Alterations of VLDL composition during alimentary lipemia

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Abstract Apoliprotein (apo) B-100-containing very low density lipoprotein (VLDL) particles secreted from the liver accumulate in plasma during alimentary lipemia. To determine whether changes of VLDL composition occur in the postprandial state that may render these lipoproteins more atherogenic, apoE, C-I, C-II, and C-III, and lipids (triglycerides, phospholipids, and cholesterol) were measured in Svedberg flotation (S_f) 60–400 (large) and S_f 20–60 (small) VLDL before and after an oral fat load. Ten normotriglyceridemic (NTG) and three hypertriglyceridemic (HTG) healthy men were given a fat-rich mixed meal (1000 kCal with 60.2 E% from fat). Triglyceride-rich lipoproteins were isolated by density gradient ultracentrifugation from plasma samples obtained before (fasting) and at 2-h intervals after the meal. VLDL was then separated from chylomicrons and their remnants by immunoaffinity chromatography using monoclonal antibodies 4G3 and 5E11, recognizing apoB-100, but not apoB-48 epitopes. Large and small VLDL isolated from the NTG group were enriched with apoE and C-I, and cholesterol, but depleted of apoC-II in the postprandial state, whereas the apoC-III, triglyceride, and phospholipid contents were essentially unchanged. The compositional changes of VLDL in HTG subjects were similar but more pronounced compared with NTG subjects. We conclude that postprandial lipemia in healthy men induces transient compositional alterations of VLDL that link these lipoprotein species to the formation of atherosclerosis.—**Björkegren, J., A. Hamsten, R. W. Milne, and F. Karpe.** Alterations of VLDL composition during alimentary lipemia. *J. Lipid Res.* 1997. **38:** 301–314.

Supplementary key words apoB-100 • apoC-I • apoC-II • apoC-III • apoE • triglyceride-rich lipoproteins

Triglyceride-rich lipoproteins (TRLs) are a heterogeneous population of lipoprotein particles. Human very low density lipoprotein (VLDL) is synthesized by the liver and contains apolipoprotein (apo) B-100 as the structural protein component (1), whereas chylomicrons that are secreted from the intestine after fat intake have apoB-48 as their structural component (2). Upon their entry into the bloodstream, TRLs acquire apoC and E by transfer from circulating plasma lipoproteins, primarily high density lipoproteins (HDL) in normotriglyceridemic (NTG) individuals (3) and in addition from VLDL in hypertriglyceridemic (HTG) subjects (4). TRL are then hydrolyzed by lipoprotein lipase (LPL), resulting in formation of smaller remnant particles. Remnant TRLs are believed to be taken up by receptor-mediated endocytosis mainly in the liver (5). The exchange of apolipoproteins between TRLs and other plasma lipoproteins in the postprandial state is likely to influence the metabolism of TRLs. ApoE has repeatedly been shown to mediate receptor binding of TRL particles both in cultured cells (6, 7) and in perfused rat liver (8). ApoC-II carried by the TRL particle is required as an activator of LPL (9). ApoC-III, on the other hand, has been considered both to counteract the lipolysis-activating function of apoC-II (10, 11) and to inhibit the receptor-mediated uptake of TRLs (8, 12, 13). Overexpression of apoC-III in transgenic mice was shown to lead to an accumulation of VLDL (14) and a decrease in the clearance rate of chylomicron remnants (15). Surprisingly, mice overexpressing apoC-II also show a hypertriglyceridemic phenotype, but this phenomenon has been hypothesized to depend on an apoC-III-like effect (16). A similar opposing effect on the receptor-mediated uptake of remnant lipoprotein particles has also been shown in vitro for apoC-I (17, 18). Accordingly, mice overexpressing apoC-I had elevated plasma triglyceride levels (19).

Both the intestinal and the liver-derived lipoproteins

Abbreviations: apo, apolipoprotein; VLDL, very low density lipoprotein(s); NTG, normotriglyceridemic; HTG, hypertriglyceridemic; LDL, low density lipoprotein(s); HDL, high density lipoprotein(s); LPL, lipoprotein lipase; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; S_f, Svedberg flotation rate; TRLs, triglyceride-rich lipoproteins.

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contribute to the triglyceridemia seen after fat intake (20, 21). However, the postprandial increase in the TRL particle number is mainly accounted for by VLDL (22, 23), particularly the large VLDL species (22). The increase of large VLDL during alimentary lipemia is even more pronounced in HTG individuals (22). Furthermore, it has been shown that large VLDL isolated from HTG individuals is more prone to receptor-mediated cellular uptake than large VLDL isolated from NTG subjects (24).

Recent studies performed in our laboratory both in humans (25) and in rats (26) have shown that VLDL accumulates in response to acute administration of a chylomicron-like triglyceride emulsion (Intralipid) due to the failure of VLDL to effectively compete for a common lipolytic pathway. A possible explanation of the preferential lipolysis of chylomicrons compared with VLDL might be differences in apolipoprotein contents between these two subspecies of TRLs. A delayed clearance of VLDL might alter the apolipoprotein and lipid composition of VLDL particles and thereby increase their potential atherogenicity. To investigate compositional features of postprandial VLDL, a method is needed to separate chylomicron and chylomicron remnant particles from VLDL. With the use of two monoclonal antibodies against apoB-100 that do not recognize apoB-48, Milne et al. (27) separated VLDL from chylomicron remnants in plasma obtained from patients with type III dyslipoproteinemia. A similar approach was used by Cohn et al. (21) who separated chylomicron remnants from VLDL in sequential plasma samples isolated during alimentary lipemia. They showed that the postprandial triglyceridemia is accounted for by chylomicrons to 80%, whereas about 90% of the alimentary lipemia-induced cholesterol accumulation in the TRL fraction is confined to VLDL (21). However, the composition of postprandial VLDL in terms of detailed analyses of its apolipoprotein and lipid contents has so far not been studied.

The aim of the present study was to investigate the apolipoprotein and lipid composition of large and small VLDL particles isolated from chylomicrons and chylomicron remnants by immunoaffinity chromatography in the fasted and fed state.

METHODS

Study subjects

Lipoproteins from 10 normolipidemic and 3 hypertriglyceridemic men aged 33 to 54 (49 ± 5 (mean ± SD)) years with apoE3/3 or apoE3/4 genotype as determined by restriction isotyping (28) were studied. All subjects gave oral informed consent to the study, which was approved by the ethics committee of the Karolinska Hospital. None of the participants was grossly obese (body mass index <34 with a mean ± SD of 25 ± 3.8 kg/m^2). Fasting plasma lipoprotein lipid concentrations are shown in **Table 1.** The major fasting plasma lipoproteins were determined by a combination of preparative ultracentrifugation and precipitation of apoB-containing lipoproteins followed by lipid analysis (29).

TABLE 1. Fasting plasma lipoprotein lipid concentrations at the time of the study

	VLDL	LDL	HDL
		mmol/l	
Cholesterol			
NTG (n = 10)	0.40 ± 0.24	3.31 ± 0.80	1.25 ± 0.50
HTG (n = 3)	0.96 ± 0.23	3.29 ± 0.72	0.85 ± 0.07
Triglycerides			
NTG (n = 10)	0.81 ± 0.39	0.36 ± 0.08	0.13 ± 0.06
HTG (n = 3)	2.43 ± 0.18	0.48 ± 0.34	0.17 ± 0.05

Values are mean ± SD; NTG, normotriglyceridemic subjects; HTG, hypertriglyceridemic subjects.

