acrylamide system using 4% to 30% polyacrylamide gels for the

separation of HDL subspecies (purchased from Dr David Rainwater, Southwest Foundation for Biomedical Research, San Antonio, TX). Thirty micrograms of protein was applied as a mixture by volume with 3 parts sample and 1 part solution of 40% sucrose with 0.01% bromphenol blue. A mixture of standard molecular weight proteins (HMW Calibration Kit; Pharmacia, Piscataway, NJ) consisting of thyroglobulin (radius, 8.50 nm), ferritin (6.1 nm), catalase (5.20 nm), lactate dehydrogenase (4.08 nm), and bovine serum albumin (3.55 nm) was included in a separate lane in each gel. The gels were stained with Coomassie G250 in perchloric acid (0.1% stain and 5% perchloric acid), destained, and stored in 7% acetic acid. The radius of the particles was assessed by densitometric scanning using an Image Master DTS densitometer with Image Master computer software (Pharmacia, Uppsala, Sweden) based on the relative distance (R_f) of migration of the standard. HDL was subdivided by size into five subclasses (HDL 2b, 5.12 to 5.72 nm Stokes radius; 2a, 4.61 to 5.12 nm; 3a, 4.37 to 4.61 nm; 3b, 4.10 to 4.37 nm; and 3c, 3.77 to 4.10 nm) as defined by Verdery et al.[26] The relative quantity of HDL within each subclass was assessed by integrating the area under the curve between R_f values defined by Verdery et al.[26] HDL particle size was calculated and expressed in arbitrary units as a single summary measure using a modification of the method described by Calabresi et al[27]: (%HDL2b × 5) + (%2a × 4) + (%3a × 3) + (%3b × 2) + (%3c × 1). The higher the score, the larger the HDL particle size.

Statistical Analyses

Changes in clinical characteristics and fasting lipid parameters before and after triglyceride-lowering treatment were tested using a repeated-measures ANOVA. Changes in the response to the fat meal and to the heparin injection pre– and post–triglyceride-lowering treatment were tested using a two-way repeated-measures ANOVA in a general linear model. This statistical approach allowed the testing of three hypotheses: (1) whether triglyceride-lowering treatment had any effect on HDL composition after allowing for the effects of the fat meal and heparin, (2) whether the fat meal/heparin had any effect on HDL composition after allowing for the effects of triglyceride-lowering treatment, and (3) whether the interaction between these two factors was significant, ie, whether the response to the fat meal/heparin was different before and after triglyceride-lowering treatment. When significant treatment effects were observed, the Student-Newman-Keuls multiple-comparison test was used to determine which levels of treatment differed from the others. Critical P values for significant differences are automatically adjusted using this test to allow for the effect of multiple comparisons on P values. Areas under the concentration curves following the oral lipid load were calculated as summary measures of the postprandial increment above baseline levels using the trapezoidal method. Changes in the area under the curve following gemfibrozil were tested using a paired t test. Spearman rank-order correlations were calculated to investigate the potential relationship between basal and postprandial lipid levels and postlipolytic HDL composition before and after triglyceride-lowering treatment. All analyses were first performed by separating type II diabetic patients and controls. Because results were essentially similar in both groups of patients, we only report results from the total group of 17 individuals. All results are expressed as the mean ± SEM.

