Postprandial Lipoprotein Responses in Hypertriglyceridemic Subjects With and Without Cardiovascular Disease

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Three groups of age- and weight-matched men (aged 40 to 70 years) without diabetes were studied: controls (n = 10), plasma triglycerides (TG) less than 180 mg/dL and no cardiovascular disease (CVD); HTG − CVD (n = 11), hypertriglyceridemic (HTG) (TG > 240 mg/dL) without CVD; and HTG + CVD (n = 10), HTG (TG > 240 mg/dL) with documented CVD. HTG + CVD subjects had higher fasting and post–oral glucose tolerance test insulin levels than the other two groups, respectively. Very–low-density lipoprotein (VLDL) + chylomicrons (CMs), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL), and three high-density lipoprotein (HDL) subfractions (HDL-L, HDL-M, and HDL-D, from least to most dense) were isolated by gradient ultracentrifugation. Fasting lipoproteins were similar in HTG groups, except for higher VLDL lipid to apolipoprotein (apo) B ratios ($P < .04$) in the HTG + CVD group. Subjects were fed a high-fat mixed meal, and lipoprotein composition was determined at 3, 6, 9, and 12 hours postprandially. Postprandial responses of the core lipids (TG and cholesterol esters [CE]) in all of the lipoprotein subfractions were similar in the two HTG groups at each time point. However, both controls and HTG − CVD subjects had increases in HDL-M phospholipid (PL) at 9 and 12 hours with no change in HDL-D PL. The HTG + CVD group, on the other hand, had no increase in HDL-M PL and had a substantial reduction in HDL-D PL. These changes resulted in significant increases in HDL-M and HDL-D PL to apo A-I ratios in both controls and HTG − CVD subjects between 6 and 12 hours, whereas there was no increase seen in the HTG + CVD group. The HTG − CVD group also had a significantly greater increase in the VLDL + CM PL to apo B ratio ($P = .038$) at 3 hours than the HTG + CVD group. This diminished amount of surface lipid per VLDL particle may account for the late decrease in the HDL-D PL to apo A-I ratio seen in HTG + CVD patients. There were no other postprandial lipid or apolipoprotein differences between the two HTG groups. We conclude therefore that the major postprandial lipoprotein abnormality in these HTG + CVD patients was a failure to increase the PL content per particle in VLDL + CM, HDL-M, and HDL-D. This abnormality could prevent the usual increase in reverse cholesterol transport seen in postprandial plasma and therefore contribute to their increased incidence of CVD. The greater insulin resistance seen in these patients also appears to contribute significantly to their CVD.

AN ELEVATED FASTING plasma cholesterol[1-4] or low-density lipoprotein cholesterol (LDL-C)[1,4,5] and reduced high-density lipoprotein cholesterol (HDL-C) are the strongest and most consistent lipid risk factors for cardiovascular disease (CVD).[1,5] Other fasting lipoprotein parameters have also been associated with CVD. These include apolipoproteins (apos) A-I, A-II, B,[6,7] and C-III,[7] as well as various HDL,[8,9] LDL,[10-12] intermediate-density lipoprotein (IDL),[13] and very–low-density lipoprotein (VLDL)[14] parameters.

The impact of fasting hypertriglyceridemia on CVD is still controversial. Many studies comparing CVD patients with controls have shown that hypertriglyceridemia is frequently observed in these patients[6,7,13,15] and is a strong univariate determinant for CVD. However, it has been difficult to show an independent association in multivariate analysis. In addition, prospective studies have had difficulty demonstrating that hypertriglyceridemia is a risk factor for CVD independently of LDL-C and HDL-C.[2,3,16] Two recent studies[17,18] have suggested that hypertriglyceridemia may contribute to CVD by increasing the risk in patients who already have an adverse LDL/HDL ratio. Two familial disorders associated with hypertriglyceridemia also add to the controversy. Some families with familial hypertriglyceridemia do not appear to have an increased risk for CVD,[19] whereas others do.[20] On the other hand, most studies of familial combined hyperlipidemia demonstrate that these individuals are at high risk for CVD.[19,20] These data suggest that only some patients with hypertriglyceridemia are at increased risk for CVD. The problem that clinicians face is how to identify which patient with hypertriglyceridemia is at an increased risk and therefore requires aggressive therapy. One of the few fasting lipid parameters that appears to identify hypertriglyceridemic (HTG) patients at risk for CVD is a high concentration of apo B in LDL (hyperapobetalipoproteinemia), even when LDL cholesterol levels are normal or low.[10] However, many HTG patients with CVD (HTG + CVD) do not have hyperapobetalipoproteinemia.[21,22] Because none of the commonly measured fasting lipoprotein measurements have proven to be helpful in identifying HTG patients at high risk, we have chosen to test the hypothesis that postprandial lipoprotein metabolism (particularly HDL metabolism) is adversely altered in HTG patients who develop CVD.

Fat ingestion in nonhyperlipidemic individuals leads to a substantial increase in plasma phospholipid (PL) and triglycerides (TG)[23] over approximately 12 hours (peak

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Submitted August 17, 1994; accepted December 26, 1994.

Supported by a grant from the American Heart Association (Tennessee Affiliate), the General Clinical Research Center (MO1 RR00211), and the Diabetes Trust Fund in Medical Center East, Birmingham, AL.

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0026-0495/95/4408-0019$03.00/0

between 3 and 5 hours). Much of this lipid is transported in enlarged chylomicron (CM) and VLDL particles with minimal increases in total apo B concentrations or the number of apo B-100 particles.[24,25] However, the number of apo B-48 (CM) particles does increase postprandially.[26] VLDL + CM total cholesterol also increases (+150%) postprandially while LDL cholesterol decreases (−37%).[25] HDL PLs,[23] total plasma apo A-I and apo A-II,[27-29] gut apo A-I secretion,[30,31] and HDL_2[32] increase postprandially (usually between 6 and 9 hours). There is a shift of both HDL_2 and HDL_3 to slightly lower densities with the addition of PL to each subfraction and an increase in the PL to protein ratio.[29,33] The magnitude of plasma TG response to fat ingestion correlates with the fasting TG(+), but is best correlated with the total mass of fasting HDL_2(−).[34,35] During postprandial lipemia, there is a shift of apo C-III and apo C-II from HDL to VLDL + CM, with a return to HDL as lipemia resolves.[34,36,37]

Fasting hypertriglyceridemia is associated with a greater number of VLDL particles in each of four VLDL subfractions and a shift in the number of particles to the lower-density subfractions.[24] After fat ingestion, there is an increase in particle size of the largest particles, but no significant increase in the number of particles (similar to non-HTG subjects). Patients with hyperapobetalipoproteinemia[38] have higher and more-prolonged lipemic responses to fat than patients with normal LDL composition regardless of whether they have fasting hypertriglyceridemia. In addition, these patients have a substantial decrease in HDL_2 cholesterol after fat ingestion, an effect not usually seen in patients without hyperapobetalipoproteinemia.

Much of the research to date concerning postprandial lipoproteins and CVD has focused on accumulation of VLDL + CM remnants in plasma because of their high atherogenic potential. In non-HTG patients, there is a significant association between the peak postprandial TG concentration[39,40] and the remnant clearance rate[39,41] with angiographic CVD. In addition, postprandial apo B-48 concentrations have been shown to correlate with progression of coronary atherosclerosis.[42] The only study that has assessed HDL subfraction composition after fat ingestion in either non-HTG patients with CVD or HTG + CVD patients used nondenaturing gradient gel electrophoresis and rocket immunoelectrophoresis (to quantify lipoprotein [Lp]A-I and LpA-I:A-II).[43] This study failed to identify any postprandial HDL parameter that correlated with CVD.

In the current study, we have focused on three aspects of postprandial HDL metabolism to determine whether reverse cholesterol transport is defective in HTG + CVD patients versus HTG subjects without CVD (HTG − CVD). First, we examined the influx of TG and its subsequent metabolic effects. A greater postprandial influx of VLDL + CM TG in CVD patients could produce a more rapid transfer of cholesterol esters (CE) from HDL into the atherogenic, apo B–containing lipoproteins while increasing TG content of HDL. CE transfer protein activity has been shown to be increased by approximately 50% in normal postprandial plasma as compared with fasting plasma.[44] Even a small increase in CE transfer at a time when remnants are not being efficiently metabolized by the liver (ie, hypertriglyceridemia) could produce dire consequences. In addition, TG-rich HDL is more rapidly cleared from the plasma.[45] This faster clearance could result in a reduced number of the acceptor particles that initiate reverse cholesterol transfer. Second, we examined apo A-I metabolism. The typical increase in postprandial apo A-I[27-29] would be expected to have a substantial beneficial impact on reverse cholesterol transfer, and a failure to increase apo A-I could have a detrimental effect. Finally, we examined PL metabolism. Influx of VLDL + CM and HDL PL leads to lipoprotein particles that are less saturated in free cholesterol (FC) and are therefore better acceptor particles for cellular FC.[46,47] If patients with CVD have a reduced influx of HDL PL, this could clearly have an adverse effect on reverse cholesterol transport.

SUBJECTS AND METHODS

Patient Population

Three groups of men between 40 and 70 years of age were recruited for this study. Nonhyperlipidemic men (controls) had a fasting cholesterol less than 5.7 mmol/L (<220 mg/dL) and fasting TG less than 2.0 mmol/L (<180 mg/dL). HTG subjects had a fasting TG between 2.7 and 6.8 mmol/L (240 to 600 mg/dL). Control and HTG − CVD subjects could have no personal history of CVD or family history of premature CVD (first-degree relatives <55 years old). HTG + CVD subjects had to have had a myocardial infarct documented by typical ECG and enzyme changes or significant coronary artery disease on cardiac catheterization. Controls were matched to HTG − CVD subjects by age (±5 years) and weight (±10% ideal body weight).

Subjects were excluded if they had been treated with thiazide diuretics, β-blockers, or hypolipidemic medications within the previous 4 weeks. None of the subjects had ever been on probucol treatment. Subjects on medications that might alter gastrointestinal motility were also excluded, as were those with previous gastrointestinal surgery or disease. Other exclusions were as follows: (1) liver disease (bilirubin > 2 mg/dL) or renal disease (proteinuria or creatinine > 2 mg/dL); (2) insulin or sulfonylurea therapy or a fasting plasma glucose more than 130 mg/dL; (3) moderate to severe hypertension (blood pressure > 140/90 mm Hg on more than one medication); (4) any cigarette smoking in the previous 6 months or greater than 2 pack-years in the previous 5 years (number of packs per day times the years of smoking; for example, if a subject smoked one-half pack of cigarettes per day for 4 years, he would be eligible to participate); (5) greater than 180% ideal body weight; (6) unstable angina, myocardial infarction, or major surgery within the previous 6 months; and (7) diet less than 25% fat in the previous 2 weeks.

Protocol

Patients were recruited from the various Lipid Metabolism Clinics at the University of Tennessee, and normal subjects were recruited by local advertising and from previous studies. Once subjects were identified, they came to the Clinical Research Center, where informed consent (approved by the University of Tennessee and the Veterans Administration Hospitals Institutional Review Boards) was obtained. Each subject's medical and exercise history was obtained, and weight, height, waist and hip circumferences, and vital signs were measured. Screening laboratory studies were then obtained (complete blood cell count, chemistry profile, urinalysis, free thyroxine, thyrotropin, standard

fasting lipid profile, and a 2-hour, 75-g glucose tolerance test with insulin levels). The nutritionist then obtained a 3-day food diary from each patient, which was analyzed by the Nutritionist III program (N-Squared Computing, Salem, OR). The nutritionist also made skinfold and arm measurements to calculate percent body fat.