Oral fat load

Participants were admitted early in the morning to the Clinical Research Unit for a mixed meal-type of oral fat tolerance test. They had been fasting for 12 h and asked to refrain from smoking during the fasting period and from alcohol intake during the preceding 3 days. The protocol for the fat tolerance test was a modification of the one used by us (22) and by Cohn et al. (20). A meal containing pasta, boiled drawn chicken breast meat, green peas, and mayonnaise was prepared. The carbohydrate contents of the pasta was 63 E% and the remaining 37 E% was provided by protein according to the producer. The total energy contents of the green peas was 8% with 60 E% as carbohydrate and the remaining 40 E% by protein according to standard food contents tables (30). The chicken breast meat was approximated to contain only protein and to have a water content of 73% by weight. The mayonnaise was prepared from soybean oil (Karlshamns Oils & Fats AB, Karlshamn, Sweden). The total energy contents of the meal was 1000 kCal with 60.2 E% from fat, 13.3 E% from protein, and 26.5 E% from carbohydrate. The meal was ingested within 10 min between 7:30 and 7:40 AM and was well tolerated by all subjects. Blood samples were obtained through an indwelling catheter inserted into an antecubital vein and were drawn before and 2, 4, 6, and 8 h after intake of the test meal.

All blood samples were drawn into precooled sterile tubes (Vacutainer, Becton Dickinson, Meylan Cedex, France) containing Na_2EDTA (1.4 mg/ml), which were immediately put on ice.

TRL subfractionation

Plasma was recovered within 30 min by low-speed centrifugation (1,750 *g*, 1°C), and kept at this temperature throughout the preparation procedure. TRLs were subfractionated by cumulative flotation in a density gradient (22). Ultracentrifugation was performed in SW40 Ti swinging bucket rotors at 40,000 rpm and 15°C (XL-70 Ultracentrifuge, Beckman Instrument, Palo Alto, CA). The gradients were first run for 32 min to float $S_f > 400$ lipoproteins. Then, two consecutive runs calculated to float S_f 60–400 (3 h 28 min) and S_f 20–60 (16 h) lipoprotein particles were made. The top 0.5 ml from each density gradient containing the respective lipoprotein subclasses was aspirated and immediately put on ice. A density 1.006 kg/l NaCl solution was used to refill the tubes before the next run. All salt solutions used to prepare the density gradients were adjusted to pH 7.4 and contained 0.02% sodium azide and 0.01% EDTA. Densities were verified to the fourth decimal place.

VLDL separation

Freshly isolated TRL fractions were subjected to immunoaffinity chromatography, using the specific monoclonal antibodies 4G3 and 5E11 against apoB-100, which do not crossreact with apoB-48 (31, 32). Preparation of immunoabsorbants was performed essentially according to Milne et al. (32). Briefly, antibodies in the form of 50% saturated ammonium sulfate precipitate of ascites were pelleted. The supernatant was discarded and the pellet containing antibodies was redissolved in, and dialyzed overnight against, a coupling buffer (0.1 M $NaHCO_3$, pH 8.0). The coupling procedure was according to the manufacturer's description (Pharmacia Fine Chemicals, Inc., Uppsala, Sweden). Typically, 20 mg of 4G3 and 20 mg of 5E11 antibodies were coupled to 5 ml CNBr-activated Sepharose. The binding capacity of the gel was 0.5–1 mg TRL protein/ml gel.

Separation of apoB-48- and apoB-100-containing lipoproteins was carried out essentially according to Cohn et al. (21). Briefly, ten 5-ml glass tubes containing 2 ml of Sepharose gel dissolved in phosphate-buffered saline (PBS, pH 7.4, 0.02% NaN_3) were centrifuged for 5 min at 1000 rpm. The supernatants were aspirated and 1–2 ml (<1 mg of total protein) of S_f 60–400 and S_f 20–60 TRL fractions were added to the tubes. The tubes were then gently mixed and rocked at +4°C overnight, or alternatively, for 2 h at room temperature. The Sepharose gel was pelleted by centrifugation for 5 min at 1000 rpm. The supernatants containing 1–2 ml of the unbound fraction were collected, and the Sepharose gel was washed with 2 ml PBS (pH 7.4, 0.02% NaN_3), rocked for 15–20 min, and after centrifugation, supernatants were again aspirated and added to the first supernatants. The washing procedure was repeated once. The TRL fraction bound to the Sepharose gel, i.e., VLDL containing apoB-100, was dissociated by the addition of 2 ml of thiocyanate (3 M NaSCn, pH 7.4) per tube. The tubes were rocked for 1 h and thereafter centrifuged, and the supernatants were collected. The gels were washed with another 2 × 2 ml of 3 M NaSCn, and these volumes were added to the first thiocyanate supernatants to a total volume of 6 ml. Finally, the gels were washed with 5 × 2 ml of PBS before storage at 4°C and later re-utilization. The protein contents of each TRL fraction were determined using the method of Lowry et al. (33) with addition of sodium dodecyl sulfate (final concentration in regent mixture 1%) to remove turbidity. The recovery of TRL added to the affinity gel was 91 ± 8.8% (mean ± SD, n = 13) as estimated by measuring the sum of recovered protein from the bound and unbound fractions. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis showed that 93 ± 4% (mean ± SD, n = 13) of total apoB-100 was found in the bound fraction. There was no contamination with apoB-48 in the bound fraction. An average of 7% of total apoB-100 in the S_f 60–400 fraction was found as a contamination in the unbound fraction in which apoB-48 represented 68 ± 7% (mean ± SD, n = 13) of total apoB. The terms large VLDL for S_f 60–400 apoB-100 lipoproteins and small VLDL for S_f 20–60 apoB-100 lipoproteins are hereafter interchangeable.

Determination of VLDL lipids and apolipoproteins

Triglycerides and phospholipids in the bound fractions were determined enzymatically (450032, Boehringer Mannheim Corporation, Indianapolis, IN; 990-54009, Wako Chemicals GmbH, Neuss, Germany). Cholesterol was either determined enzymatically (14 350, Merck, Darmstadt, Germany) or by a chemical method (34), the latter if very low concentrations were expected. ApoB and E were quantified by SDS-PAGE (35), whereas C apolipoproteins were determined by urea gel electrophoresis essentially according to Kane (36). Briefly, 100–200 µl of the S_f 60–400 and S_f 20–60 TRL fractions were delipidated in two different tubes containing methanol–diethylether. The delipidations were always performed without any delay, i.e., mixed fractions were delipidated immediately after ultracentrifugation and VLDL fractions just after immunoaffinity chromatography. From the bound TRL fractions two

portions of 750–3000 μl (30–100 μg of total protein) of the S_f 60–400 and S_f 20–60 lipoprotein fractions were delipidated in methanol–diethylether.

