Delayed clearance of postprandial large TG-rich particles in normolipidemic carriers of LPL Asn291Ser gene variant

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Abstract **The carrier frequency of Asn291Ser polymorphism of the lipoprotein lipase (LPL) gene is 4–6% in the Western population. Heterozygotes are prone to fasting hypertriglyceridemia and low high density lipoprotein (HDL) cholesterol concentrations especially when secondary factors are superimposed on the genetic defect. We studied the LPL Asn291Ser gene variant as a modulator of postprandial lipemia in heterozygote carriers. Ten normolipidemic carriers were compared to ten control subjects, who were selected to have similar age, sex, BMI, and apolipoprotein (apo)E-phenotype. The subjects were given a lipid-rich mixed meal and their insulin sensitivity was determined by euglycemic hyperinsulinemic clamp technique. The two groups had comparable fasting triglycerides and glucose utilization rate during insulin infusion, but fasting HDL cholesterol was lower in carriers (1.25 ± 0.05 mmol/L) than in the control subjects (1.53 ± 0.06 mmol/L, $P = 0.005$). In the postprandial state the most pronounced differences were found in the very low density lipoprotein 1 (VLDL1) fraction, where the carriers displayed higher responses of apoB-48 area under the curve (AUC), apoB-100 AUC, triglyceride AUC, and retinyl ester AUC than the control subjects. The most marked differences in apoB-48 and apoB-100 concentrations were observed late in the postprandial period (9 and 12 h), demonstrating delayed clearance of triglyceride-rich particles of both hepatic and intestinal origin. Postprandially, the carriers exhibited enrichment of triglycerides in HDL fraction. Thus, in normolipidemic carriers the LPL Asn291Ser gene variant delays postprandial triglyceride, apoB-48, apoB-100, and retinyl ester metabolism in VLDL1 fraction and alters postprandial HDL composition compared to matched non-carriers.**—Mero, N., L. Suurinkeroinen, M. Syvänne, P. Knudsen, H. Yki-Järvinen, and M-R. Taskinen. **Delayed clearance of postprandial large TG-rich particles in normolipidemic carriers of LPL Asn291Ser variant.** ***J. Lipid Res.*** **1999.** 40: **1663–1670.**

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Lipoprotein lipase (LPL) functions as a key enzyme in triglyceride (TG) hydrolysis providing free fatty acids for tissues (1). Postheparin plasma LPL activity correlates positively with high density lipoprotein (HDL) cholesterol concentration and inversely with plasma TG concentration (2). High plasma TG and low HDL cholesterol concentrations associate with early coronary artery disease (CAD) (3, 4). The familial occurrence of this lipid phenotype in some CAD patients has initiated the search for underlying genetic defects. The LPL gene is one of the obvious candidates, and more than 60 mutations of the gene have so far been described (5, 6).

The LPL gene Asn291Ser variant in exon 6 is a common abnormality and has a carrier frequency of 4–6% (7, 8). In vitro this gene variant results in 25–50% loss of the catalytic activity of LPL (9, 10). In heterozygotes LPL activity has been decreased in some (9, 10) but not all studies (11). The fasting serum lipoprotein profile has been highly variable; heterozygote carriers of this variant show decreased HDL and elevated TG concentrations in the presence of other factors modulating lipid metabolism (7, 8, 10–12). It has been suggested that the carrier status of Asn291Ser polymorphism (7, 13, 14) or other mutations of the LPL gene (15, 16) increase the risk for CAD.