RESULTS

Fasting, Postprandial, and Postheparin Plasma Lipid Levels

Fasting lipid levels before and after treatment with gemfibrozil are presented in Table 1. There was a significant reduction in triglyceride levels after triglyceride-lowering therapy (54% reduction, $P < .001$) and a significant increase in HDL cholesterol (19%, $P = .004$). However, there was no change in fasting plasma apo A-1 or FFA concentrations posttreatment. In diabetic subjects, fasting glucose and HbA_{1c} did not change during the 3 months of triglyceride-lowering treatment (glucose, 8.29 ± 0.70 mmol/L baseline and 9.94 ± 1.62 mmol/L posttreatment; HbA_{1c}, 8.26% ± 0.50% pretreatment and 8.44% ± 0.65% posttreatment). There was a significant reduction in the triglyceride response (area under the curve above basal level from 0 to 300 minutes) to the fat meal posttreatment ($P < .001$; Fig 1). Injection of heparin at the peak of the lipid response to the fat meal (time 300 minutes) resulted in a substantial decrease in plasma triglycerides both pretreatment (1.7-fold) and posttreatment (2.2-fold, $P < .05$). The magnitude of the postheparin decrease in triglycerides (315 v 300 minutes) tended to be reduced following triglyceride-lowering treatment ($P = .06$ by paired t test). Plasma FFA levels did not increase substantially above baseline during the fat meal (0 to 300 minutes), but the area under the FFA response was nevertheless significantly reduced posttreatment ($P < .01$; Fig 1). Injection of heparin at the peak of the lipid response to the fat meal (300 minutes) resulted in a marked increase in plasma FFA levels both before and after triglyceride-lowering treatment. However, the postheparin increase (315 v 300 minutes) in plasma FFA concentrations was significantly reduced after triglyceride-lowering treatment ($P = .02$).

Table 1. Clinical Characteristics and Fasting Plasma Lipid Parameters Before and After Triglyceride-Lowering Treatment

Parameter	Pretreatment	Posttreatment	P*
Age (yr)	52.6 ± 2.4	—	
Gender (male/female)	11/6	—	
Body mass index (kg/m²)	29.3 ± .09	28.7 ± 0.9	.02
Triglycerides (mmol/L)	4.01 ± 0.48	1.75 ± 0.23	<.001
Cholesterol (mmol/L)	5.1 ± 0.3	4.8 ± 0.3	.22
HDL cholesterol (mmol/L)	0.83 ± .03	0.99 ± .06	.004
Apo A-1 (mg/dL)	98.7 ± 5.3	109.2 ± 6.4	.30
FFA (mmol/L)	0.72 ± .06	0.64 ± 0.06	.25

*Repeated-measures ANOVA.

Postheparin Plasma Lipases

The 3-month treatment with gemfibrozil had no significant effect on postheparin HL (9.44 ± 0.67 μmol FFA/mL · h pretreatment v 9.63 ± 0.78 μmol FFA/mL · h posttreatment, $P = .62$) or LPL activity (9.12 ± 0.48 μmol FFA/mL · h pretreatment v 8.64 ± 0.85 μmol FFA/mL · h posttreatment, $P = .29$).

HDL Density and Compositional Changes Postprandially and Postheparin

There was a nonsignificant shift to a lighter density of the major HDL peak (HDL3) postprandially. Pretreatment, the peak HDL density shifted from 1.110 ± 0.003 g/mL at baseline to 1.105 ± 0.003 g/mL postprandially (P = NS). Density changes comparing fasting and postprandial levels in the postgemfibrozil studies were similar. Postheparin, there were major changes in the density of HDL ($P < .001$). The HDL peak shifted to the left into the lighter HDL2 density range and HDL3 was no longer evident as a separate peak. The postheparin HDL peak