To determine apoB and E, the protein pellets were solubilized in 100 μl of sample buffer (2% SDS, 0.15 M sodium phosphate, 5% mercaptoethanol, 12.5% glycerol and bromphenol blue (0.025 mg/ml) at pH 6.8) and denatured at 80°C for 10 min. ApoB-100 from LDL was used as a reference protein and for standard curve dilutions (35). To determine apoCs another pellet was solubilized in 50–100 μl of a urea-containing loading buffer (6 M urea, 42 mM Tris, 46 mM glycine and bromphenol blue (0.025 mg/ml), pH 9.81) and thereafter directly applied to a 10% polyacrylamide gel together with insulin, which was used for standard curve dilutions. One part of insulin (Actrapid, Novo Nordisk A/S, Gentofte, Denmark) was mixed with two parts of the urea-containing buffer to a final insulin concentration of 1 μg/μl. Subsequently, 1, 2, 5, 7.5, 10, 12, and 15 μl of the insulin standard were run on a separate urea gel, first at 60 V for 10 min and then at 100 V for 1 h.

After electrophoresis all gels were fixed in a glass Petri dish using 12% trichloroacetic acid for 30 min, then stained with 0.2% Coomassie G-250 (Merck) in methanol–water–acetic acid 5:5:1 overnight and finally destained in ethanol–water–acetic acid 12:81:7 for 12 h with three to four changes of destainer. Gels with standard curves were stained and destained in the same Petri dish as gels containing samples. A laser densitometer (Ultroscan XL, LKB Pharmacia, Bromma, Sweden) was used to scan the gels and to subsequently evaluate the contents of apoCs, apoE, and apoB on the respective gels (Gelscan XL 2400 Software).

To determine the relative chromogenicity of insulin in comparison with that of apoC-I, C-II, and C-III, the dye uptake of the respective protein was compared with a protein determination made by amino acid analysis. Plasma drawn from healthy volunteers was incubated with Intralipid (Pharmacia, Uppsala, Sweden) in vitro at room temperature for 2 h. The density of the plasma containing Intralipid was then increased to 1.10 kg/l by addition of solid NaCl, and the Intralipid particles, now enriched with apoCs, were harvested from the top of the tube ($S_f > 400$) after 32 min of ultracentrifugation at 40,000 rpm (SW 40Ti, 1°C) as previously described. After delipidation as previously described, 0.5–2 mg of the exchangeable apolipoproteins was solubilized in an urea-containing buffer (10 mM Tris, 6 M Urea, pH 8) and applied to a 1 ml Resource-Q-column (Pharmacia) connected to a fast protein liquid chromatography system (FPLC, LCC-500, Pharmacia). Elution was made with 10 mM Tris, 6 M urea, 0.5 M NaCl, pH 8.0, at a rate of 0.5 ml/min. The eluent was collected in 0.5-ml fractions and monitored at 280 nm to indicate the boundaries of the apolipoprotein peaks. Fractions containing pure apoC-I, C-II, and C-III as determined by inspection on urea gels were subjected to amino acid analysis. Volumes estimated to contain 4–8 μg of each apoC species were evaporated under a gentle stream of N_2. A volume of 100 μl of the eluent buffer was treated in the same way to provide a background sample. The dried protein samples were hydrolyzed by constant boiling HCl under vacuum, in the presence of one crystal of phenol at 110°C for 24 h using a Pico Tag Work station from Waters (Milford, MA). After hydrolysis the samples were dried and dissolved in citric acid buffer (0.1 M, pH 2.2) containing α-amino-β-guanido propionic acid-HCl as an internal standard. The amino acid analysis was performed on a Biotronic LC5000 amino acid analyzer linked to a Shimadzu C-R2AX integrator. The background sample was subtracted from the amino acid contents of the samples. Aliquots of the apoC fractions (containing 5–15 μg of protein) subjected to amino acid analysis were also applied in duplicate to an urea gel together with the insulin standard for determination of the chromogenicity of each protein. The gels were stained with Coomassie G-250, and the dye uptake was recorded by laser densitometry as described. The chromogenicities of apoC-I, C-II, and C-III were 1/3.9, 1/4.8, and 1/6.2, respectively, compared with the insulin standard. Pure apoE was treated similarly and the chromogenicity after the analytic SDS-PAGE was 1/2.6 compared with apoB-100.

Calculations and statistical methods

Conventional methods were used for calculating means and standard deviations. The statistical significance of differences in lipoprotein concentrations or composition between postprandial time points and the fasting state was tested by paired two tailed *t*-test. Associations between lipoprotein parameters were determined by calculation of Pearson correlation coefficients. The individual values of skewed parameters were log transformed prior to statistical computations (37).

RESULTS

Postprandial responses of plasma triglycerides and TRL apoB-48 and apoB-100 concentrations

Plasma triglycerides peaked at 3 h after the ingestion of the mixed meal in the NTG group, whereas the peak of plasma triglycerides seemed to occur 1 h later in the

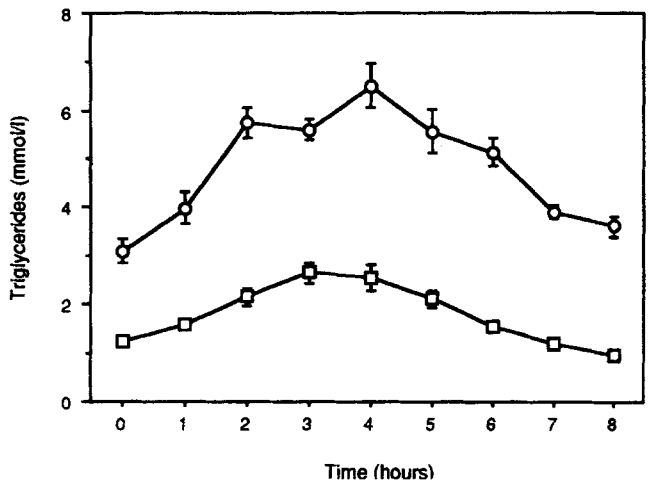

Fig. 1. Line plots of plasma triglyceride responses to ingestion of the mixed meal in healthy normotriglyceridemic (NTG, □) and hypertriglyceridemic (HTG, ○) subjects. The meal, containing pasta, boiled drawn chicken breast meat, green peas, and mayonnaise, was given to 10 NTG and 3 HTG men aged 33 to 54 (49 ± 5 (mean ±SD)) years with apoE3/3 or apoE3/4 genotype. The total energy contents of the meal was 1000 kCal with 60.2 E% from fat, 13.3E% from protein, and 26.5 E% from carbohydrate. Blood samples were obtained through an indwelling catheter inserted into an antecubital vein and were drawn before and hourly for 8 h after intake of the test meal. Values are mean ± SEM with SEM indicated by bars.

HTG individuals (**Fig. 1**). Plasma triglycerides returned to fasting levels or even lower by 8 h after fat intake in both the NTG group and the HTG individuals.