Because LPL is the rate-limiting enzyme for the hydrolysis of TG, LPL activity is critical for normal clearance of postprandial triglyceride-rich lipoprotein (TRL) particles. Two previous studies in carriers of the LPL Asn291Ser variant have reported excessive postprandial lipemia (8, 17). However, it is unclear whether endogenous or exogenous TRL particles or both are responsible for the lipemia. The aim of this study was to determine the effect of Asn291Ser variant in the LPL gene as a modulator of postprandial lipemia and insulin sensitivity measured as whole body glucose uptake, in carriers with normal fasting lipid profile compared to control subjects. As the measurement of postheparin plasma LPL activity or fasting lipid levels do not necessarily reveal the carrier status at

Abbreviations: apo, apolipoprotein; Asn291Ser, Asn291→Ser polymorphism of the LPL gene; LPL, lipoprotein lipase; AUC, area under the curve; BMI, body mass index; TG, triglyceride.

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the individual level, we wanted to examine whether a fat loading test could be used to discover lipid abnormalities in the carriers of this genetic defect.

SUBJECTS AND METHODS

Subjects

The study population included 10 healthy carriers of Asn291Ser polymorphism in the LPL gene, who had been identified in our previous studies (18). All carriers had normal concentrations of fasting plasma lipids measured at a screening visit. Each normolipidemic carrier was selected to have a non-carrier control subject with normal fasting lipid concentrations and with comparable age, sex, BMI, and apoE phenotype (E3/3 or E3/4). These control subjects were chosen from previous screenings of normal cohorts and from the laboratory staff. The subjects underwent three tests in random order on separate occasions: an oral fat loading test, an euglycemic hyperinsulinemic clamp, and measurement of postheparin plasma lipolytic enzyme activities. All carriers and control subjects were healthy, non-obese, and used no medications. Each subject was interviewed by the same investigator about lifestyle factors; no one consumed a special diet, and the amount of physical activity and alcohol consumption were recorded. Thyroid, renal, and liver diseases were excluded by routine laboratory tests. Subject characteristics are given in **Table 1**. Each subject gave his/her informed consent, and the study protocol was approved by the Ethics Committee of Department of Medicine, Helsinki University Central Hospital.

Measurements

Solid-phase minisequencing. A minisequencing method (19) was used to screen for the point mutations A→G at position 291 of the LPL gene. The known point mutation Asn291Ser in exon 6 of the human LPL gene was amplified by PCR using an upstream PCR primer 5′-(ATC TTG GTG TCT CTT TTT TAC CC)-3′ and biotinylated downstream PCR primer 5′-(AGT CTT CAG GTA CAT TTT GCT GCT)-3′. The detection primer 5′-(CAA TCT GGG CTA TGA GAT CA)-3′ was used for determining allelic variants with an A at the second position of codon 291. The PCR was carried out as described (20) in a total volume of 50 μl. The cycles of denaturation (1 min at 95°C), annealing (1 min at 54°C), and elongation (2 min at 72°C) were repeated 32 times. For each minisequencing reaction, a 10-μL aliquot of the PCR mixture and 40 μL 20 mm sodium phosphate buffer, pH 7.5, containing 0.1% Tween 20 were added to microtitration wells (Labsystems, Helsinki, Finland) that had been coated with streptavidin. The samples were incubated with gentle shaking at 37°C for 1.5 h and washed five times with 350 μl 40 mm Tris-HCl, pH 8.8, 1 mm EDTA, 50 mm NaCl, 0.1% Tween 20, at room temperature. The wells were treated once with 100 μL 50 mm NaOH for 5 min at room temperature and washed five times as above. A reaction mixture consisting of 0.2 μm detection primer and 0.4 μm [^{3}H]dATP (TRK 625, 37 Ci/mmol) to detect A at the second position of codon 291 or 0.4 μm [^{3}H]dGTP (TRK 627, 37 Ci/mmpl) to detect G at this position was added to each well. The samples were incubated at 50°C for 10 min and washed as above. The microtitration plates were treated with 60 μl of 50 mm NaOH for 5 min at room temperature and the eluted radioactivity was measured in a liquid scintillation counter.