Each subject returned at 7:00 AM on the appointed day after a 14-hour fast. The test meal consisted of a milkshake, eggs, bread, butter, and cheese containing 50 g fat/m^2 body surface area, and was 55% fat, 30% carbohydrate, and 15% protein with 600 mg cholesterol/1,000 cal and a polyunsaturated to saturated fat ratio of 0.3. Aqueous vitamin A (60,000 U/m^2 body surface) was added to the milkshake to "label" chylomicrons and their remnants.[48]

Blood was drawn for lipoprotein compositional analysis and determination of lecithin cholesterol acyltransferase (LCAT) activity and VLDL and IDL retinol ester concentrations before the meal (8:00 AM) and 3, 6, 9, and 12 hours afterward. These times were chosen because preliminary studies indicated that VLDL + CM level peaks between 4 and 7 hours, HDL TG level peaks at 3 hours, and HDL PL and apo A-I peak between 6 and 12 hours after a fat load.[27-29] Postheparin lipase activities were measured during fasting and 6 and 12 hours postprandially. The 12-hour postprandial lipase determination was performed on the same day that lipoprotein compositional analyses were obtained. Subjects then returned twice over the next 2 weeks for determination of additional postheparin lipase activities at 7:30 AM (fasting) and 6 hours after the same test meal. Each lipase test was conducted at least 3 days apart to negate any effect from the previous heparin injection.

Lipoprotein Compositional Analysis

Lipoprotein separation by ultracentrifugation. Lipoproteins were isolated and analyzed as described previously[49] using our gradient-ultracentrifugation/high-performance liquid chromatography (HPLC) technique, except for recently reported revisions that were made to shorten the ultracentrifugation time.[50] Briefly, blood was drawn after a 12-hour fast into tubes containing EDTA and immediately placed on ice. Plasma was separated from blood cells by centrifugation at 4°C. NaN_3 (.02%) and α-tocopherol were added to the plasma to prevent bacterial contamination and lipid oxidation. Nine milliliters of plasma was increased to a density of 1.27 g/mL with 4.5 g KBr and added to 40-mL Quik-seal ultracentrifuge tubes (Beckman Instruments, Houston, TX). A second layer (NaCl 0.3%, EDTA 1.0 mmol/L, Tris 10 mmol/L, and NaN_3 0.1%, pH 8.5, containing 0.5 μg/mL glutathione and protease inhibitors, 6-aminohexanoic acid 100 mmol/L, benzamidine HCl 5 mmol/L, and phenylmethylsulfonyl fluoride 1.0 mmol/L) increased to a density of 1.22 g/mL with KBr was added to the centrifuge tube. Finally, the tube was filled with the above-described buffer without KBr (d = 1.006 g/mL) to a final volume of approximately 40 mL. The tubes were sealed and centrifuged to 6.0 × 10^{11} radian^2 · s^{-1} at 70,000 rpm (361,000 × *g*) at 15°C in a 70 Ti rotor (~3 hours and 15 minutes).

Ultracentrifuge tubes were emptied by pumping the effluent out of the top of the tube and collecting 1-mL fractions. The fractions were pooled into VLDL, IDL, LDL, and three HDL subfractions designated L, M, and D (lowest to highest density). These correspond roughly to HDL_{2b}, HDL_{2a+3a}, and HDL_{3b+3c}, respectively. As previously described,[49] each lipoprotein subfraction has been characterized by its usual density distribution and protein composition. LDL contained predominately cholesterol as the core lipid component, and the protein composition was greater than 99% apo B by sodium dodecyl sulfate (SDS) gradient gels. VLDL and IDL, on the other hand, contained apo C particles along with apo B and were more enriched in TG, relative to cholesterol, than LDL (~80% TG in VLDL and 50% in IDL). The major protein in each HDL subfraction was apo A-I, and the subfractions were subdivided based on apo A-II to A-I ratio. HDL-M had the highest apo A-II to A-I ratio and a medium buoyant density (d = 1.11 to 1.16 mg/mL), and both HDL-L (least dense) and HDL-D (most dense) had substantially lower apo A-II to A-I ratios.

Apolipoprotein analysis by HPLC. One milliliter of VLDL and each HDL pool and 2 mL IDL were delipidated first with 5 mL hexane/isopropanol (3:2) and then with 4 mL hexane alone. Human insulin, either Novolin-R (Novo Nordisk Pharmaceuticals, Princeton, NJ) or Humulin-R (Eli Lilly, Indianapolis, IN) (100 to 300 μg), was added as an internal standard to each sample before delipidation. The hexane layer was removed by suction, and the aqueous layer was concentrated (not dried) by speed-vacuum. Proteins were solubilized in 3 mol/L guanidine HCl in Tris buffer (described earlier, without the protease inhibitors) and injected onto the HPLC column. A Vydac (Hesperia, CA) reversed-phase C18 column (4.6 × 250 mm, no. 218ATP54) was used for analysis, and proteins were eluted with an acetonitrile/water gradient (containing 0.1% trifluoroacetic acid [TFA]) of 28% to 61% at 1%/min and a flow rate of 1.2 mL/min. The column was heated to 50°C in a water bath.

Enzymatic and chemical assays. Total cholesterol (reagent #352100; Sigma Diagnostics, St Louis, MO), FC (reagent #139 050; Boehringer Mannheim Diagnostics, Indianapolis, IN), TG (reagent #339-50; Sigma Diagnostics), and PLs (reagent #271-54008; Wako Pure Chemical Industries, Osaka, Japan) were assayed with commercially available kits using a 96-well microtiter plate reader. Each assay used standards obtained from the College of American Pathologists if available. Triplicates of both normal and elevated controls (provided with each kit) and a pooled plasma control prepared locally and stored at −80°C were assayed with each plate. KBr present in lipoprotein subfractions did not interfere with the results of these assays (except for FC in HDL-D). This laboratory participates in the laboratory quality-control testing program sponsored by the Centers for Disease Control for the total cholesterol and TG assays. The Centers for Disease Control does not provide unknowns for the FC or PL assays.

Apo B content of LDL was determined[49,51] by diluting an aliquot of LDL 1:5 with water and then mixing 100 μL diluted LDL with 100 μL 100-mmol/L SDS, 0.2N NaOH. Lowry reagents were added, and absorption was measured at 640 nm. Bovine serum albumin, in similar reagents, was used as a standard. Apo B concentrations in VLDL and IDL were determined by precipitating apo B with 50% isopropanol/water.[49,52] Three aliquots (100 μL) of each sample were washed twice with 1.0 mL isopropanol (IPA)/water and once with hexane/IPA (3:2). Apo B was pelleted by centrifugation (2,500 rpm for 20 minutes), and the supernatant containing soluble proteins and salt was poured off each time. The pellet was dried under vacuum and resolubilized in 100 μL 100-mmol/L SDS, 0.2N NaOH. This required incubation overnight at 37°C. One hundred microliters of water was added, and then the Lowry reagents as described earlier.

Other Assays

Plasma glucose level was measured by the glucose oxidase method on a Beckman Glucose Analyzer 2 (Fullerton, CA). Insulin level was measured with a double-antibody radioimmunoassay using Corning Medical Insulin reagents (Corning Medical, Medfield, MA). Free fatty acid levels were measured by an enzymatic colorimetric method using Wako NEFA C reagents.

Plasma Cholesterol Esterification Activity (LCAT activity)

This assay was adapted from an assay described by Patsch et al.[53] Fifty microliters of concentrated buffer (.15 mol/L NaCl, 100

mmol/L Tris, and 10 mmol/L EDTA) was added to 500 μl fresh plasma and kept on ice or in the refrigerator at all times. The sample was divided into two aliquots of 250 μL each. One 250-μL aliquot was left on ice while the other was incubated at 37°C for exactly 6 hours. Each aliquot was assayed in triplicate for FC. The change in FC was used to calculate absolute cholesterol esterification activity (micromoles per liter per hour). Each sample was incubated twice on consecutive days, and all samples from the same patient were analyzed in the same assay within 1 week of incubation.

Postheparin Lipase Activities

Five milliliters of blood was drawn into a clot tube 15 and 30 minutes after intravenous heparin (60 U/kg). The serum was separated from red blood cells and stored at −80°C until analysis. The method used has been described by Gamlen and Muller.[54] Briefly, sera were diluted 1:1 with 0.2 mol/L Tris HCl, pH 8.0, and analyzed with no NaCl and with 0.5 mol/L NaCl. One unit of heparin was added to 50 μL sera. Incubation was initiated by addition of substrate, [^{14}C]triolein with 0.36 mmol/L albumin, at a final concentration of 2.5 mmol/L, 0.05 μCi. The assay mixture was incubated for 10 minutes at 37°C. The reaction was terminated[55] by addition of chloroform:methanol:hexane followed by potassium bicarbonate. An aliquot of the upper phase was analyzed by scintillation spectrometry using hydrofluor. Percent recovery of radioactivity was used to calculate activity as micromoles of added substrate hydrolyzed per milliliter of serum per hour. Total lipase activity was activity observed without added salt, and hepatic lipase activity was activity observed in the presence of 0.5 mmol/L NaCl. Lipoprotein lipase activity was the difference between the total and the hepatic lipase. The highest value of the two measured values (15 and 30 minutes) was used in all analyses for each lipase.

Carotid Ultrasonography

Carotid atherosclerosis was quantified by B-mode ultrasound in two ways.[56,57] First, diagnosis was based on the presence or absence of disease as reflected by the maximum plaque (media + intima) thickness over a 30-mm wall segment on each side of each carotid artery. Individual boundaries between (1) the intima and lumen and (2) the media and adventia were located to a precision of 0.067 mm. Individual determinations of intima plus media thickness were made at 1-mm intervals along each segment of the arteries using a computer-controlled measurement station. A thickness greater than 2.0 mm was required to diagnosis a definite atherosclerotic plaque, and a thickness of less than 1.3 mm was considered normal.

Second, using the same image frames defined in the previous section, the mean wall thickness was obtained on all subjects to correlate severity of atherosclerosis with lipoprotein parameters. The mean wall thickness was determined from 32 measurements 1 mm apart in each of the left and right carotid arteries (internal, bifurcation, and common). All measurements were made from the far wall and included the intima and media. These measurements provide the global score for the degree of atherosclerosis used in this report.

Statistics

Two standard deviations from the mean of each lipoprotein parameter has been established as the normal limit for this laboratory. These limits were determined using unrelated, nonhyperlipidemic (total cholesterol < 240 mg/dL and total TG < 200 mg/dL) men and women recruited as controls for previous studies. The men (n = 34) and women (n = 34) had mean ages of 49.9 years (range, 24 to 67) and 46.2 (range, 26 to 69), respectively. Men were 129% (range, 89% to 223%) and the women 134% (range, 89% to 187%) of ideal body weight. Both groups were predominately white (men, 9% black; women, 26% black).

Data are presented as the mean ± SEM unless otherwise stated. Differences between groups were determined by ANOVA using the NPAR1WAY procedure in SAS (Statistical Analysis System; SAS Institute, Cary, NC). TG concentrations are frequently skewed, so we tested total plasma TG and VLDLTG determinations for normality. None of the three study groups could be shown to have distributions different from normal. Therefore, parametric analysis was appropriate. If variances of the two groups proved to be unequal, then Satterthwaite's approximation for reducing the degrees of freedom was used. Differences over time within the same group were determined by paired differences also using SAS.