Fasting and postprandial plasma concentrations of apoB-48 and apoB-100 in $S_f > 400$, S_f 60–400, and S_f 20–60 fractions in the NTG group and the HTG individuals are shown in **Fig. 2.** The apoB-48 concentration had increased significantly in the $S_f > 400$, S_f 60–400, and S_f 20–60 fractions in the NTG group at 2–4 h and had returned to baseline at 8 h after the intake of the mixed meal. Of note, the fasting and postprandial plasma concentrations of apoB-48 isolated from NTG subjects in the $S_f > 400$ fraction were approximately one-tenth of the apoB-48 concentrations in the S_f 60–400 or the S_f 20–60 fractions. The apoB-48 concentrations in the $S_f > 400$ and S_f 60–400 fractions in HTG individuals were approximately 2-fold higher but showed a similar postprandial pattern compared with the NTG group.

The apoB-100 concentration in the $S_f > 400$ fraction was very low and responded to the oral fat load in a similar way as the apoB-48 concentration in both NTG and HTG individuals. The apoB-100 concentration increased in the S_f 60–400 fraction, and the increment was similar to the one seen for apoB-48 in this fraction, both for HTG individuals and in the NTG group. Of note, the mean plasma level of apoB-100 was approximately 10-fold higher than the mean plasma concentration of apoB-48 in the S_f 60–400 fraction at all time points. A small increase of apoB-100 in the S_f 20–60 fraction occurred between 2 and 4 h postprandially in the NTG group ($P < 0.05$, n = 10) and was followed by a return to baseline during the last 4 postprandial hours. In contrast, the apoB-100 concentration in the S_f 20–60 fraction of HTG individuals dropped during the first 2 h of the postprandial period ($P < 0.01$, n = 3) and then returned to baseline during the last 6 postprandial hours.

Lipid and apolipoprotein composition of fasting and postprandial S_f 60–400 VLDL particles

The apolipoprotein and lipid contents of large and small NTG and HTG VLDL particles isolated from chylomicrons and their remnants by immunoaffinity chromatography in the fasting state and 2, 4, 6, and 8 h after ingestion of the mixed meal are shown in **Fig. 3** and **Fig. 4.** The large VLDL particles isolated at 2 and 4 h into the postprandial period from NTG individuals contained on average 75% more apoE and apoC-I molecules compared with the corresponding VLDL fraction isolated from fasting plasma ($P < 0.01$, n = 10). These changes reverted to fasting levels by the end of the postprandial period. In contrast, despite an increase of the S_f 60–400 apoC-II concentration (data not shown), the apoC-II content of large NTG VLDL particles were approximately 50% lower after 4 postprandial hours. The apoC-III_1 content of large NTG VLDL particles were higher after the initial 2 postprandial hours ($P < 0.001$, n = 10), but returned to the fasting level during the following 2 h and remained unchanged thereafter. The apoC-III_2 content of the large VLDL particles, on the other hand, were relatively unaffected by the oral fat intake.

Overall, it seemed that both the fasting and postprandial large VLDL isolated from HTG individuals contained more apoE and C-I molecules and about equal numbers of apoC-II, apoC-III_1, and apoC-III_2 molecules per VLDL particle compared with VLDL isolated from the NTG individuals. In some respects the postprandial compositional changes of large HTG VLDL were similar to the ones observed for NTG VLDL, but there were some interesting differences. The peak of the apoE contents of large VLDL seemed to occur 2 h later in HTG compared with NTG subjects. Furthermore, the difference in apoC-I content of large VLDL isolated in the fasting state from HTG and NTG individuals seemed to gradually disappear during the postprandial period due to a drastic decline in the apoC-I content of large HTG VLDL in the later part of the postprandial phase. The apoC-II content of large VLDL decreased during the

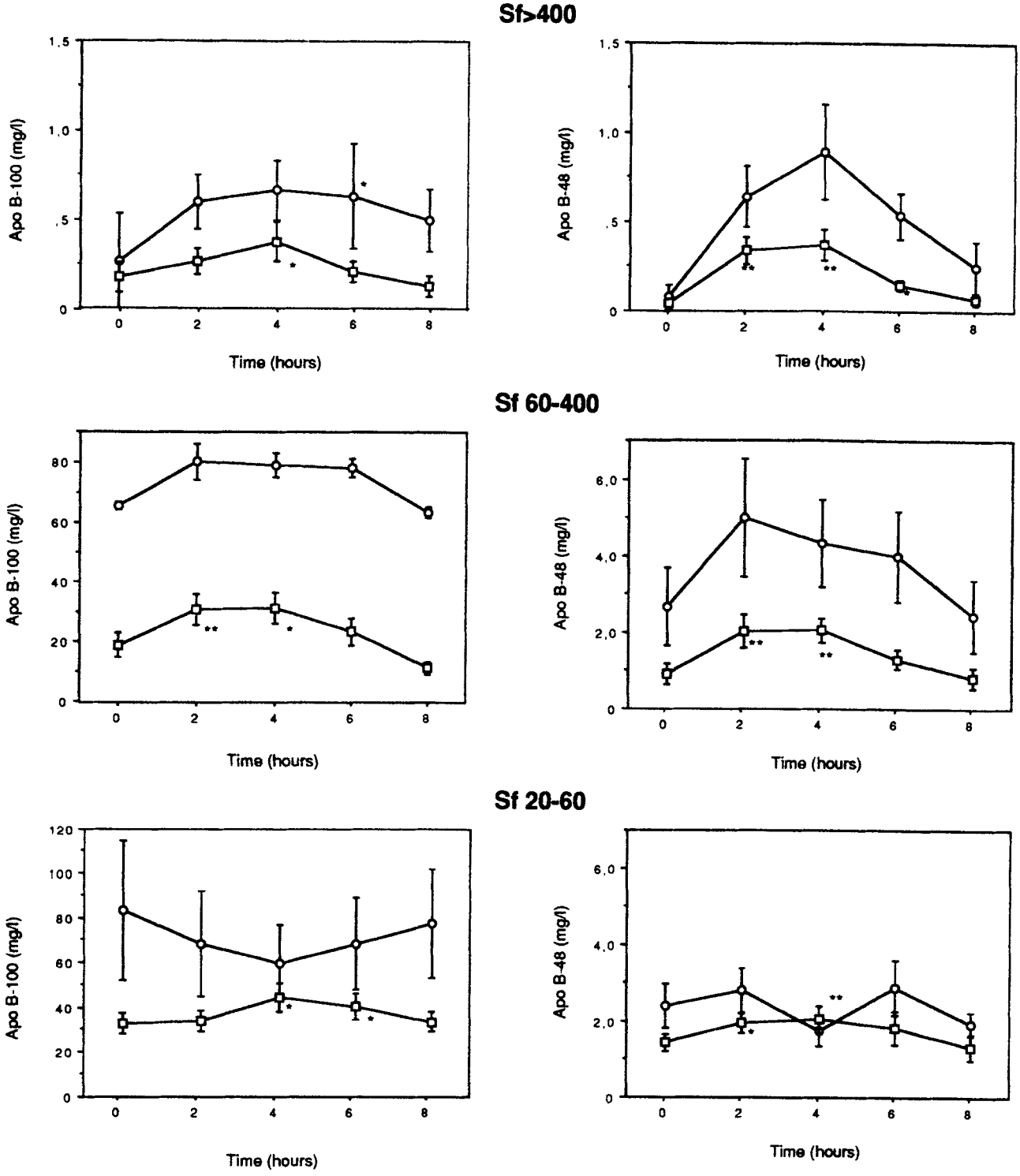

Fig. 2. Line plots of changes in plasma apoB-100 and apoB-48 concentrations in $S_f > 400$, S_f 60–400, and S_f 20–60 lipoprotein fractions in response to ingestion of the mixed meal. Blood samples were drawn before and 2, 4, 6, and 8 h after intake of the test meal. Triglyceride-rich lipoproteins (TRLs) were subfractionated by cumulative flotation in a density gradient (22). The apoB-100 and apoB-48 contents of TRL subfractions were quantified by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (35). The left panel shows line plots of plasma apoB-100 concentrations in normotriglyceridemic (NTG, □) and hypertriglyceridemic (HTG, ○) subjects and the right panel shows line plots of plasma apoB-48 concentrations in the same subjects. Values are mean ± SEM with SEM indicated by bars; * $P < 0.05$ and ** $P < 0.01$ compared with fasting concentrations.