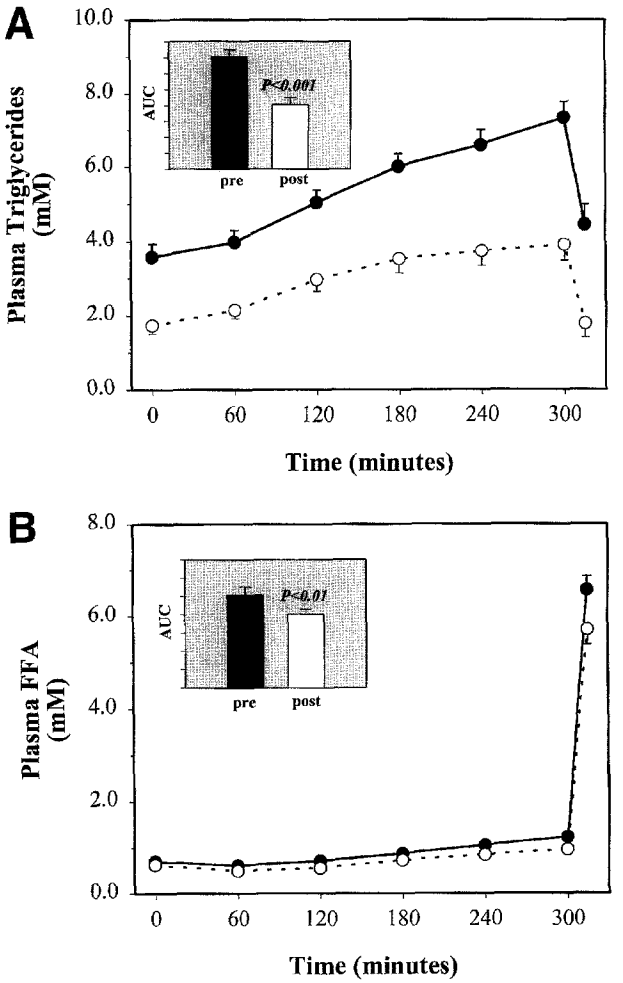

Fig 1. Plasma triglycerides (A) and FFA (B) in response to the fat meal and heparin injection. Studies were performed before (●) and after (○) triglyceride-lowering treatment. A high-fat meal was ingested at time 0 minutes, and intravenous heparin was administered at 300 minutes. Inset, pretreatment *v* posttreatment area above basal (ie, incremental area) under the concentration curve between 0 and 300 minutes.

density was identical pretreatment and posttreatment (1.086 ± 0.003 g/mL).

The proportion of triglycerides in HDL significantly increased postprandially and the proportion of cholesteryl ester was reduced both pretreatment and posttreatment (Table 2). Heparin administration resulted in a large reduction in the triglyceride content of HDL and a large increase in the FFA content of HDL. The FFA content of HDL increased from an undetectable level preheparin to 9% to 13% of total HDL weight postheparin. There was a significant effect of triglyceride-lowering treatment on reducing the triglyceride content of HDL ($P = .002$) postprandially and postheparin, whereas the postlipolytic increase in HDL FFA levels was similar before and after triglyceride-lowering treatment. No substantial change could be observed in the free cholesterol or phospholipid content of HDL following the fat meal/heparin and triglyceride-lowering treatment. There was also no significant interaction between the effect of triglyceride-lowering treatment and the response to the fat meal/heparin, suggesting that although the treatment had significant effects on some aspects of HDL composition, the response to the fat meal and heparin was not modified by the triglyceride-lowering treatment.

Changes in the proportion of HDL subfractions during the fat meal/heparin test and pretreatment and posttreatment are presented in Table 3. There were no major postprandial changes in any of the HDL subfractions either pretreatment or posttreatment. There were significant global effects of the fat meal and heparin on the relative proportion of HDL2b and HDL3a ($P = .04$), but multiple comparisons did not show a significant difference between each of the time points. There was a significant increase in the proportion of HDL3c following administration of heparin ($P < .001$), but a significant interaction was noted as triglyceride-lowering treatment substantially reduced the postlipolytic production of these small HDL3c particles ($P < .001$). Pretreatment, the HDL score (a crude index of average HDL size) was significantly reduced after heparin (from 293 ± 13 to 226 ± 10 U, $P < .05$), but this reduction became insignificant posttreatment, suggesting that the triglyceride-lowering treatment may have modified the response of the HDL size to heparin.