The Logistic procedure in SAS was used to determine which fasting parameters were independent discriminators of CVD between the two HTG groups. Only the 17 variables that were shown to have group differences by ANOVA ($P < .1$) were used in the different models. A maximum of four variables were used in each model because of the limited number of subjects.

RESULTS

Patient Population—Demographic, Diet, Hormone, and Enzyme Measurements

Thirty-one subjects were studied: 10 controls, 11 HTG − CVD, and 10 HTG + CVD (Table 1). There were no differences in age, percent ideal body weight, or body mass index. The HTG − CVD group had a higher percent body fat and waist to hip ratio than controls, but the HTG + CVD group was not different from the other two groups. The HTG − CVD group consumed significantly less total calories, simple sugars, and dietary fiber than controls, whereas the HTG + CVD had an intermediate intake of total calories and a higher percentage of protein intake than controls. There were no significant dietary differences between the two HTG groups, and all three groups had similar percent total fat, percent carbohydrate, and cholesterol intakes. Two subjects from each group drank alcoholic beverages more than once per week. All except one subject ingested less than 15 g ethanol per day. One HTG − CVD subject ingested an average of 71 g ethanol daily. All intake of alcoholic beverages was stopped 2 weeks before these studies.

HTG + CVD subjects had higher fasting plasma glucose levels than controls, whereas both HTG groups had higher glucose concentrations after a standard, 75-g oral glucose tolerance test (Table 1). Several subjects in each HTG group had oral glucose tolerance tests that fulfilled diagnostic criteria for diabetes mellitus or glucose intolerance, even though they had no personal or family history of diabetes mellitus and had normal fasting plasma glucose concentrations. However, HTG + CVD subjects had significantly higher fasting and postprandial insulin concentrations and higher insulin to glucose ratios than either the controls or HTG − CVD subjects. There were no differences between the groups in fasting (Table 1) or postprandial (data not shown) free fatty acid levels.

There were no differences in fasting lipoprotein lipase or hepatic lipase activities. However, LCAT activity was significantly higher in the HTG + CVD group than in either of the other two groups (Table 1).

Table 1. Demographic, Diet, Hormone, and Enzyme Measurements

Characteristic	Controls	HTG − CVD	*P* v Controls	HTG + CVD	*P* v Controls	*P* v HTG − CVD
No. of subjects	10	11		10		
Age (yr)	57.2 ± 2.9	54.6 ± 2.8		56.9 ± 2.8		
%IBW	122 ± 7	122 ± 6		124 ± 7		
BMI (kg/m² × 100)	28.9 ± 1.9	29.6 ± 1.7		29.2 ± 1.2		
Body fat (%)	21.1 ± 2.8	27.2 ± 1.3	.055	25.9 ± 1.2		
WHR	0.932 ± 0.013	0.974 ± 0.014	.044	0.953 ± 0.015		
Dietary intake						
kcal	2,577 ± 175	1,742 ± 168	.003	2,150 ± 120	.064	.062
Cholesterol (mg)	312 ± 57	279 ± 51		271 ± 32		
Simple sugars (g)	105 ± 14	54 ± 9	.006	77 ± 10		
Dietary fiber (g)	20.2 ± 2.9	11.9 ± 1.5	.019	14.2 ± 1.7		
Protein (%)	16 ± 1	18 ± 1		20 ± 2	.042	
Carbohydrate (%)	49 ± 2	47 ± 3		48 ± 2		
Fat (%)	35 ± 2	33 ± 2		32 ± 2		
Fasting glucose (mmol/L)	5.3 ± 0.2	5.6 ± 0.1		5.9 ± 0.2	.03	
PP glucose sum*	9.0 ± 1.3	14.5 ± 1.6	.022	19.2 ± 2.2	.002	
Fasting insulin (pmol/L)	87 ± 19	62 ± 13		170 ± 26	.019	.001
PP insulin sum*	1,458 ± 228	2,310 ± 486		4,032 ± 624	.002	.043
Fasting insulin to glucose ratio	16.5 ± 3.6	10.9 ± 2.2		27.9 ± 3.6	.039	.0006
PP insulin to glucose ratio	60.5 ± 7.1	68.2 ± 11.5		112.2 ± 15.3	.009	.034
Fasting FFA (mEq/L)	0.509 ± 0.041	0.557 ± 0.041		0.559 ± 0.039		
LCAT (μmol/L/h)	59 ± 5	65 ± 5		89 ± 7	.005	.017
LPL (μmol/mL/h)	24.4 ± 5.9	14.6 ± 3.4		13.3 ± 4.1		
HL (μmol/mL/h)	7.28 ± 1.35	3.03 ± 0.54		4.96 ± 2.19		
Carotid score (mm)	1.98 ± 0.14	2.11 ± 0.13		2.17 ± 0.15		

Abbreviations: FFA, free fatty acid; LPL, lipoprotein lipase; HL, hepatic lipase; PP, postprandial; %IBW, percent ideal body weight; BMI, body mass index; WHR, waist to hip ratio.

*PP sum minus fasting level after a 2-hour, 75-g oral glucose tolerance test.

The carotid score as determined by carotid ultrasonography tended to be higher in HTG groups, but these differences were not significant (Table 1). Definite plaques were seen in all three groups.

Fasting Lipoprotein Composition

There were no differences in standard lipoprotein measurements between the two HTG groups (Table 2). Both HTG groups had higher total plasma cholesterol, TG, apo B, and apo C concentrations than controls. However, all three groups had similar total apo A-I and apo A-II levels, as well as similar LDL/HDL cholesterol ratios. The HTG − CVD group did have a higher apo A-II to A-I ratio than the controls.

As expected, all VLDL lipids and apolipoproteins were higher in HTG groups as compared with controls (Table 3). However, VLDL particle composition (lipid ratios and lipid to apo B ratios) of the HTG − CVD group was normal, whereas VLDL particles of HTG + CVD subjects were enriched in all lipids when adjusted for apo B levels. This lipid enrichment of VLDL was one of the two significant fasting lipoprotein differences between HTG groups (the other being the reduced HDL-D apo A-II concentrations discussed later).

Table 2. Total Plasma Lipoproteins and Apolipoproteins (fasting)

Plasma Component	Controls	HTG − CVD	*P* v Controls	HTG + CVD	*P* v Controls	*P* v HTG − CVD
Total cholesterol (mmol/L)	4.65 ± 0.26	6.13 ± 0.44	.012	6.49 ± 0.54	.01	
TG (mmol/L)	1.00 ± 0.15	3.41 ± 0.40	.0001	4.02 ± 0.63	.0009	
PL (mmol/L)	2.13 ± 0.11	2.67 ± 0.28		3.51 ± 0.39	.006	.09
LDL-C (mmol/L)	2.82 ± 0.16	2.87 ± 0.34		2.90 ± 0.31		
HDL-C (mmol/L)	1.22 ± 0.10	1.03 ± 0.05		1.06 ± 0.05		
Apolipoproteins (mg/dL)						
B	78 ± 5	102 ± 8	.03	102 ± 10	.07	
A-I	143 ± 8	137 ± 5		145 ± 6		
A-II	40 ± 3	45 ± 1		43 ± 2		
C-III	9.3 ± 0.9	23.1 ± 2.2	.0001	23.7 ± 2.6	.0003	
C-II	3.4 ± 0.6	6.1 ± 0.8	.018	5.7 ± 0.7	.032	
C-I	6.7 ± 0.5	10.1 ± 0.8	.004	10.2 ± 1.4	.035	
LDL-C/HDL-C	2.47 ± 0.23	2.71 ± 0.26		2.74 ± 0.26		
Apo B/A-I (molar)	0.029 ± 0.002	0.039 ± 0.003	.024	0.036 ± 0.004	.09	
Apo A-II/A-I (molar)	0.45 ± 0.02	0.54 ± 0.01	.002	0.48 ± 0.03		.08
Apo C-II/C-III (molar)	0.40 ± 0.06	0.28 ± 0.02	.074	0.26 ± 0.01	.042	

Abbreviations: LDL-C, LDL cholesterol; HDL-C, HDL cholesterol.

Table 3. VLDL, IDL, and LDL Lipids and Apolipoproteins (fasting)

Parameter	Controls	HTG − CVD	*P* v Controls	HTG + CVD	*P* v Controls	*P* v HTG − CVD
VLDL						
Total cholesterol (mmol/L)	0.34 ± 0.05	1.47 ± 0.16	.0001	1.71 ± 0.34	.003	
FC (mmol/L)	0.21 ± 0.03	0.78 ± 0.08	.0001	0.85 ± 0.16	.002	
CE (mmol/L)	0.15 ± 0.04	0.70 ± 0.11	.0003	0.87 ± 0.19	.005	
TG (mmol/L)	0.54 ± 0.09	2.36 ± 0.27	.0001	2.73 ± 0.44	.0007	
PL (mmol/L)	0.23 ± 0.04	0.81 ± 0.12	.0007	1.12 ± 0.20	.001	
Total mass (mg/dL)	97 ± 16	373 ± 40	.0001	439 ± 71	.0009	
Apolipoproteins (mg/dL)						
B	3.59 ± 1.58	14.49 ± 1.91	.0003	9.98 ± 1.67	.013	.09
C-III	2.79 ± 0.54	13.59 ± 1.98	.0002	14.18 ± 2.02	.0003	
C-II	0.852 ± 0.162	3.959 ± 0.624	.0005	3.49 ± 0.61	.002	
C-I	0.749 ± 0.173	3.818 ± 0.621	.0005	3.95 ± 0.79	.003	
FC/PL (molar)	0.85 ± 0.04	1.39 ± 0.43		0.80 ± 0.08		
CE/TG (molar)	0.28 ± 0.04	0.30 ± 0.04		0.30 ± 0.03		
ApoC-II/C-III (molar)	0.34 ± 0.02	0.32 ± 0.03		0.26 ± 0.02	.014	.1
FC/apo B (molar)	4,742 ± 1,150	3,223 ± 312		5,466 ± 923		.041
CE/apo B (molar)	3,067 ± 643	2,684 ± 301		5,213 ± 881	.08	.021
TG/apo B (molar)	12,830 ± 2,541	10,103 ± 1,302		17,541 ± 2,541		.014
PL/apo B (molar)	5,490 ± 1,171	3,316 ± 527	.08	7,393 ± 1,391		.023
IDL						
Total cholesterol (mmol/L)	0.28 ± 0.05	0.75 ± 0.08	.0001	0.80 ± 0.13	.002	
FC (mmol/L)	0.13 ± 0.03	0.31 ± 0.03	.0001	0.34 ± 0.05	.0009	
CE (mmol/L)	0.17 ± 0.02	0.43 ± 0.06	.0002	0.47 ± 0.08	.005	
TG (mmol/L)	0.18 ± 0.03	0.50 ± 0.08	.002	0.60 ± 0.09	.001	
PL (mmol/L)	0.12 ± 0.01	0.29 ± 0.04	.0008	0.37 ± 0.07	.003	
Total mass (mg/dL)	47 ± 6	117 ± 12	.0001	134 ± 17	.0006	
Apolipoproteins (mg/dL)						
B	3.63 ± 0.63	11.61 ± 1.49	.0003	11.61 ± 2.14	.005	
C-III	0.62 ± 0.12	2.75 ± 0.58	.004	2.71 ± 0.39	.0004	
C-II	0.19 ± 0.06	0.69 ± 0.14	.005	0.59 ± 0.10	.004	
C-I	0.25 ± 0.08	0.81 ± 0.16	.009	0.761 ± 0.14	.008	
FC/PL (molar)	1.08 ± 0.07	1.47 ± 0.46		0.97 ± 0.09		
CE/TG (molar)	0.96 ± 0.11	1.01 ± 0.20		0.83 ± 0.12		
ApoC-II/C-III (molar)	0.29 ± 0.04	0.27 ± 0.02		0.24 ± 0.02		
FC/apo B (molar)	2,101 ± 298	1,732 ± 341		1,775 ± 256		
CE/apo B (molar)	2,643 ± 301	2,456 ± 591		2,404 ± 342		
TG/apo B (molar)	3,068 ± 521	3,087 ± 1,023		3,576 ± 837		
PL/apo B (molar)	1,932 ± 234	1,669 ± 447		2,262 ± 681		
LDL						
Total cholesterol (mmol/L)	2.82 ± 0.16	2.87 ± 0.34		2.90 ± 0.31		
FC (mmol/L)	0.91 ± 0.03	0.85 ± 0.10		0.80 ± 0.08		
CE (mmol/L)	1.91 ± 0.13	2.00 ± 0.23		2.11 ± 0.26		
TG (mmol/L)	0.18 ± 0.01	0.29 ± 0.03	.011	0.37 ± 0.06	.011	
PL (mmol/L)	0.84 ± 0.04	0.79 ± 0.11		0.99 ± 0.09		
Total mass (mg/dL)	286 ± 13	301 ± 30		328 ± 26		
Apo B (mg/dL)	71 ± 5	76 ± 8		80 ± 8		
FC/PL (molar)	1.08 ± 0.04	1.39 ± 0.40		0.86 ± 0.09	0.034	
CE/TG (molar)	11.17 ± 0.69	7.96 ± 1.47	.07	7.22 ± 1.32	.016	
FC/apo B (molar)	721 ± 47	626 ± 31	.1	551 ± 38	.012	
CE/apo B (molar)	1,523 ± 145	1,430 ± 93		1,513 ± 197		
TG/apo B (molar)	140 ± 15	241 ± 38	.03	269 ± 50	.032	
PL/apo B (molar)	672 ± 51	583 ± 56		715 ± 93		