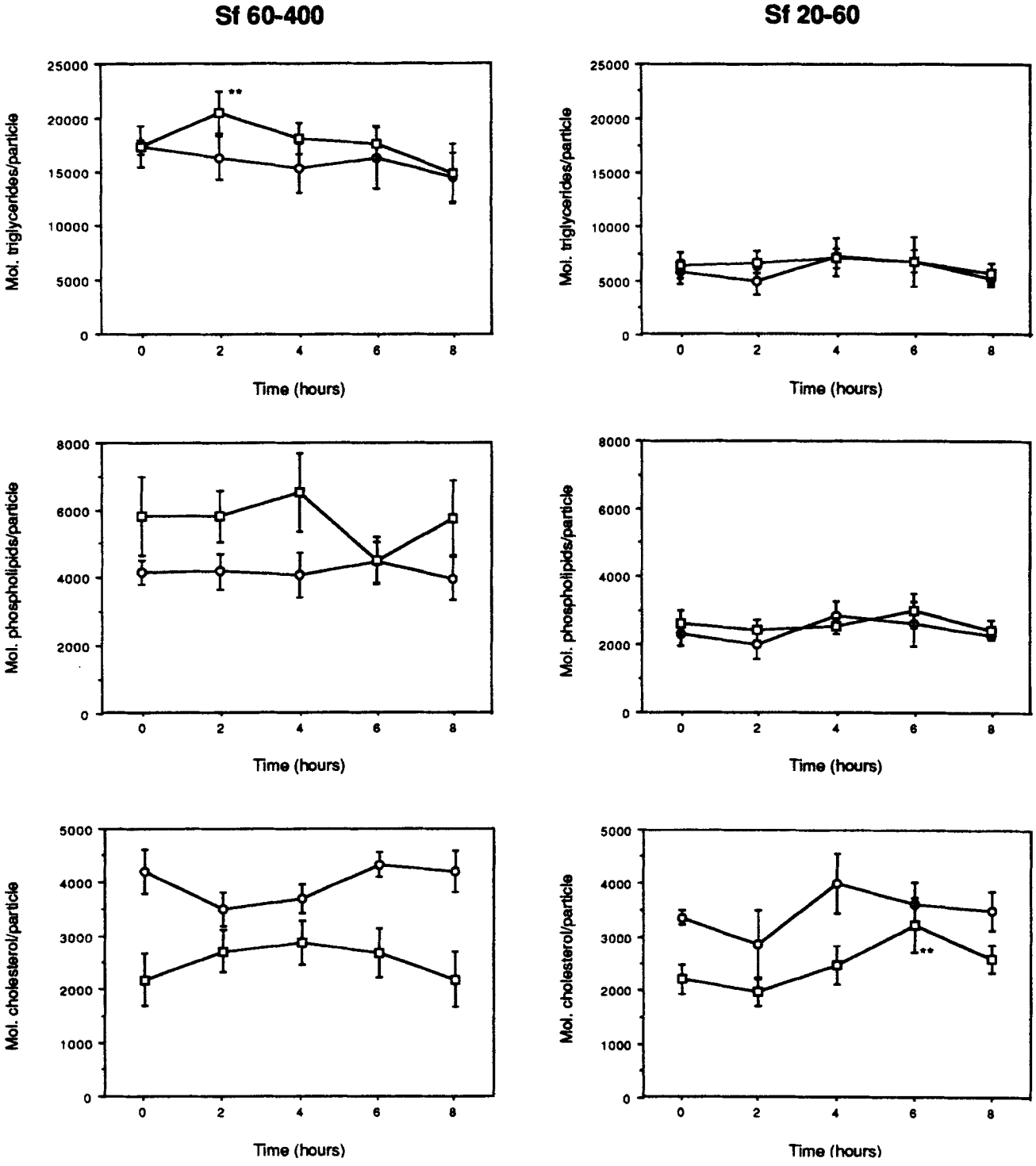

Fig. 3. Line plots of changes in VLDL lipid contents (number of lipid molecules per particle) in S_f 60–400 and S_f 20–60 lipoprotein fractions in response to ingestion of the mixed meal. Blood samples were drawn before and 2, 4, 6, and 8 h after intake of the test meal. Triglyceride-rich lipoproteins (TRLs) were subfractionated by cumulative flotation in a density gradient (22). VLDL was then isolated from chylomicrons and chylomicron remnants by immunoaffinity chromatography, using the specific monoclonal antibodies 4G3 and 5E11 against apoB-100 (31, 32). The apoB contents of the retained fractions (VLDL) were quantified by SDS-PAGE (35). The left panel shows line plots of the lipid contents of VLDL in the S_f 60–400 fraction isolated from normotriglyceridemic (NTG, □) and hypertriglyceridemic (HTG, ○) subjects, respectively, and the right panel shows line plots of the lipid contents of VLDL in the S_f 20–60 fraction isolated from the same subjects. Values are mean ± SEM with SEM indicated by bars; * $P < 0.05$ and ** $P < 0.01$ compared with the fasting sample.