Figure 2 illustrates these results and also compares postprandial and postheparin changes in HDL triglycerides, HDL3c, and the HDL score (values at 315 minutes minus values at 300 minutes) pretreatment and posttreatment. Triglyceride-lowering treatment had no effect on the postprandial-postheparin change in HDL triglyceride. The postprandial-postheparin change in the proportion of HDL3c, on the other hand, was significantly reduced posttreatment (from 14% to 8.5%, $P < .005$), suggesting that fewer small HDLs were produced by stimulation of lipolysis posttreatment. Finally, the postprandial-postheparin reduction in the HDL score was substantially attenuated by triglyceride-lowering treatment (from −47 to −27 U of score, $P < .005$).

Correlations Between Postheparin HDL and Other Lipid and Lipoprotein Parameters

The postheparin HDL score correlated inversely with the fasting HDL triglyceride concentration ($r = -.34$, $P = .02$), fasting plasma triglyceride level ($r = -.55$, $P < .001$), and total area under the postprandial plasma triglyceride concentration curve ($r = -.49$, $P = .005$). Similar associations were observed with the postlipolytic proportion of HDL3c. In addition, the magnitude of reduction in the postprandial HDL triglyceride concentration with triglyceride-lowering treatment correlated negatively with the change in the postheparin HDL score. This suggests that the greater the reduction in postprandial triglyceride enrichment of HDL posttreatment, the fewer the small HDL particles formed postheparin. There were no significant correlations between plasma postheparin lipase activities or the LPL/HL ratio versus changes in HDL composition in response to the fat load or heparin.

DISCUSSION

We have demonstrated that heparin-induced lipolysis of HDL results in the generation of small, light HDL particles in hypertriglyceridemic individuals. The magnitude of reduction

Table 2. HDL Composition by Weight (%) Before and After Triglyceride-Lowering Treatment

Parameter	HDL-Protein	HDL-TG	HDL-FC	HDL-CE	HDL-PL	HDL-FFA
Pretreatment						
Fasting (0 min)	49.7 ± 1.8	16.8 ± 1.7	4.0 ± 0.6	8.9 ± 0.5	20.6 ± 1.2	ND
Postprandial (300 min)	44.5 ± 2.2§	22.9 ± 2.3§	4.0 ± 0.6	7.2 ± 0.4§	21.4 ± 1.2	ND
Postheparin (315 min)	43.7 ± 2.1	13.6 ± 1.8‖	3.6 ± 0.5	6.4 ± 0.5‖	22.3 ± 1.5	11.1 ± 1.3
Posttreatment						
Fasting (0 min)	51.3 ± 1.3	10.7 ± 0.8¶	4.4 ± 0.4	10.9 ± 0.5¶	22.5 ± 1.5	ND
Postprandial (300 min)	48.0 ± 1.9§	17.9 ± 2.0§¶	4.1 ± 0.4	8.6 ± 0.5§	21.4 ± 1.5	ND
Postheparin (315 min)	45.5 ± 1.7	8.7 ± 1.3‖¶	4.5 ± 0.5	8.1 ± 0.4	23.9 ± 1.6	11.4 ± 1.1
P						
Pretreatment *v* posttreatment*	.20	.002	.35	.004	.51	—
Lipid load + heparin effects†	<.001	<.001	.68	<.001	.04	—
Interaction‡	.44	.71	.52	.23	.41	—

NOTE. *P* values were obtained by 2-way repeated-measures ANOVA. Abbreviations: TG, triglycerides; FC, free cholesterol; CE, cholesterol ester; PL, phospholipids; ND, not detected. Pairwise multiple comparisons were performed when a significant effect of triglyceride-lowering treatment on the fat load/heparin was noted using the Student-Newman-Keuls test.

*Difference in the mean values pretreatment and posttreatment after allowing for the effects of time (fasting, postprandial, and postheparin).

†Difference in the mean values among the different periods (fasting, postprandial, and postheparin) after allowing for the effects of the triglyceride-lowering treatment.

‡Interaction between the effect of triglyceride-lowering treatment and the fat load/heparin on HDL composition.