IDL had a normal particle composition in both HTG groups, even though there was a twofold to threefold excess of all lipids and apolipoproteins in this subfraction (Table 3). LDL composition and mass were similar in HTG groups, but there was a twofold increase in TG in both HTG groups as compared with controls. This resulted in a decrease in their CE/TG ratios and an increase in their TG/apo B ratios (Table 3).

Similar to LDL, there was a twofold to threefold increase in TG

in each of the three HDL subfractions (Table 4) in both HTG groups. Both FC and CE in HDL-L and HDL-M were slightly lower in HTG subjects versus controls, but only the reduction in HDL-M CE in HTG − CVD subjects was statistically significant. Total cholesterol in HDL-D, on the other hand, tended to be higher in HTG groups. Similarly, there were significant reductions in apo A-I in HDL-L of HTG subjects, whereas there were significant increases in apo A-I in HDL-D. Apo A-II was similar in all three groups in each of the HDL subfractions, except that it was higher in HDL-D of HTG − CVD subjects than in either controls or the HTG + CVD group. This increase in apo A-II produced a significant increase in the apo A-II to A-I ratio in this subfraction. This was the most significant fasting HDL difference between HTG groups.

Table 4. HDL Lipids and Apolipoproteins (fasting)

Parameter	Controls	HTG − CVD	*P* v Controls	HTG + CVD	*P* v CVD	*P* vControls	*P* v HTG − CVD
HDL-L							
Total cholesterol (mmol/L)	0.36 ± 0.05	0.31 ± 0.03		0.31 ± 0.03			
FC (mmol/L)	0.12 ± 0.02	0.11 ± 0.01		0.09 ± 0.01			
CE (mmol/L)	0.25 ± 0.04	0.21 ± 0.04		0.21 ± 0.02			
TG (mmol/L)	0.04 ± 0.01	0.08 ± 0.02	.021	0.10 ± 0.03	.044		
PL (mmol/L)	0.24 ± 0.03	0.15 ± 0.03	.021	0.21 ± 0.03		.09	
Total mass (mg/dL)	66 ± 8	48 ± 14	.056	59 ± 5			
Apolipoproteins (mg/dL)							
A-I	18.9 ± 2.2	8.9 ± 1.2	.0008	11.7 ± 1.6	.017		
A-II	4.4 ± 0.7	3.1 ± 0.4		4.4 ± 0.5		.07	
C-III	1.6 ± 0.2	1.6 ± 0.2		1.9 ± 0.2			
C-II	0.41 ± 0.05	0.27 ± 0.05	.08	0.32 ± 0.04			
C-I	1.18 ± 0.14	0.87 ± 0.08	.06	1.12 ± 0.14			
FC/PL (molar)	0.53 ± 0.04	1.04 ± 0.26	.08	0.49 ± 0.08		.07	
CE/TG (molar)	7.49 ± 1.09	4.21 ± 0.97	.036	3.69 ± 0.97	.018		
Apo A-II/A-I (molar)	0.39 ± 0.04	0.61 ± 0.04	.003	0.62 ± 0.04	.002		
FC/apo A-I (molar)	19.2 ± 1.9	39.4 ± 6.2	.009	26.6 ± 5.5			
CE/apo A-I (molar)	36.7 ± 3.0	81.0 ± 15.4	.017	56.9 ± 9.0	.055		
TG/apo A-I (molar)	5.7 ± 0.8	37.8 ± 13.6	.041	28.3 ± 9.8	.048		
PL/apo A-I (molar)	36.5 ± 1.7	51.1 ± 8.4		53.6 ± 6.3	.025		
HDL-M							
Total cholesterol (mmol/L)	0.67 ± 0.05	0.52 ± 0.03	.011	0.57 ± 0.03	.09		
FC (mmol/L)	0.16 ± 0.01	0.14 ± 0.01		0.14 ± 0.02			
CE (mmol/L)	0.49 ± 0.04	0.38 ± 0.02	.016	0.43 ± 0.02			
TG (mmol/L)	0.05 ± 0.01	0.12 ± 0.02	.013	0.15 ± 0.04	.038		
PL (mmol/L)	0.57 ± 0.04	0.47 ± 0.05	.09	0.63 ± 0.05		.03	
Total mass (mg/dL)	203 ± 13	190 ± 8		203 ± 13		.09	
Apolipoproteins (mg/dL)							
A-I	86 ± 6	80 ± 4		85 ± 3			
A-II	28 ± 3	30 ± 1		30 ± 1			
C-III	3.3 ± 0.3	4.2 ± 0.4		4.1 ± 0.5			
C-II	1.32 ± 0.25	0.93 ± 0.11		1.04 ± 0.19			
C-I	3.4 ± 0.2	3.6 ± 0.3		3.69 ± 0.46			
FC/PL (molar)	0.29 ± 0.03	0.39 ± 0.10		0.23 ± 0.03			
CE/TG (molar)	11.79 ± 1.10	5.20 ± 1.12	.0005	4.80 ± 1.02	.0002		
Apo A-II/A-I (molar)	0.53 ± 0.02	0.61 ± 0.01	.009	0.57 ± 0.02			
FC/apo A-I (molar)	5.4 ± 0.6	5.0 ± 0.3		4.6 ± 0.6			
CE/apo A-I (molar)	16.5 ± 0.7	13.6 ± 0.5	.002	14.2 ± 0.7	.029		
TG/apo A-I (molar)	1.5 ± 0.1	4.3 ± 0.9	.011	4.9 ± 1.3	.035		
PL/apo A-I (molar)	18.8 ± 0.7	16.6 ± 1.5		20.7 ± 1.5		.07	
HDL-D							
Total cholesterol (mmol/L)	0.17 ± 0.01	0.20 ± 0.02		0.20 ± 0.02			
TG (mmol/L)	0.02 ± 0.00	0.06 ± 0.01	.013	0.07 ± 0.02	.054		
PL (mmol/L)	0.15 ± 0.01	0.17 ± 0.01		0.21 ± 0.01	.011		
Total mass (mg/dL)	67 ± 4	87 ± 5	.006	88 ± 6	.012		
Apolipoproteins (mg/dL)							
A-I	38 ± 2	48 ± 3	.017	48 ± 4	.044		
A-II	7.2 ± 0.5	11.8 ± 0.8	.0001	8.6 ± 0.8		.01	
Apo A-II/A-I (molar)	0.31 ± 0.02	0.41 ± 0.02	.004	0.30 ± 0.03		.015	
TC/apo A-I (molar)	13.0 ± 0.6	11.9 ± 0.5		11.8 ± 0.7			
TG/apo A-I (molar)	1.3 ± 0.1	3.2 ± 0.6	.014	3.5 ± 0.9	.038		
PL/apo A-I (molar)	11.1 ± 0.5	10.5 ± 1.0		12.6 ± 1.3			

Compared with controls, both HTG groups had a lower total mass of HDL-L and a higher total mass of HDL-D, with TG enrichment of the core lipid in all three HDL subfractions (Table 4). HDL-L composition was affected the most by hypertriglyceridemia, with substantial increases in lipid to apo A-I ratios and apo A-II to A-I ratios. These changes suggest a decrease in the number of HDL-L particles, particularly those particles containing only apo A-I, with an enlargement of the remaining particles by addition of TG. However, these changes in HDL-L did not help to identify those HTG subjects with CVD.

Postprandial Lipoprotein Changes—Core Lipids

HTG groups were found to have significantly different core lipid postprandial responses only in the IDL subfraction. As expected, there were highly significant (~twofold) increases in VLDL + CM TG in all three patient groups (Fig 1). Controls peaked between 3 and 6 hours, whereas both HTG groups peaked at 6 hours. Increases in IDL TG were temporally similar to VLDL + CM TG but of smaller magnitude. However, IDL TG had decreased below baseline by 9 hours and continued to decrease at 12 hours in HTG + CVD subjects, whereas levels were still significantly higher than baseline in HTG − CVD patients at 9 hours. This reduction in IDL TG was the only statistically significant core lipid difference ($P = .019$) between the two HTG groups. However, a similar phenomenon was seen in LDL, where controls and HTG − CVD subjects had small increases in LDL TG at 3 and 6 hours, whereas the HTG + CVD group had a steady decline in TG (which reached significance at 12 hours). All three groups had increases in VLDL + CM CE at 9 hours, whereas there were significant reductions in IDL and LDL CE by 6 hours.

No significant differences of HDL core lipids were found in HTG groups. There was a small increase in TG concentration between 3 and 6 hours, with a subsequent decline (frequently to < initial concentration at 12 hours) in all three groups and in all three subfractions (Fig 2). There was little change in CE concentrations in HDL-L in any of the groups. However, there were significant reductions in CE in HDL-M (nadirs at 6 hours), with a return to baseline by 12 hours. Total cholesterol changes in HDL-D were similar to HDL-M CE, except that HTG groups had not returned to preprandial levels by 12 hours.

CE/TG ratio changes were almost identical in the two HTG groups. There were substantial reductions in VLDL + CM CE/TG ratio with the postprandial influx of TG in all three groups, but with some delay in HTG groups (Fig 3). This change in core composition in VLDL led to significant reductions in HDL-M CE/TG ratio (~50%) over the same time course. The decline in HDL-M CE/TG ratio in both HTG groups was less dramatic in both absolute and percentage terms, suggesting less net transfer of CE and TG in these subjects. This may have been due to their much lower preprandial CE/TG ratios initially.