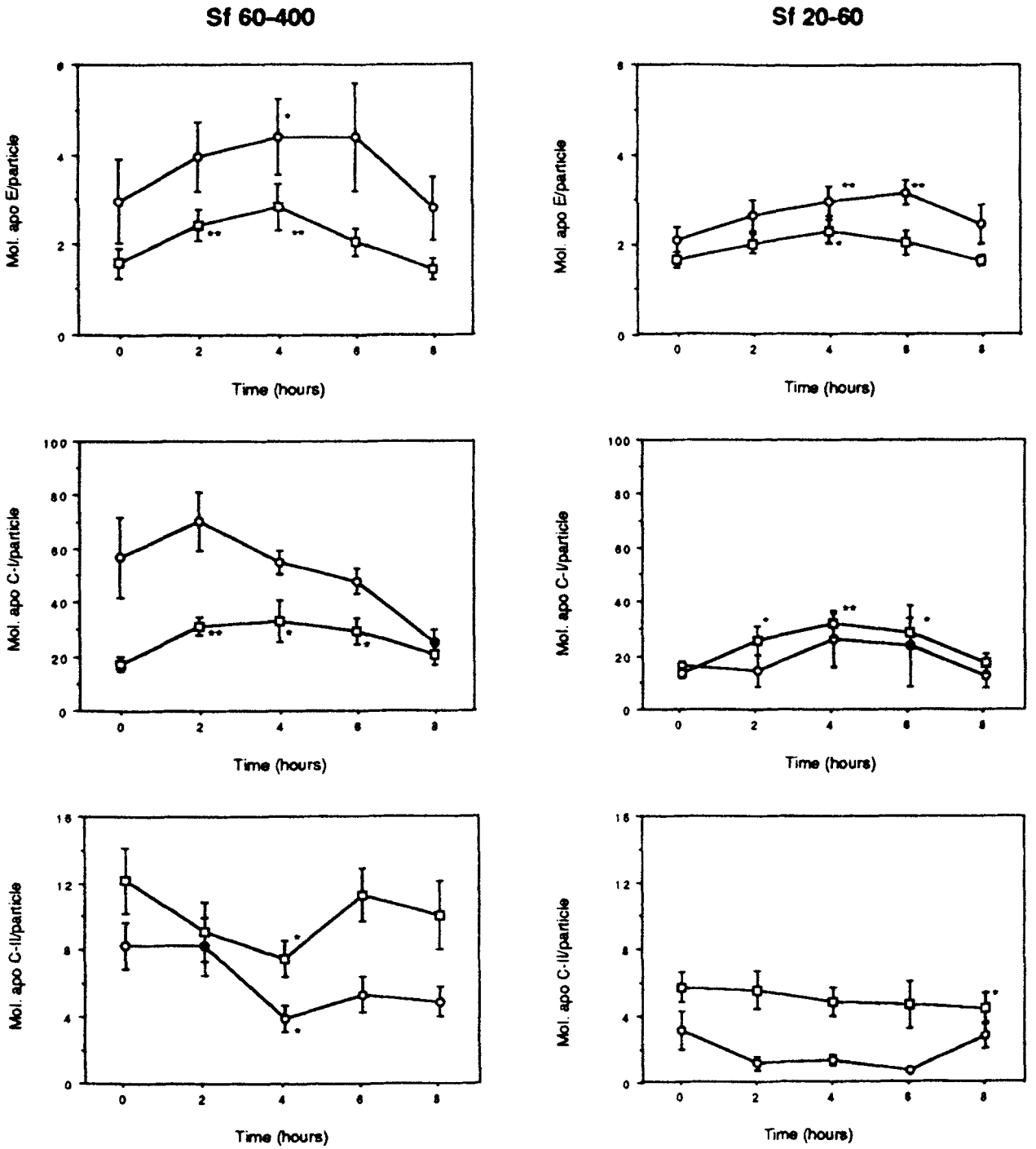

Fig. 4. Line plots of changes in VLDL apolipoprotein contents (number of apolipoprotein molecules per particle) in S_f 60–400 and S_f 20–60 fractions in response to ingestion of the mixed meal. Blood samples were drawn before and 2, 4, 6, and 8 h after intake of the test meal. Triglyceride-rich lipoproteins (TRLs) were subfractionated by cumulative flotation in a density gradient (22). VLDL was then isolated from chylomicrons and chylomicron remnants by immunoaffinity chromatography, using the specific monoclonal antibodies 4G3 and 5E11 against apoB-100 (31, 32). The apoB and E contents of the retained fractions (VLDL) were quantified by SDS-PAGE (35), whereas apoCs were determined by urea gel electrophoresis, essentially according to Kane et al. (36). The left panel shows line plots of the apolipoprotein contents of VLDL in the S_f 60–400 fraction isolated from normotriglyceridemic (NTG, □) and hypertriglyceridemic (HTG, ○) subjects, respectively, and the right panel shows line plots of the apolipoprotein contents of VLDL in the S_f 20–60 fraction isolated from the same subjects. Values are mean ± SEM with SEM indicated by bars; * $P < 0.05$ and ** $P < 0.01$ compared with the fasting sample.

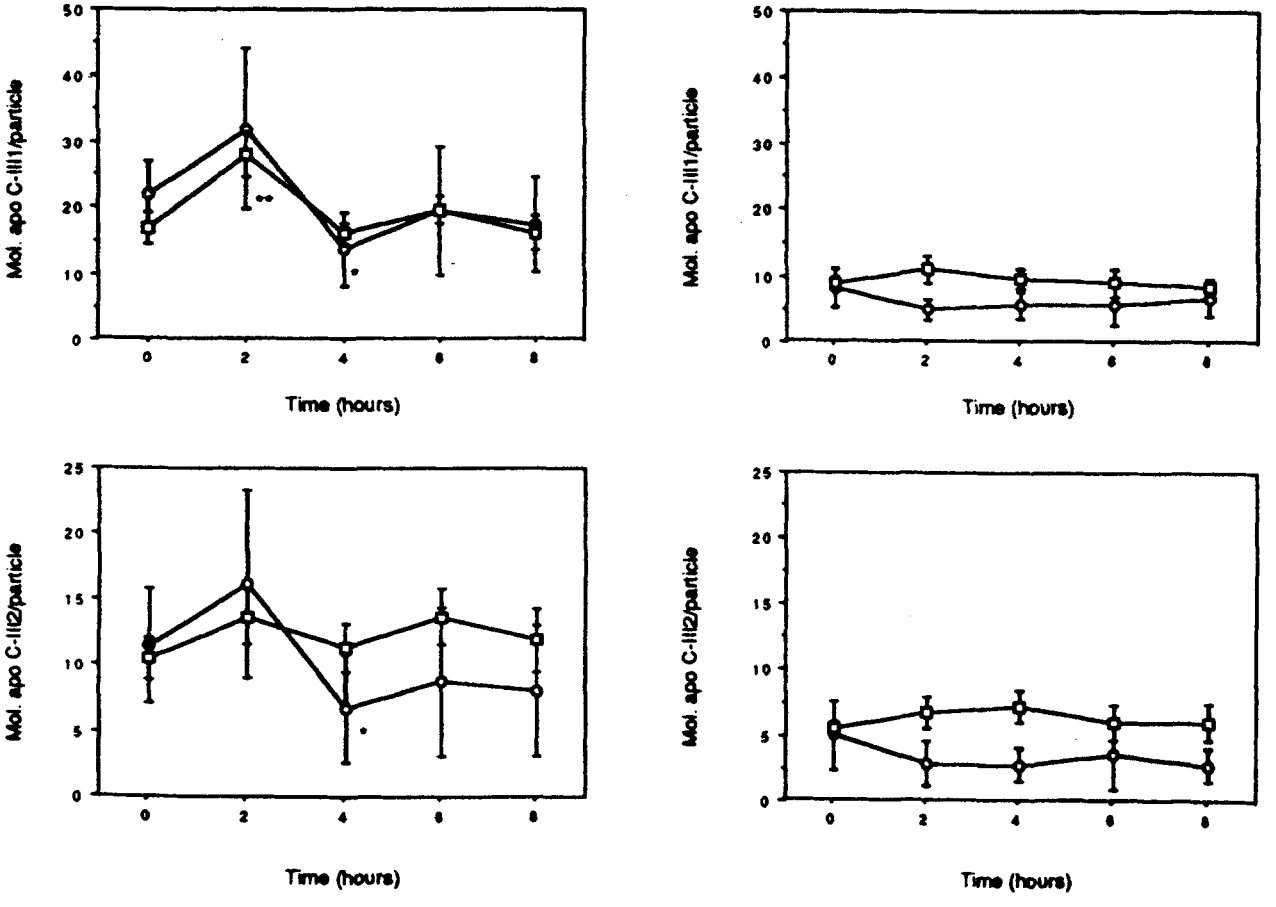

Fig. 4.

initial 4 postprandial hours and never attained the fasting level in HTG subjects, in contrast to the NTG group.