$P < .05$: §0 *v* 300 minutes, ‖300 *v* 315 minutes, ¶Pretreatment *v* posttreatment (corresponding time points).

in HDL particle size after heparin correlated with plasma and HDL triglycerides. There was a smaller heparin-induced change in HDL size (ie, fewer small HDLs formed after heparin) after 3 months' triglyceride-lowering treatment. The larger the reduction in postprandial triglyceride enrichment of HDL posttreatment, the smaller the postheparin reduction in HDL size and formation of small HDL particles. These data suggest that triglyceride levels in plasma and in HDL may significantly determine the reduction in HDL size following intravascular lipolysis. No major differences were detected between control subjects and subjects with type II diabetes. The results for diabetic and nondiabetic subjects have therefore been pooled and analyzed as a single group. The reduction in HDL size in hypertriglyceridemia is therefore not specific to type II diabetes, and is a function of the hypertriglyceridemia frequently associated with diabetes and other insulin-resistant states.

It has been proposed that triglyceride enrichment of HDL renders the HDL particle vulnerable to lipolysis by HL, which reduces the size of HDL particles.[15] CETP transgenic mice have small triglyceride-rich HDL, from which apo A-1 is shed more rapidly than from the HDL of control mice.[28] Anti-LPL antibodies infused into monkeys produced marked hypertriglyceridemia, an increase in HDL triglyceride content, a reduction in HDL cholesterol and plasma apo A-1 levels, and a marked increase in the fractional catabolic rate (FCR) of HDL.[29] Transgenic mice with coexpression of hypertriglyceridemia (overexpression of the human apo CIII gene) and CETP have triglyceride-rich small HDLs with an increase in the HDL

Table 3. Proportion of HDL Subfractions Expressed as a Percentage of Total HDL Protein and HDL score Before and After Triglyceride-Lowering Treatment

Parameter	HDL2b	HDL2a	HDL3a	HDL3b	HDL3c	HDL Score
Pretreatment						
Fasting (0 min)	4.6 ± 2.7	25.5 ± 3.8	35.4 ± 1.4	31.1 ± 3.6	6.1 ± 1.7	290 ± 12
Postprandial (300 min)	6.0 ± 3.0	26.4 ± 3.5	31.9 ± 1.6	30.1 ± 3.7	7.1 ± 1.8	293 ± 13
Postheparin (315 min)	1.2 ± 0.7	11.9 ± 2.6‖	27.0 ± 2.5	34.8 ± 2.5	26.6 ± 3.8‖	226 ± 10‖
Posttreatment						
Fasting (0 min)	5.5 ± 1.6	28.2 ± 3.1	31.5 ± 1.2	29.4 ± 2.6	5.7 ± 1.4	290 ± 9
Postprandial (300 min)	5.3 ± 1.5	28.4 ± 3.4	33.3 ± 1.5	28.2 ± 2.7	4.9 ± 1.4	302 ± 9
Postheparin (315 min)	3.5 ± 1.0	21.8 ± 3.3¶	33.2 ± 1.8	28.3 ± 2.8	13.2 ± 2.1‖¶	274 ± 9¶
P						
Pretreatment *v* posttreatment*	.33	.30	.62	.42	.11	.12
Lipid load + heparin effects†	.04	<.001	.04	.40	<.001	<.001
Interaction‡	.49	.02	.002	.24	<.001	.003

NOTE. *P* values were obtained by 2-way repeated-measures ANOVA. Pairwise multiple comparisons were performed when a significant effect of treatment on the fat load/heparin was noted using the Student-Newman-Keuls test.

*Difference in the mean values pretreatment and posttreatment after allowing for the effects of time (fasting, postprandial, and postheparin).

†Difference in the mean values among the different periods (fasting, postprandial, and postheparin) after allowing for the effects of the triglyceride-lowering treatment.

‡Interaction between the effect of triglyceride-lowering treatment and the fat load/heparin on the proportion of HDL subfractions.

$P < .05$: §0 *v* 300 minutes, ‖300 *v* 315 minutes, ¶pretreatment *v* posttreatment (corresponding time points).