Postprandial Lipoprotein Changes—Surface Lipids

There were significant increases in VLDL + CM PL in all three groups (Fig 4), with patterns similar to VLDL + CM TG. Controls and HTG − CVD groups had increases in

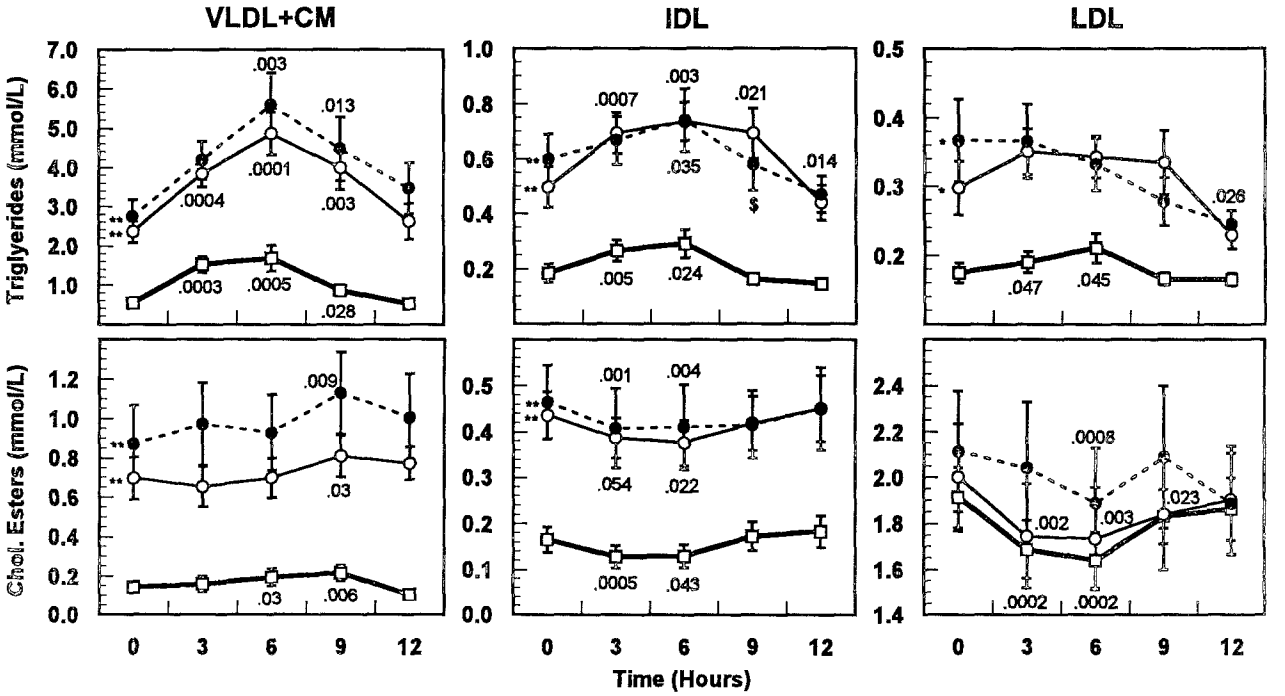

Fig 1. Postprandial responses of VLDL + CM, IDL, and LDL TG and CE to a high-fat meal in non-HTG men without CVD (controls, □), HTG−CVD men (○), and HTG + CVD men (●). Numbers are *P* values for paired differences within each group between time zero (fasting) and the postprandial time point next to the number. *$P < .05$, **$P < .01$: Fasting values of controls *v* HTG group. ‡Significant difference ($P < .05$) in changes from time zero between the two HTG groups.

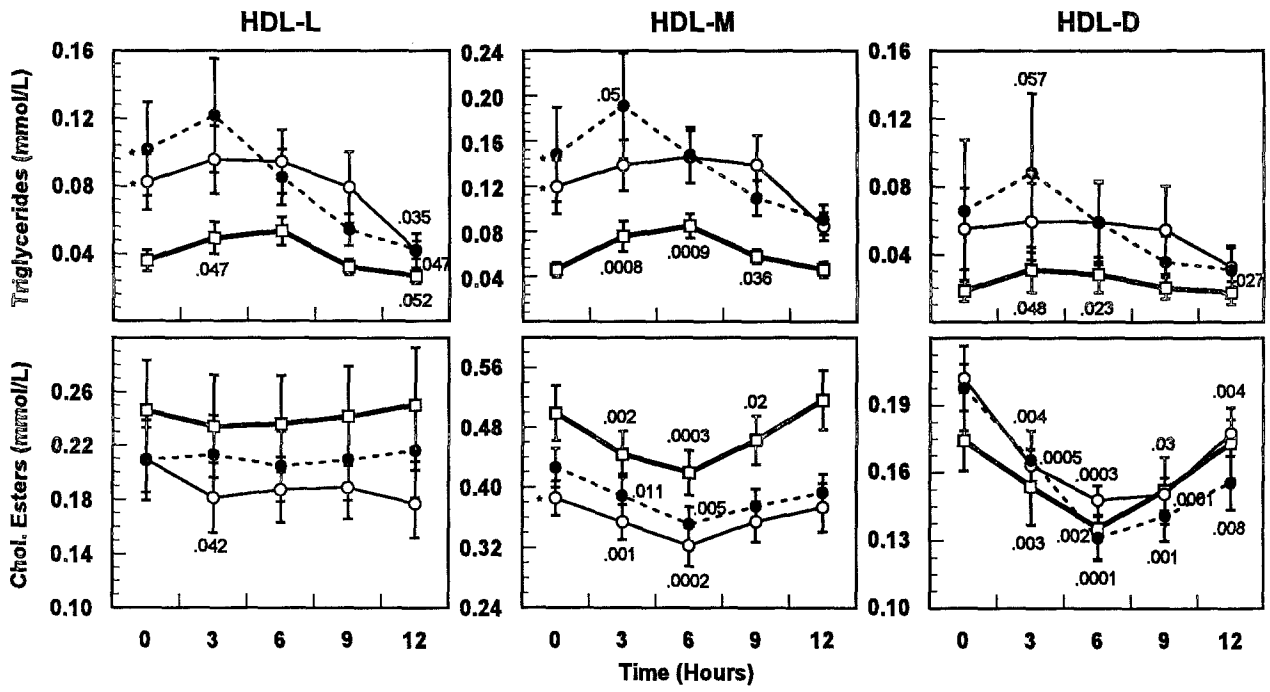

Fig 2. Postprandial responses of HDL-L and HDL-M TG and CE and HDL-D TG and total cholesterol in the three study groups. Note that total cholesterol is shown for HDL-D because FC could not be measured in this subfraction by the methodology used. Symbols are as in Fig 1.

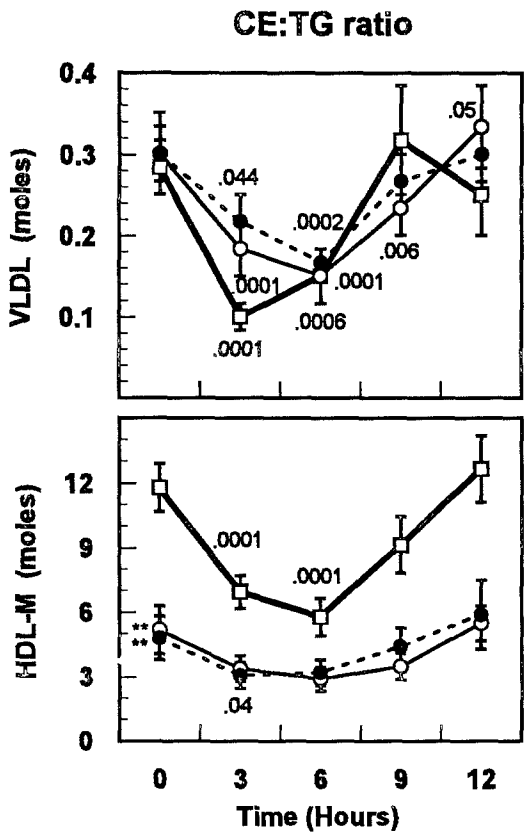

Fig 3. Postprandial responses of VLDL + CM and HDL-M molar ratio of CE to TG in the three study groups. Symbols are as in Fig 1.

IDL PL but no change in LDL PL. The HTG + CVD group, on the other hand, had no change in IDL PL but had a significant decrease in LDL PL. FC concentrations in VLDL + CM, IDL, and LDL generally followed the same patterns as the PL (Fig 4). However, LDL FC/PL ratio decreased by 4.9% to 7.8% in the two HTG groups between 6 and 9 hours ($P < .025$).

HDL-L PL increased significantly at 6 and 9 hours in controls, but did not increase in either HTG group (Fig 5). However, both controls and HTG − CVD subjects had highly significant late increases in PL in HDL-M, but again there was no increase in HDL-M PL in the HTG + CVD group. Finally, HDL-D PL decreased in all three groups, but this decrease was especially pronounced and prolonged in the HTG + CVD group. At 6 ($P = .017$) and 9 ($P = .029$) hours postprandially, there were significantly greater reductions from baseline in HDL-D PL in HTG + CVD subjects than in the HTG − CVD group. This decrease in HDL-D PL in the HTG + CVD group was not accompanied by a decrease in apo A-I (Fig 6), even though both control and HTG − CVD groups had significant postprandial reductions in HDL-D apo A-I. These changes resulted in reductions in HDL-D PL to apo A-I ratios in HTG + CVD subjects, but significant increases in PL to apo A-I ratios in control and HTG − CVD groups (Fig 7). Differences between these two groups were significant at 9 ($P = .017$) and 12 ($P = .012$) hours postprandially. HDL-M had similar changes in PL to apo A-I ratios, with significant increases in controls and HTG − CVD subjects but no change in the HTG + CVD group. Once again, the change from baseline was significantly different between the two HTG groups at 12 hours ($P = .033$).