The lipid composition of large VLDL also exhibited changes after the oral fat load. A transient 5–10% increase of the triglyceride contents was observed after 2 h in the NTG subjects ($P < 0.01$, n = 10). The cholesterol content of large VLDL was nearly 2-fold higher in HTG than in NTG subjects in the fasting state. However, during the postprandial period, the cholesterol content of large NTG VLDL particles transiently rose by 50%, whereas the cholesterol contents of large HTG VLDL particles seemed instead to transiently decrease by 25%.

Lipid and apolipoprotein composition of fasting and postprandial S_f 20–60 VLDL particles

The apolipoprotein and lipid contents of large and small NTG and HTG VLDL particles isolated from chylomicrons and their remnants by immunoaffinity chromatography in the fasting state and 2, 4, 6, and 8 h after ingestion of the mixed meal are shown in Figs. 3 and 4. Similar to the S_f 60–400 fraction, the mean apoE and apoC-I contents of small NTG VLDL had increased by 30% and 100%, respectively ($P < 0.05$ and $p < 0.001$), at 4 h after oral fat intake and returned to fasting levels by the end of the postprandial period. The apoC-II content of small NTG VLDL decreased slowly throughout the postprandial phase and was 20% lower at the end of the postprandial period compared with the fasting level ($P < 0.05$, n = 10). The contents of apoC-III_1, apoC-III_2, triglycerides, and phospholipids of small NTG VLDL did not change in response to the fat intake. In contrast, the cholesterol content of small NTG VLDL was unaffected during the first 2 h of the postprandial period but then increased by 60% during the following 4 h ($P < 0.01$, n = 10).

The compositional changes of small HTG VLDL in response to the oral fat intake showed a similar pattern compared with the changes observed for small NTG VLDL. As recorded for large VLDL, the number of apoE molecules per small VLDL seemed to be higher both in the fasting state and throughout the postprandial period in HTG compared with NTG individuals and the postprandial peak occurred 2 h later. Interestingly, the apoC-II content of small HTG VLDL de-

creased more rapidly and drastically by 50% than the apoC-II contents of small NTG VLDL. The numbers of both apoC-III_1 and apoC-III_2 molecules contained in small HTG VLDL particles were around 50% of fasting numbers during most of the postprandial period and attained levels that were considerably lower than those of small NTG VLDL. In contrast, the triglyceride and phospholipid contents of small HTG VLDL were similar to small NTG VLDL, both in the fasting state and throughout the postprandial period. However, the choesterol content of small HTG VLDL were approximately 50% higher compared with small NTG VLDL in the fasting state, whereas the difference seemed to diminish in the postprandial state due to the absence of a cholesterol-enrichment of postprandial small HTG VLDL.

Correlations between fasting plasma levels of triglycerides or VLDL particle numbers and compositional changes of VLDL particles induced by alimentary lipemia

Changes in the contents of apoE, apoC-I, apoC-II, and cholesterol occurring 4 to 6 h after the ingestion of the test meal were found to characterize the postprandial VLDL. In search for determinants of postprandial alterations in the fasting plasma sample, correlation coefficients were calculated between the changes of the apoE, apoC-I, apoC-II, and cholesterol contents of VLDL and the fasting plasma level of triglycerides, or the corresponding S_f 60–400 or S_f 20–60 apoB-100 levels. Second, relations were sought between changes in apolipoprotein and lipid contents in the respective VLDL fractions. The HTG individuals were not included in these analyses. The fasting plasma triglyceride concentration correlated significantly with the increase in apoE molecules per large VLDL particle and negatively with the drop in the apoC-II content of small VLDL occurring during the first 4 postprandial hours ($r = 0.69$, $P < 0.05$, and $r = -0.67$, $P < 0.05$, respectively, n = 10). In contrast, there were no significant correlations between fasting plasma apoB-100 concentrations in the S_f 60–400 and S_f 20–60 fractions and the changes of the apoE, apoC-I, apoC-II, or cholesterol contents of the corresponding VLDL particles.

The increase in the number of cholesterol molecules contained in the large VLDL particles that occurred during the first 6 postprandial hours, correlated positively with the concomitant increase in the particle contents of apoE ($r = 0.68$, $P < 0.05$, n = 10). Interestingly, the increase in cholesterol contents of small VLDL between baseline and 6 h after intake of the test meal also tended to correlate with the simultaneous increase in apoE ($r = 0.61$, $P = 0.06$, n = 10). Otherwise, no correlations between change in VLDL constituents were statistically significant.

DISCUSSION

The present study deals with transient changes in VLDL composition induced by alimentary lipemia. Both large and small VLDL particles were found to contain more apoE, apoC-I, and cholesterol molecules, and fewer apoC-II molecules. In addition, VLDL particles isolated at 2 h into the postprandial period contained more apoC-III_1 and apoC-III_2 molecules than VLDL particles isolated at any other time point. These VLDL alterations were seen in both NTG and HTG subjects.

Monoclonal antibodies directed against two apoB-100 epitopes have previously been used to isolate postprandial lipoproteins by immunoaffinity chromatography from a crude fraction of TRLs (21, 23). The same technique was also used to characterize postabsorptive VLDL from type III subjects (32, 38). However, until the present study, compositional studies of VLDL of different particle size had not been performed. We used the immunoaffinity chromatography protocol of Cohn et al. (21), with some minor modifications, whereas other design features differed significantly from that study. In particular, we isolated subfractions of VLDL by applying an initial separation of the TRL fractions according to particle size using density gradient ultracentrifugation prior to the immunoaffinity chromatography. The rationale for separate characterizations of large and small VLDL particles was primarily the fact that these lipoprotein species are metabolized differently during alimentary lipemia (22).