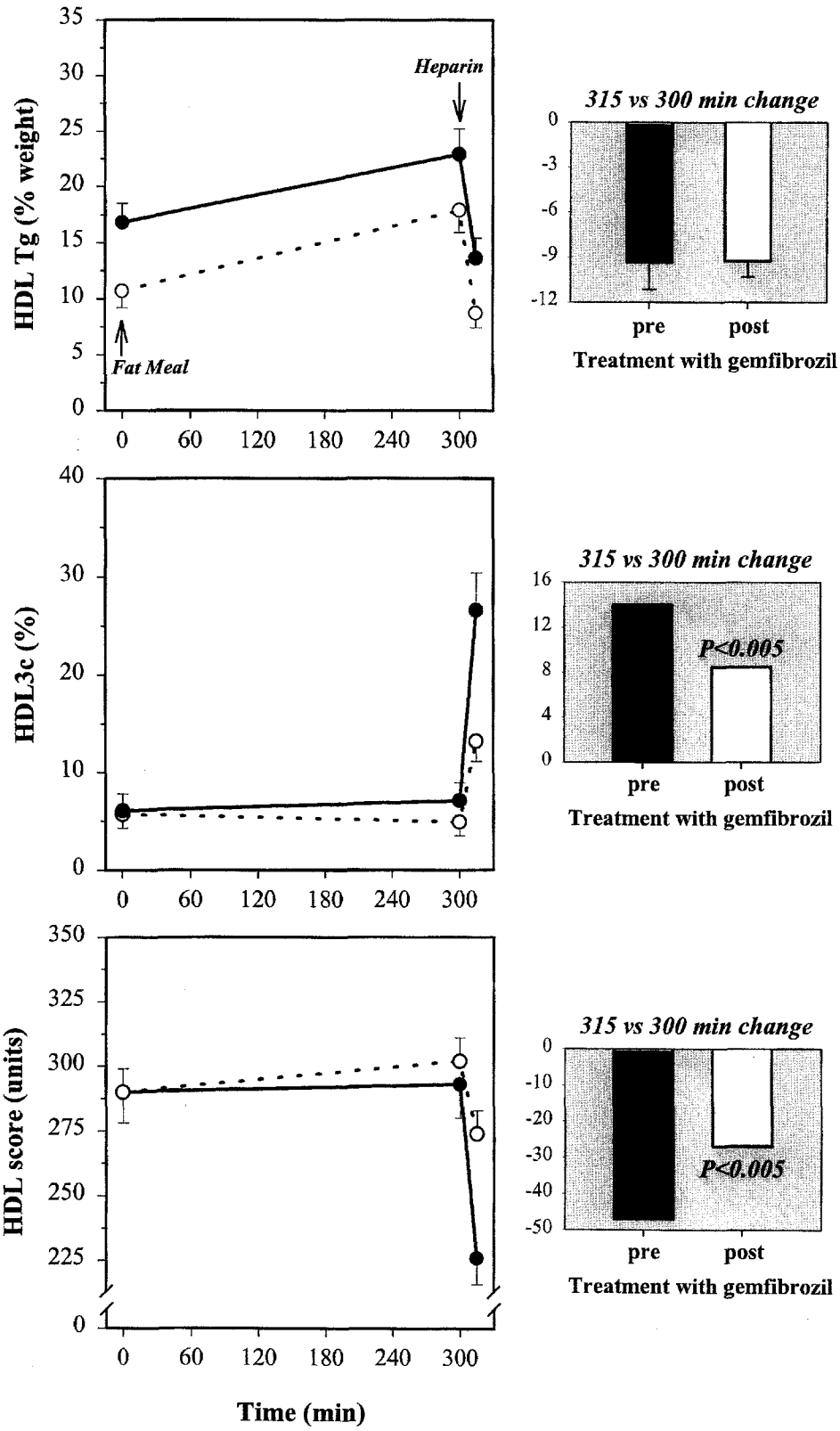

Fig 2. HDL compositional change during the fat meal/heparin before and after triglyceride-lowering treatment. A high-fat meal was ingested at 0 minutes and IV heparin was administered at 300 minutes (left). Studies were performed before (●) and after (○) 3 months of triglyceride-lowering treatment. Graphs at right illustrate the postprandial-postheparin change in HDL triglycerides, HDL3c, and the mean HDL size (score) values at 315 minutes minus values at 300 minutes pretreatment and posttreatment. Changes in HDL3c and the HDL score could not be normalized, and pretreatment *v* posttreatment values were tested using the Wilcoxon signed-rank test. The median is thus presented for these 2 variables. The mean ± SEM is presented for HDL triglycerides.

cholesteryl ester FCR.[30] Increased renal clearance of apo A-1 occurs when HDL that is cholesterol-depleted and relatively enriched with triglyceride interacts with plasma LPL and HL.[31] In the latter study, the investigators postulated that a reduction in HDL size resulting from such interactions leads to an increased apo A-1 dissociation from HDL. Clay et al[32] demonstrated that triglyceride-enriched HDL incubated with HL promotes not only a significant reduction in HDL triglycerides

but also an enhanced transfer of cholesteryl esters out of the particles and a marked loss of apo A-1 from the HDL fraction.

Subjects in the present study were moderately hypertriglyceridemic at baseline. The HDL was significantly enriched with triglycerides and smaller in size at baseline compared with HDL in normolipidemic individuals previously described by members of our group.[12] There was also a paucity of HDL2 and a predominance of HDL3 compared with the levels in normolipidemic controls.[12] These HDL findings have been described previously in hypertriglyceridemic humans[11,12,16,17] and HuCII-ICETP transgenic mice[30] and are typical of the hypertriglyceridemic state. It has been proposed that these abnormalities are due to increased exchange of core triglycerides in very–low-density lipoprotein for core cholesteryl esters in HDL, with subsequent hydrolysis of HDL triglycerides by plasma lipases.[30] This results in a diminution of HDL size, which has been associated with an increased FCR for apo A-1.[33,34]