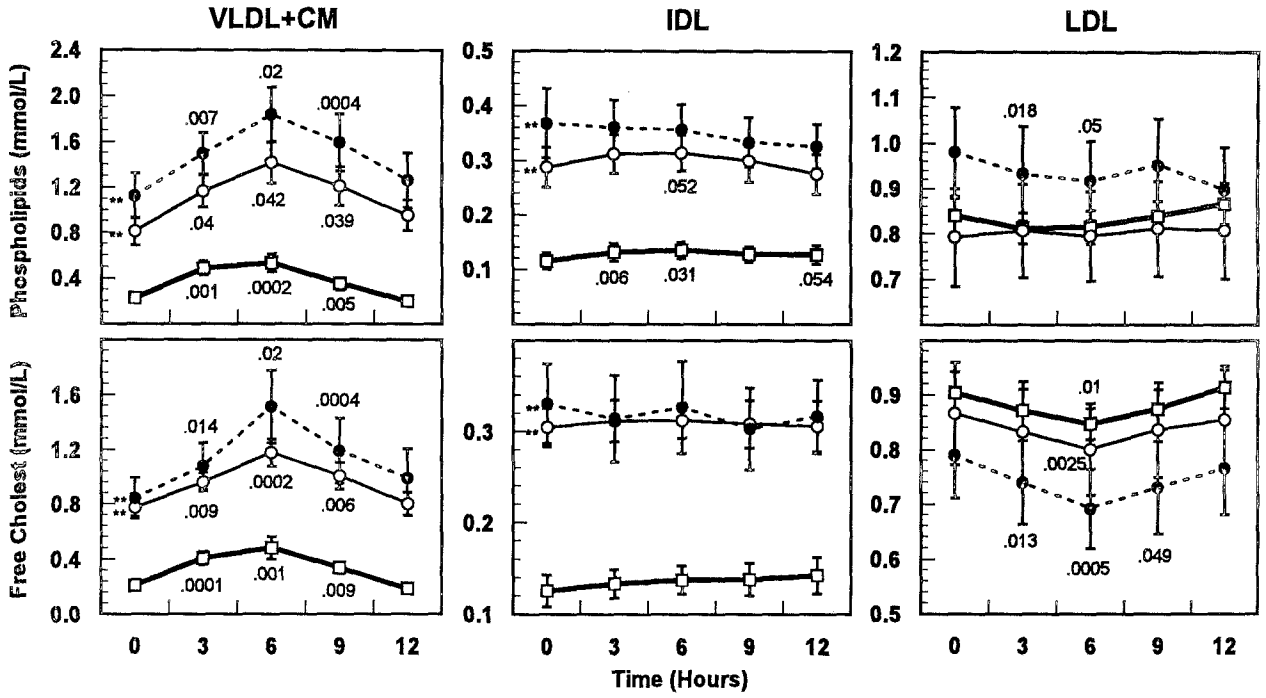

Fig 4. Postprandial responses of VLDL + CM, IDL, and LDL PL and FC in the three study groups. Symbols are as in Fig 1.

It is likely that HDL PL originated in the VLDL + CM subfraction. As noted earlier, all three patient groups had significant increases in VLDL + CM PL postprandially. This influx of PL resulted in significant increases in both the VLDL + CM and IDL PL to apo B ratios in controls and HTG − CVD subjects between 3 and 9 hours, but there was no increase seen in the HTG + CVD group (Fig 7). The change in VLDL + CM PL to apo B ratio at 3 hours was significantly less in the HTG + CVD group than in HTG − CVD subjects ($P = .038$). The change in VLDL + CM PL to apo B ratio at 3 hours tended to be correlated with the change in HDL-D PL to apo A-I ratio at 9 hours ($r = .346$, $P = .12$) in the combined HTG groups.

HDL-L and HDL-M FC changes generally followed those of the PL. However, there were 10% to 29% reductions in HDL-L FC/PL ratios in all three groups between 6

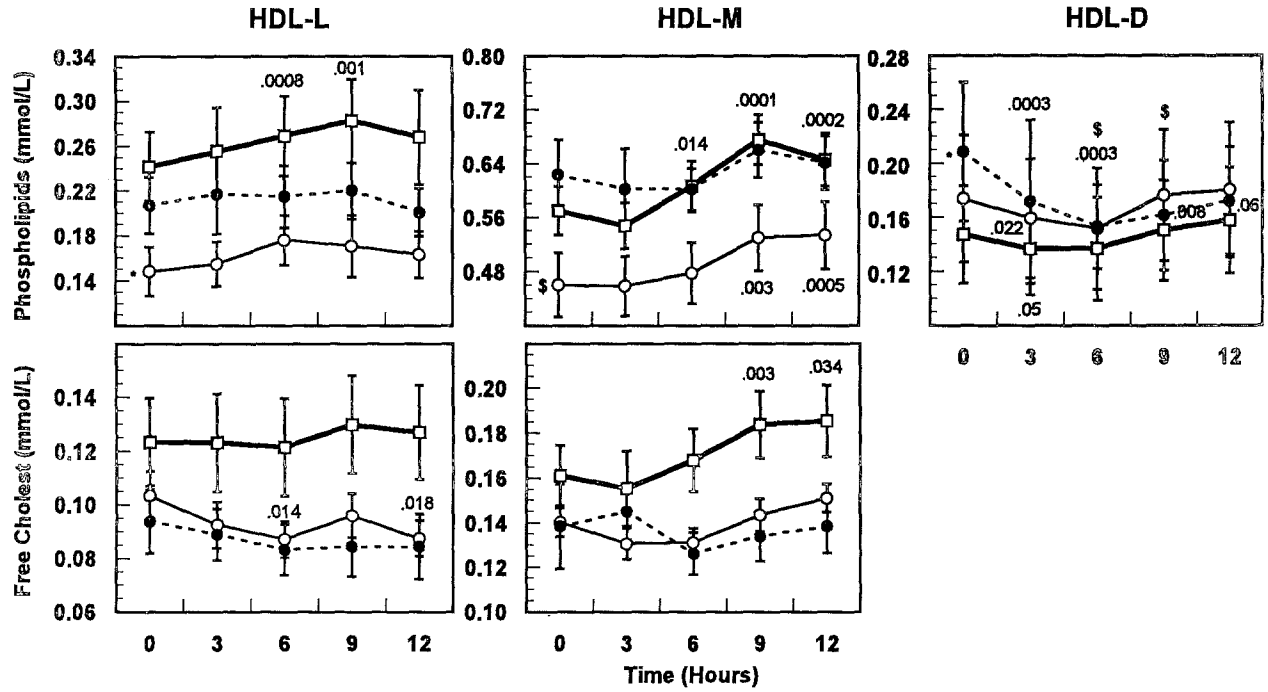

Fig 5. Postprandial responses of HDL-L, HDL-M, and HDL-D PL and FC in the three study groups. Symbols are as in Fig 1.

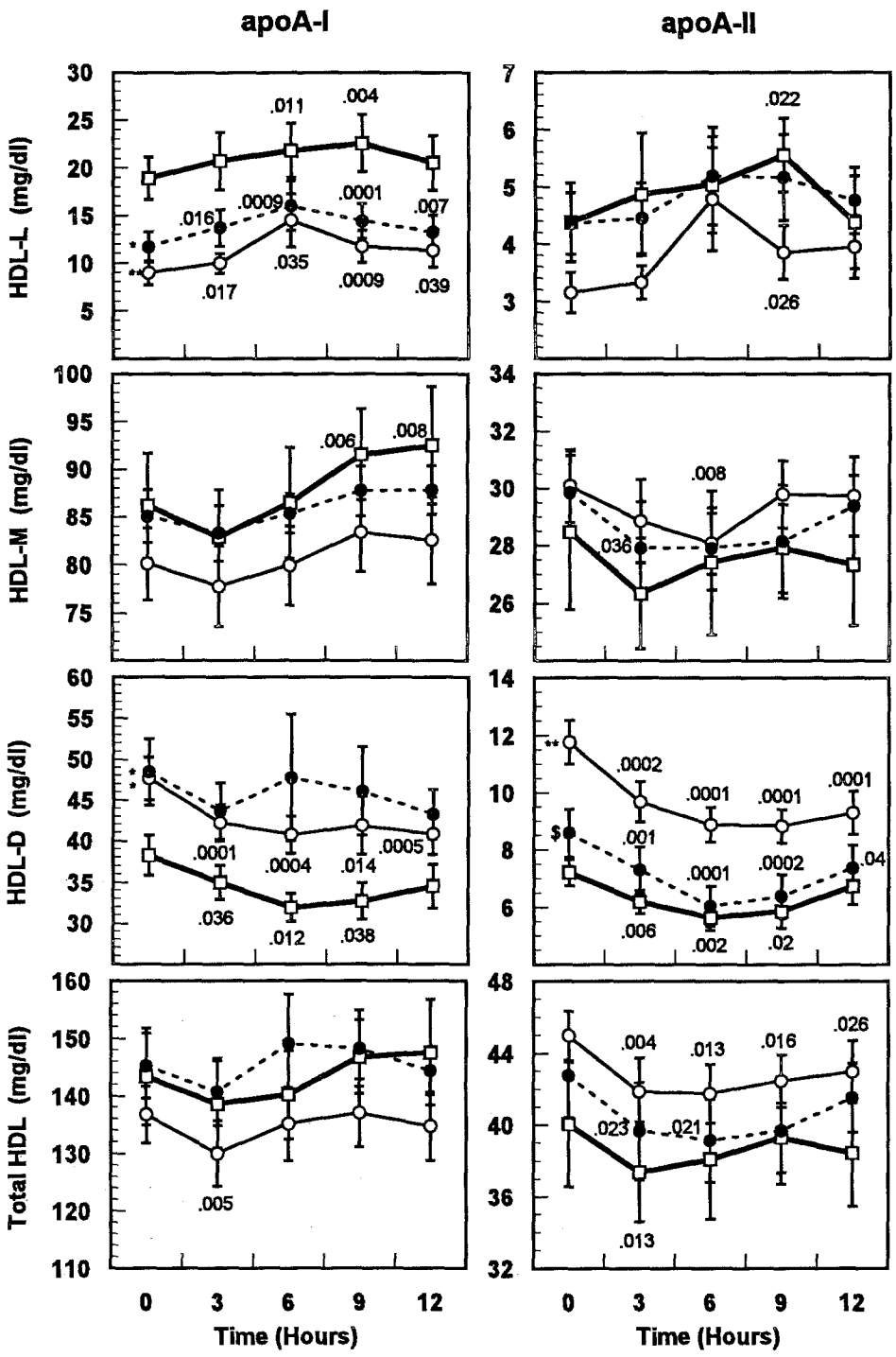

Fig 6. Postprandial responses of apo A-I and apo A-II in HDL-L, HDL-M, HDL-D, and total HDL in the three study groups. Symbols are as in Fig 1.

and 9 hours ($P < .03$). Only HTG − CVD subjects had a reduction in the HDL-M FC/PL ratio (6 hours, −9.4%, $P = .032$).

Postprandial Apolipoprotein Changes

There were no significant differences in postprandial apolipoprotein changes between HTG − CVD and HTG + CVD subjects. VLDL + CM apo B increased postprandially only in controls, whereas IDL apo B decreased slightly in controls and HTG − CVD groups (Fig 8). LDL apo B decreased significantly in all three groups, with a nadir at 6 hours (Fig 8). Total apo B was essentially unchanged postprandially (Fig 8).

There were similar increases in HDL-L apo A-I in all three groups, with only modest increases in apo A-II (Fig 6). HDL-M apo A-I increased late in all three groups, but this increase was significant only in controls. However, HDL-M apo A-II tended to decrease postprandially. As noted earlier, HDL-D apo A-I decreased in controls and HTG − CVD subjects but remained unchanged in the

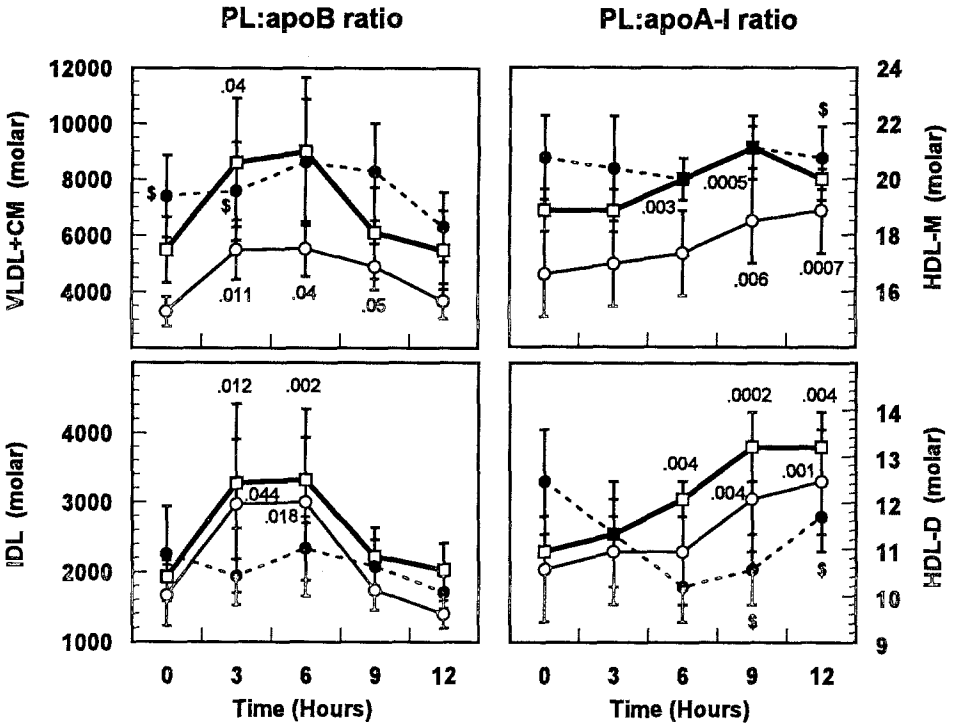

Fig 7. Postprandial responses of PL to apo B ratios in VLDL + CM and IDL and postprandial responses of PL to apo A-I ratios in HDL-M and HDL-D in the three study groups. Symbols are as in Fig 1.