The apoE enrichment of VLDL during alimentary lipemia may have several implications for the metabolism of the VLDL particle. First, Ji et al. (39) have shown that the apoE content of TRL particles are a determinant of the binding to heparan sulphate proteoglycans (HSPG) in vitro. Recently, the same authors established the role of HSPG in lipoprotein remnant metabolism in vivo by showing that an intravenous injection of heparinase retarded both the plasma clearance and liver uptake of TRL remnants in mice (40). Accordingly, it could be hypothesized that the postprandial apoE enrichment of VLDL particles enhances lipoprotein retention, if and when the particle enters the arterial wall. Second, a number of studies have indicated that apoE is of vital importance for the recognition of remnant lipoproteins by the LDL-receptor. Gianturco and colleagues (24) have suggested that apoE is involved in the LDL receptor-mediated uptake of large VLDL and later demonstrated that apoE, and not apoB, is necessary and sufficient for the binding of large VLDL to the LDL receptor (41). The physiological significance in vivo of these findings was first indicated by Yamada et al. (42, 43) who showed that the presence of apoE on VLDL particles influenced their elimination and conversion to LDL in a rabbit model. More recently, an increase of

the apoE content of apoB-containing lipoproteins purified by immunoaffinity chromatography from human plasma was shown to enhance the affinity of the lipoprotein to the LDL receptor (44). The metabolism of normolipidemic VLDL through the interaction with the LDL receptor is also stimulated by addition of exogenous apoE-3 (45). Furthermore, mice overexpressing apoE show an increased clearance of apoB-containing lipoproteins (46). Third, the LDL receptor-related protein (LRP) mediates uptake of TRL remnant-like particles, provided they are enriched with excess apoE (47). Due to the need for a surplus of apoE for LRP-mediated endocytosis of TRL remnants, a secretion–recapture model for remnant uptake by the liver has been described (48). According to this model, TRL remnants trapped in the sinusoidal space are exposed to high concentration of newly secreted apoE, and by this mechanism the LRP-mediated binding and endocytosis is made possible. Theoretically, the apoE enrichment of postprandial VLDL, as demonstrated here, could stimulate the binding and uptake of these lipoproteins by LRP in the liver. Fourth, and more speculatively, apoE has recently been proposed to have a role as a non-competitive regulator of the lipolysis of TRL through a mechanism by which apoE mediates binding of LPL to triglyceride-poor lipoproteins, thus forming enzyme-inhibitor complexes not allowing LPL to complex with TRL particles (49).

The concomitant apoC-I enrichment and apoC-II depletion of postprandial VLDL observed in the present study may also influence the LDL receptor interaction of the lipoprotein particle. Sehayek and Eisenberg (18) have shown that addition of exogenous apoE-3 to VLDL enhanced its cellular uptake in vitro. The facilitated uptake could be inhibited by addition of exogenous apoC, of which apoC-I had the strongest inhibitory effect (18). Recent studies using immunoaffinity-purified apoB-containing lipoproteins, devoid of soluble apolipoproteins, to which exogenous apolipoproteins were added and the lipid contents modulated by incubation with a triglyceride emulsion, have indicated that triglycerides, apoC-II and apoC-III act individually to modulate the lipoprotein receptor-recognition. As expected, a marked elevation of the apoC-II and C-III contents almost completely abolished the interaction of the triglyceride-enriched apoB-containing lipoproteins with the LDL receptor (50), whereas apoC-I had no effect in this respect. Furthermore, VLDL from transgenic mice overexpressing human apoC-I, in contrast to VLDL isolated from transgenic mice overexpressing apoC-II or apoC-III, seemed to bind HSPG normally (19), strongly suggesting a difference between the apoE opposing effect of apoC-I and that of other apoCs. Indeed, we have recently found that VLDL isolated after intravenous administration of a chylomicron-like triglyceride emulsion in humans is enriched with apoE and C-I and depleted of apoC-II molecules (J. Björkegren, F. Karpe, P. Tornvall, and A. Hamsten, unpublished data), compositional changes very similar to the ones presently observed in vivo after an oral fat load. Despite the apoC-I enrichment, the cellular processing of these VLDL particles by the LDL-receptor on fibroblasts was unchanged or even slightly enhanced.

The changes of the apoC contents of VLDL during the postprandial period seemed to be specific. First, the apoC-III content of VLDL, known to have an opposing effect on the apoE function (15), was, except for the early increase in large VLDL, surprisingly unaffected by the oral fat load. Second, apoC-II decreased, in contrast to apoC-I which increased. It is difficult to evaluate the net effect of these specific and, from a metabolic point of view, opposing modulations of the apoC contents in the context of cell surface recognition of apoE-enriched postprandial VLDL. We hypothesize that the parallel apoC-I and apoE enrichment, together with the apoC-II depletion of VLDL, yields an average lipoprotein particle that has facilitated binding to HSPG in the arterial wall and thereby a slightly facilitated cellular processing compared with postabsorptive VLDL. It is evident that an increase or decrease of any apolipoprotein or lipid constituent of a certain VLDL fraction could either reflect a homogeneous increase/decrease of that particular constituent in all VLDL particles contained in the fraction or an in- or out-flux of VLDL particles of a different composition from the liver or from another VLDL fraction. Indeed, the latter is more probable, as it has been shown that VLDL consists of a heterogeneous population of particles, differing not only in size but also in compositional features across the size distribution (51, 52).

HDL is the major donor of apoC-II during transient triglyceridemia in NTG subjects, whereas VLDL is a major donor of apoC-II in the HTG situation. Transfer of apoC-II is necessary for the activation of LPL in vitro (9). Tornoci and co-workers (4) have shown that the total amount of apoC-II is divided into exchangeable and non-exchangeable pools and that, accordingly, all apoC-II cannot be used for activation of LPL. It could therefore be speculated that the lower apoC-II contents of postprandial VLDL, as demonstrated in the present study, could be explained by transfer of apoC-II molecules from VLDL to newly secreted triglyceride-rich chylomicrons. This transfer could be particularly prominent in the HTG individuals as S_f 20–60 and S_f 60–400 apoC-II concentrations decreased during the postprandial period in contrast to the NTG group where the apoC-II content of the corresponding fractions increased. Theoretically, a chylomicron particle can bind up to 37 LPL molecules (53), indicating that one apoC-II is needed for each LPL site. A situation may therefore

occur in which VLDL particles, particularly in the HTG situation, are poorly lipolyzed due to lack of apoC-II.

The early transient increase of apoC-III_1 and C-III_2 contents of exclusively large VLDL particles may be implicated in the accumulation of this lipoprotein species during alimentary lipemia (22). Indeed, apoC-III is known to counteract both the apoE-mediated receptor clearance of TRLs (15) as well as the apoC-II-mediated lipolysis of TRLs (54). Conversely, Maeda and co-workers (55) have shown that apoC-III-deficient mice are protected from postprandial hypertriglyceridemia. Theoretically, the early apoC-III accumulation and the depletion of apoC-II of large VLDL could explain part of the preferential lipolysis of chylomicrons and the ensuing postprandial accumulation of large VLDL (25). Studies on chylomicron and chylomicron remnant composition are warranted to further address this issue.

The compositional changes of VLDL accumulating in the postprandial state may have implications for atherogenesis. The cholesterol enrichment of small VLDL might be of particular interest in this respect. Indeed, it has been shown that small cholesteryl ester-rich VLDL particles are related to global severity and rate of progression of coronary atherosclerosis (56, 57). The cholesterol and apoE-enriched VLDL particles formed in the postprandial state have features in common with a fraction of VLDL suggested to be remnant lipoproteins (58) and to some extent to β-VLDL, which could provide a cellular basis for assigning an atherogenic role to postprandial VLDL (59, 60). Furthermore, Rapp and colleagues (61) have recently isolated large VLDL particles from the vascular wall using immunoaffinity chromatography with compositional features closely similar to the ones described for postprandial large VLDL in the present work.

In summary, the alterations of VLDL particle composition induced by alimentary lipemia as presented in this study could add to our understanding of postprandial VLDL metabolism and suggest a link between postprandial modification of VLDL composition and formation of atherosclerosis.

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