Postprandially, there was an accentuation of the fasting abnormalities. Postprandial plasma triglycerides increased in proportion to fasting triglycerides as previously described.[11-15] HDL triglyceride content also increased postprandially, with a slight reduction in cholesteryl ester. There were no changes in HDL particle size postprandially, although HDL triglyceride enrichment was associated with a slight shift to a lighter density. Plasma FFAs also increased postprandially, but HDL FFA still could not be detected with our assay.

Gemfibrozil therapy resulted in a significant reduction in plasma and HDL triglycerides and an elevation in HDL cholesterol. Gemfibrozil has been shown by others to cause a number of direct and indirect changes affecting HDL metabolism, including an increase in LPL and HL activities,[35] although no consensus has emerged with respect to the effects on plasma or tissue lipase activities.[36] In the present study, we were unable to demonstrate any significant increase in either heparin-releasable LPL or HL activities. Gemfibrozil has been shown to cause an increase in the synthetic rates of apo A-1 and apo A-II[37] and to reduce apo CIII expression.[38] Others have shown no effect of gemfibrozil on CETP activity but a decrease in HDL-dependent transfer of cholesteryl ester due to alterations in the physicochemical characteristics of HDL.[39,40] The present study was not designed to determine the precise mechanism of the effect of gemfibrozil on the observed changes in HDL. Gemfibrozil was used as a means to reduce plasma triglycerides so that the effect of the latter on heparin-induced HDL transformation could be studied in the same subjects at different plasma triglyceride levels. However, the correlations between the HDL particle size changes and the changes in plasma and HDL triglycerides suggest that the lipid composition of HDL may play an important role in determining HDL metabolism. In the present study, it is also important to note that while fasting and postprandial triglycerides were significantly reduced by gemfibrozil, the levels were not normalized and a number of subjects remained mildly hypertriglyceridemic after gemfibrozil therapy (ie, fasting triglycerides > 1.5 mmol/L). Our study was not designed to compare postheparin HDL changes in hypertriglyceridemic individuals versus normotriglyceridemic controls. In addition, since we studied only hypertriglyceridemic individuals, we were unable to determine the threshold value below which postheparin changes in HDL may be minimal. However, the studies by O'Meara et al[12] showed no significant changes in subjects with maintained plasma triglycerides less than 2.26 mmol/L in the fasting and postprandial state.