HTG + CVD group (Fig 6). There were significant reductions of HDL-D apo A-II in all three groups. These changes in apo A-I and apo A-II produced significant reductions in apo A-II to A-I ratios (data not shown) in HDL-M and HDL-D, suggesting a postprandial reduction in "apo A-I + A-II" particles in both HDL-M and HDL-D. There were significant postprandial reductions in total apo A-II in all three groups, but total apo A-I was essentially unchanged except for a slight reduction at 3 hours in the HTG − CVD group (Fig 6).

Total apo C-III increased significantly in all three groups between 3 and 9 hours, whereas total apo C-II increased slightly only in the HTG + CVD group. Both apo C-III and apo C-II increased in VLDL + CM but decreased in IDL and total HDL postprandially. These changes led to significant reductions in the apo C-II to C-III ratio in VLDL + CM and IDL but no change in HDL (data not shown).

Postprandial Retinol Palmitate

Postprandial VLDL + CM retinol palmitate concentrations were significantly lower in the HTG + CVD group than in controls at 3 hours (Fig 9). Retinol palmitate concentrations in the HTG − CVD group appeared to be higher than in the HTG + CVD group. However, if retinol palmitate concentration is corrected for particle number by dividing by the VLDL + CM apo B concentration, it is apparent that retinol palmitate metabolism is similar in the two HTG groups (Fig 9). The retinol palmitate to apo B ratio was significantly lower in the HTG + CVD group than in controls at all postprandial time points.

Best Parameters for Identifying CVD in HTG Subjects

By logistic analysis, the only fasting parameter that was independently associated with the presence of CVD was the fasting insulin to glucose ratio ($P = .0163$). Multiple models were tested containing the fasting insulin to glucose ratio and two or three other fasting demographic, metabolic, or lipid parameters that had been found to have a significant difference ($P < .1$) between the two HTG groups (Tables 1 to 4). A total of 17 parameters were ultimately tested.

The change in HDL PL to apo A-I ratio was the most consistent postprandial lipoprotein parameter that correlated with the presence of CVD. There were significant group differences in the change in PL to apo A-I ratio seen in two HDL subfractions (HDL-M and HDL-D) at two different time points (9 and 12 hours postprandially). The fasting insulin to glucose ratio and the change in HDL-D PL to apo A-I ratio at 9 hours were significantly correlated in HTG subjects ($r = -.490$, $P = .027$). However, this correlation was primarily due to HTG + CVD subjects (HTG − CVD, $r = -.015$; HTG + CVD, $r = -.335$, $P = .34$). These parameters were also correlated in control subjects ($r = -.589, P = .073$).

Figure 10 depicts individual data for these two parameters in each study subject. Seven of 10 HTG + CVD patients but none of the HTG − CVD patients had a fasting insulin to glucose ratio more than 25. Similarly, 70% of HTG + CVD patients but only one of 11 HTG − CVD patients had a change in the HDL-D PL to apo A-I ratio at 9 hours less than +1.0. If only patients with an insulin to glucose ratio greater than 25 were selected for treatment, then none of the HTG − CVD subjects would receive

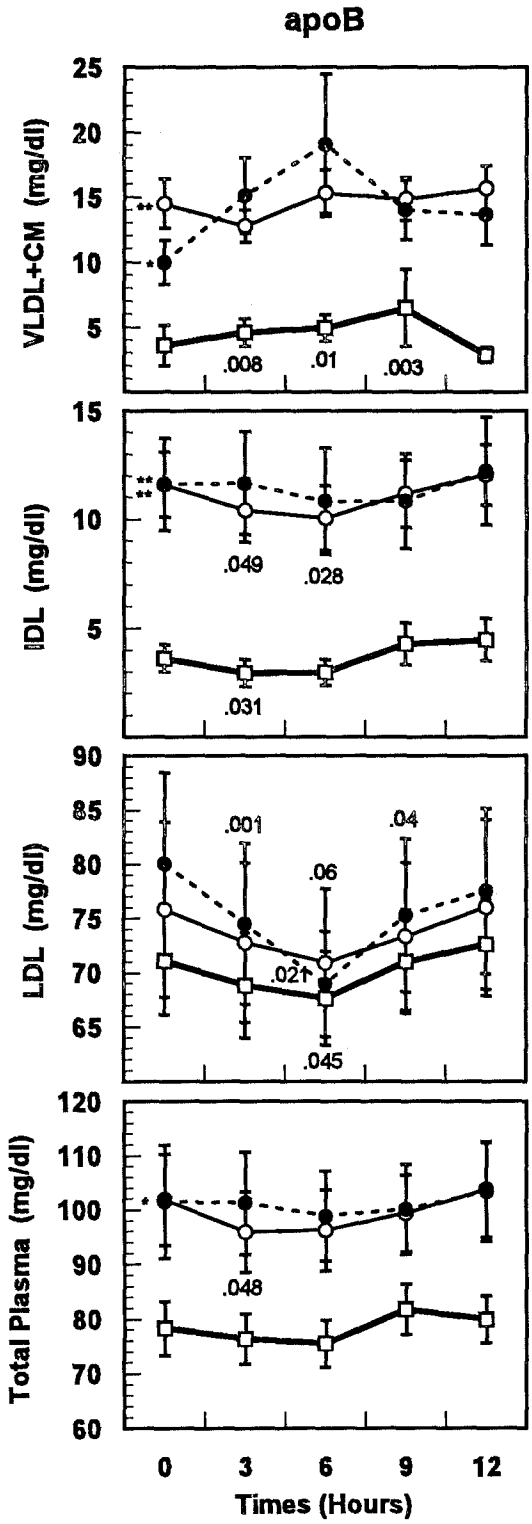

Fig 8. Postprandial responses of apo B in VLDL + CM, IDL, LDL, and total plasma in the three study groups. Symbols are as in Fig 1.

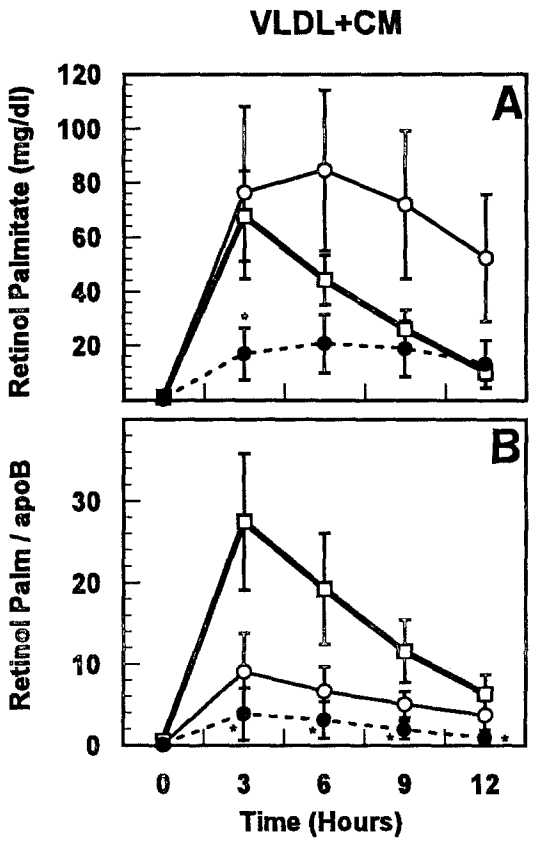

Fig 9. Postprandial responses of retinol palmitate concentrations (A) and retinol palmitate to apo B mass ratios (B) in VLDL + CM. *Significant difference ($P < .05$) between the mean values of the control group *v* the HTG + CVD group at this time point. There were no significant differences between HTG groups. Symbols are as in Fig 1.

potentially needless therapy and three HTG + CVD patients would be denied essential therapy. One of these three HTG + CVD subjects would be identified by measuring postprandial HDL PL to apo A-I ratios, but two subjects had insulin to glucose ratios and postprandial lipoprotein changes within these guidelines. To identify 100% of the HTG + CVD subjects with these two parameters, cutoff points for the insulin to glucose ratio would have to be reduced to 19 and the change in PL to apo A-I ratio increased to +3.5. However, these cutoff points would then dictate that six of 11 HTG − CVD subjects should receive therapy. If only an insulin to glucose ratio greater than 19 was used, then 80% of HTG + CVD subjects and 82% of HTG − CVD subjects would be appropriately treated.

DISCUSSION

This study was designed to identify postprandial lipoprotein parameters that distinguish HTG + CVD men from HTG − CVD men. Standard lipid determinations of total cholesterol, TG, HDL cholesterol, LDL cholesterol, and total plasma apolipoprotein levels have not been found to identify HTG patients at risk unless hyperapobetalipoproteinemia is present.[10,21,22] Our data corroborate these findings. It has been proposed that postprandial lipoprotein abnormalities may be present that predispose some individuals to atherosclerosis.[39-43] We therefore designed this study to examine parameters that reflect postprandial reverse cholesterol transport in HTG patients. We tried to reduce

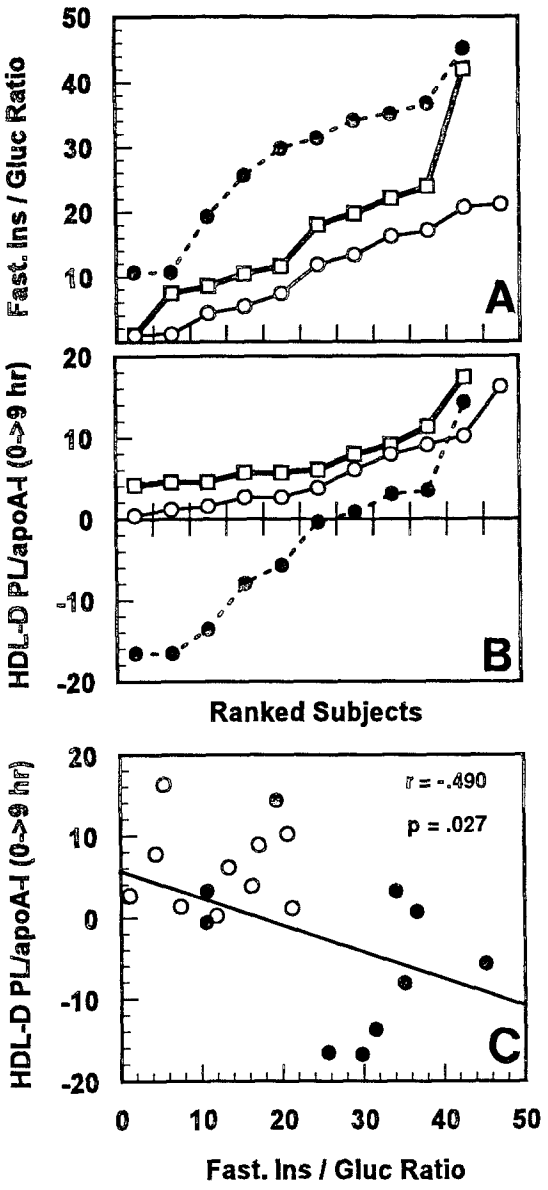

Fig 10. Individual ranked values (lowest to highest) for each subject in the three groups. (A) Fasting insulin to glucose ratio (mean of 2 determinations 3 weeks apart); (B) Postprandial change after 9 hours in the HDL-D PL to apo A-I molar ratio; (C) Scatterplot of the two parameters showing only HTG subjects. Symbols are as in Fig 1.

the influence of other major risk factors for CVD by eliminating those subjects with severe hypertension, diabetes mellitus, or a heavy smoking history. The groups were well matched for age and percent ideal body weight. The presence of CVD was determined on hard clinical evidence (documented infarct or by angiography), and those subjects without CVD had to have a negative personal and family history for premature CVD or diabetes mellitus and were devoid of any CVD symptoms. All three groups did have mild carotid atherosclerosis (consistent with their age and sex) based on computer-generated ultrasound data. Carotid scores for the HTG + CVD group were slightly but not significantly higher than those of the other two groups.

Hypertriglyceridemia is associated with insulin resistance,[58,59] which may contribute to lipoprotein dysfunction and/or atherosclerosis. Both HTG groups had some glucose intolerance, with elevations of post–oral glucose tolerance test plasma glucose levels. HTG + CVD patients also had significantly higher fasting plasma glucose concentrations. However, the HTG + CVD group had fasting and postprandial hyperinsulinemia that was significantly higher than either of the other two groups. In fact, the fasting insulin to glucose ratio was the best fasting plasma discriminator for CVD. These findings are similar to those of Inchiostro et al,[60] who demonstrated that patients with non–insulin-dependent diabetes mellitus with CVD had greater insulin resistance than such patients without CVD. These findings are also consistent with the general hypothesis that insulin resistance is an important independent risk factor for CVD.[61]

Both fasting lipoprotein lipase and hepatic lipase tended to be lower in both HTG groups. However, lipase activities were similar in the two HTG groups, and there were no significant changes in lipase activities postprandially in any of the groups (data not shown). The HTG + CVD group did have significantly higher fasting LCAT activities than either controls or the HTG − CVD group. LCAT is the enzyme that converts FC to CE in the plasma. This reaction occurs primarily on HDL because apo A-I is the major activator of this enzyme. However, these CE are then transferred from HDL to the other lipoproteins with the assistance of CE transfer protein. LCAT activity is believed to be critical to reverse cholesterol transport because the PL surface of lipoproteins would rapidly become saturated with FC from cellular membranes if this FC was not esterified and moved into the core of the particles. Similar to previous reports,[62] LCAT activity tended to increase postprandially in all three groups over the first 9 hours, but none of these changes reached statistical significance and there were no group differences.

As expected, fasting lipoprotein composition was similar in the two HTG groups. There were no differences in the usual fasting measurements. In fact, total apo B, LDL cholesterol, HDL cholesterol, and the LDL/HDL ratio were almost identical in the two HTG groups, whereas apo A-I was actually higher in the HTG + CVD group. The major compositional difference that we identified was larger VLDL particles (higher lipid to apo B ratios), with a tendency toward fewer particles (lower apo B) in the HTG + CVD group. This is a significant observation because familial combined hyperlipidemia, which is usually associated with CVD, typically has an increase in VLDL apo B (more particles) but normal lipid to apo B ratios.[63] These data therefore suggest that our patients did not have familial combined hyperlipidemia. There were no differences seen in IDL or LDL composition, and only minor differences were seen in HDL composition (lower HDL-M PL and HDL-D apo A-II in the HTG + CVD group). Most importantly, there were no increases in LDL apo B concentrations, indicating that these patients did not have hyperapobetalipoproteinemia.

Postprandial movement of TG and CE was similar in the

two HTG groups in all six lipoprotein subfractions. Non-HTG controls had postprandial increases in TG in all six subfractions, whereas CE increased only in VLDL + CM and decreased in IDL, LDL, and HDL, similar to previous reports.[25] HTG groups, on the other hand, had delayed increases in VLDL + CM and IDL TG, typical of subjects with HTG,[48] and minimal or no increases in LDL or HDL TG. Despite these differences in TG changes, CE changes seen in HTG groups were similar to those of controls. Genest et al[38] reported a decrease in HDL_2 (similar to our HDL-L) total cholesterol only in patients with hyperapobetalipoproteinemia. We also saw little change in either HDL-L FC or CE in any of our patient groups. However, we did see significant reductions in HDL-M CE and HDL-D total cholesterol in all three groups. These changes are consistent with other reports of postprandial reductions in total HDL cholesterol in both non-HTG[64] and HTG[64] subjects. There were no differences in the disappearance of retinol palmitate (a surrogate for chylomicron CE) between the two HTG groups. In fact, VLDL + CM retinol palmitate was lower in the HTG + CVD group than in the HTG − CVD group. These data indicate that CM remnant accumulation was not responsible for the excess CVD in HTG + CVD patients.

Total plasma apo B did not change substantially in any group postprandially, which is consistent with previous studies.[24,25] However, we did find that LDL apo B decreased significantly in both non-HTG and HTG subjects, and there was also a slight reduction in IDL apo B. Our control group did have a significant increase in VLDL + CM apo B, probably due to an increase in apo B-48 as reported by Peel et al[26] (also in non-HTG subjects). However, neither HTG group had a significant increase in their already high VLDL apo B concentrations, although HTG + CVD patients did tend to have a postprandial increase. None of these apo B changes helped to differentiate patients with CVD.

These data demonstrate that the first aspect of reverse cholesterol transport that we wished to investigate (TG influx, clearance, and transfer to HDL) was similar in the two HTG groups and therefore could not explain their differences in CVD.

We did not find an increase in total plasma apo A-I, as previously reported,[27-29] in either control or HTG groups. However, we did find an increase in HDL-L and HDL-M apo A-I with a reduction in HDL-D apo A-I in controls. These results would suggest that even though there was no net increase in total HDL apo A-I, there was a postprandial shift of apo A-I from the more-dense to less-dense HDL particles in non-HTG subjects. This type of shift was not seen by Karpe et al[43] using gradient gel techniques. Both of our HTG groups also had an increase in HDL-L apo A-I, but the apo A-I increase in HDL-M was not significant in either of these groups. The only substantial apolipoprotein difference between HTG groups was that the HTG + CVD group did not have a significant postprandial decrease in HDL-D apo A-I, whereas both the HTG − CVD group and controls had substantial reductions. This will be discussed further later. HDL-M, HDL-D, and total plasma apo A-II decreased substantially (especially HDL-D) in all three groups postprandially, suggesting reductions in LpA-I:A-II particles in both of these subfractions. Again, Karpe et al[43] were unable to demonstrate these changes using immunoassays for LpA-I and LpA-I:A-II. These data indicate that postprandial changes in apo A-I and apo A-II do not help to identify HTG subjects with CVD, with the possible exception of HDL-D apo A-I. Therefore, the second aspect of reverse cholesterol transport (influx of apo A-I) appears to be similar in the two HTG groups.

All three groups had highly significant increases in VLDL + CM PL, as expected,[23] with both HTG groups having greater absolute increases than controls, as well as delayed peaks (6 hours). However, controls had a slight decrease in HDL-D PL (3 hours), with a subsequent increase in HDL-M and HDL-L PL. The HTG − CVD group also had a small reduction in HDL-D PL at 3 hours, but only their HDL-M had an ensuing increase in PL. HTG + CVD patients, on the other hand, had a rapid substantial decrease in HDL-D PL, which stayed suppressed for the entire 12-hour monitoring period. In addition, this group had no significant increase in either HDL-M or HDL-L PL. These differences were even more clearly seen when PL to apo A-I ratios were examined. Both controls and HTG − CVD subjects had significant increases in PL to apo A-I ratios in both HDL-D and HDL-M. However, HTG + CVD patients actually had a reduction in the HDL-D PL to apo A-I ratio. Similarly, both controls and HTG − CVD subjects had significant early postprandial increases in VLDL + CM and IDL PL to apo B ratios, whereas there were no changes seen in these ratios in the HTG + CVD group. The change in the HDL-D PL to apo A-I ratio 9 hours after fat ingestion proved to be the parameter that best discriminated HTG subjects with CVD from those without CVD. Using a cutoff point of +1.0 molar-ratio change in this parameter, 70% of the HTG + CVD group but only 9% of the HTG − CVD were below this level. When this parameter was combined with the fasting insulin to glucose ratio, an algorithm could be designed that would appropriately recommend therapy in approximately 80% of these HTG patients. This algorithm could represent a substantial improvement over the current situation, in which there are few scientifically validated guidelines for treatment of these HTG patients. Of course, this algorithm will have to be tested prospectively before it can be used clinically.

These studies suggest a possible mechanism for accelerated atherogenesis in these patients. We propose that normally VLDL + CM and IDL particles enlarge postprandially by adding more PL (and TG) per apo B molecule. As the TG in these particles is removed by lipolysis, the excess PL is released and forms small HDL particles ("pre-beta HDL") by associating with apo A-I. These particles would be recovered in the HDL-D subfraction. The apo A-I for these particles could be derived from the same CM particles that released the PL or could be released by metabolism of larger HDL particles.[66] These small HDL particles would then be available to adsorb FC from cells and begin the process of reverse cholesterol transport.[66] Generation

of these FC-rich, small HDL particles would be expected to stimulate LCAT activity, causing propagation of CE with a subsequent shift of these small particles into the larger and more buoyant HDL-M and HDL-L subfractions.[66] This shift would be identified by a shift of apo A-I from HDL-D into HDL-M and HDL-L. However, if VLDL + CM and IDL particles do not enlarge in size postprandially (as was seen in HTG + CVD patients), then there would be insufficient quantities of PL transferred into the HDL-D subfraction and thus inadequate substrate for the removal of cellular FC. This would result in minimal increased production of CE in HDL and limited movement of apo A-I out of the HDL-D density range. Clearly, a deficiency of appropriate cholesterol acceptor particles would greatly retard reverse cholesterol transport and therefore could easily contribute to the severe CVD seen in these patients.

We therefore conclude that it is the third aspect of reverse cholesterol transport (PL influx and transfer to HDL) that is defective in these HTG patients, and that this deficiency is at least partially responsible for their severe CVD. Insulin resistance appears to be another factor contributing to their CVD, but the mechanistic connection between these two parameters cannot be determined by these studies. It may be that PL metabolic dysfunction and insulin resistance are both common phenomena in HTG subjects, and when these occur simultaneously they result in especially aggressive atherogenesis.

ACKNOWLEDGMENT

We thank Charles M. Mansbach, MD, for critical review of the manuscript and helpful suggestions.

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