As noted previously by O'Meara et al,[12] the small HDL particles generated postheparin were light, floating in the HDL2 density range in the ultracentrifuge. The LDL peak seen in the gradient density ultracentrifugal profile also shifted to a lighter density postheparin.[12] Forte et al[41] demonstrated the formation of small (5.8 nm diameter) spherical particles after heparin-induced lipolysis in hypertriglyceridemic subjects. They also found that heparin-induced lipolysis resulted in higher flotation rates of HDL. In the present study, plasma FFAs increased to supraphysiological levels postheparin and the HDL FFA content increased dramatically, contributing substantially to HDL lipid composition by weight. It is notable (Table 2) that the HDL FFA concentration was similar in postheparin samples pretreatment and posttreatment with the triglyceride-lowering agent, suggesting that the FFA content of HDL did not contribute to the difference in postheparin response pretreatment and posttreatment. This degree of particle enrichment with FFAs, accompanied by a decrease in protein, is sufficient to substantially decrease the HDL density.[42] Chung et al[43] found that 81% of serum FFAs are associated with plasma lipoproteins in the serum of hypertriglyceridemic subjects following heparin-induced lipolysis, with an 18- to 29-fold greater molar ratio of FFA to lipoprotein cholesterol in postlipolysis versus prelipolysis serum. They detected a greater increase in the FFA content of HDL versus LDL, consistent with the role of HDL as the primary acceptor of lipolytic surface remnants enriched with FFA. It is also apparent from the figures in the report by Chung et al[43] that there was a marked reduction in HDL density following lipolysis, although this was not discussed by them. We suggest therefore that the apparent paradoxical behavior of the postheparin HDL particles by ultracentrifugation and nondenaturing gel electrophoresis is best explained by the abnormal enrichment of lipoproteins with FFA, when released by the lipolysis of triglycerides in TRL and HDL.

Heparin administered at this dose causes a marked release of endothelial lipases into the circulation, resulting in supraphysiological levels of circulating lipase and marked lipolysis of plasma lipoproteins. HDL particles isolated postheparin are not normally measurable in the circulation of healthy normolipidemic humans, and therefore, the question of the physiological relevance of studying HDL particles modified in vivo by intravenous heparin needs to be addressed. However, one could argue that lipolysis occurring in the capillary microcirculation could result in the formation of particles having similar characteristics, which could then be rapidly cleared from the circulation. In addition, the dramatic changes in HDL size, density, and composition noted 15 minutes following intravenous heparin in the present study may occur with triglyceride-rich HDL to a lesser extent over a more protracted period, ultimately resulting in similar long-term effects on HDL.

In summary, we have shown that the postprandial increase in triglyceride levels in hypertriglyceridemic subjects is associated with an increased production of small HDL particles when lipolysis is stimulated, and that lipid-lowering therapy can contribute to favorably reduce this postprandial production of small HDL particles. The relative stability of postprandial HDL

in individuals with low triglyceride levels may be an important factor in determining the plasma concentration of HDL cholesterol. We have recently shown that small lipolytically modified human HDL particles are cleaved more rapidly from the circulation when injected into rabbits, compared with nonlipolytically modified triglyceride-rich HDL particles.[44] We conclude that the susceptibility of HDL particles to transformation by in vivo lipolysis can be modified in an individual by triglyceride-lowering therapy, presumably by altering the lipid composition of the particle and thereby decreasing the susceptibility to lipolysis-induced transformation.

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