TABLE 1. Subject characteristics and parameters of glucose metabolism

	Carriers	Controls
Males/females	5/5	5/5
Age, yr	38 ± 5	40 ± 4
BMI, kg/m^2	24.3 ± 1.0	23.5 ± 0.9
Fat, %	28 ± 2	26 ± 1
Glucose, mmol/L	5.4 ± 0.2	4.8 ± 0.2
C-peptide, nmol/L	0.79 ± 0.16	0.50 ± 0.06
M value, mg/lean body weight * min	6.3 ± 0.7	7.8 ± 0.9
Insulin, mU/L	5.7 ± 0.8	6.4 ± 1.0

Carriers, heterozygote for the Asn→Ser variant in the LPL gene; BMI, body mass index. Data are means ± SE or number of subjects. Differences are calculated with Kruskal-Wallis test. $P > 0.05$ for each variable between carriers and controls.

Oral fat loading test. The study was performed at 7.30 am after an overnight 10–12 h fast. The subjects were asked to abstain from alcohol intake for at least 2 preceding days. A lipid-rich mixed meal consisting of bread, butter, cheese, sliced sausage, a boiled egg, fresh paprika, soured whole milk, orange juice, and coffee was served to the subjects (21). The meal contained 63 g fat, 490 mg cholesterol, with a P/S ratio of 0.08, 25 g carbohydrate, and 35 g protein. During the meal, 150 000 IU of vitamin A (retinyl palmitate, Leiras Oy, Turku, Finland) was administered to label intestinal TRL particles with retinyl esters. After the meal the participants were allowed to drink only water until the last sample was collected. Blood samples were drawn from an indwelling catheter placed in an antecubital vein before and 3, 6, 9, and 12 h after the lipid-rich meal. Venous blood was collected into tubes containing EDTA and plasma was separated within 20 min by low-speed centrifugation. Samples were protected from light and kept at 4°C before and after centrifugation.

Density gradient ultracentrifugation. The density of plasma samples used for density gradient ultracentrifugation was adjusted to d 1.10 kg/L with saline and preservatives (aprotinin 50 IU/mL and phenylmethylsulfonyl fluoride 1 mmol/L) were added. Four mL of plasma was placed in 13.4 mL tubes (Ultra-Clear, Beckman Inc., Palo Alto, CA) and overlayered carefully with 3.0 mL of d 1.065 and d 1.020 kg/L, and 2.8 mL d 1.006 kg/L NaCl solutions. Ultracentrifugation was performed in a SW40 Ti swinging bucket rotor at 40,000 rpm and at 15°C in a Beckman Optima LC ultracentrifuge. The $S_f > 400$ fraction representing chylomicrons was isolated after a run of 32 min and collected by aspirating the top fraction of 1.0 mL. The tube was refilled with d 1.006 kg/L NaCl solution. Thereafter, ultracentrifugation was continued under the same conditions and the S_f 60–400 fraction (VLDL1) was isolated in a run of 3 h 28 min. The sample was aspirated and the tube was refilled as previously. To separate the S_f 20–60 lipoproteins (VLDL2) and intermediate density lipoproteins (IDL, S_f 12–20), centrifugation was continued for 17 h and the fractions were separated as described in detail by Karpe and Hamsten (22) and Karpe et al. (23). Low density lipoproteins (LDL, S_f 0–12) and HDL were recovered from the same tube by aspiration, and the final concentrations were corrected with a dilution coefficient. Aliquots of the isolated fractions for apolipoprotein B-48 (apoB-48) and apolipoprotein B-100 (apoB-100) determinations were frozen immediately at −80°C.

Measurements of apoB-48 and apoB-100. Concentrations of apoB-48 and apoB-100 were analyzed in density gradient ultracentrifugation fractions. Briefly, delipidated aliquots of samples were dissolved in buffer and run in 3.5–20% sodium dodecyl sulfate polyacrylamide gel electrophoresis according to the method of Karpe and Hamsten (22) with slight modifications (21). Intragel and intergel coefficients of variation (CV) for apoB-48 were 3.3% and 13.7%, and for apoB-100 2.9% and 10.5%, respectively. The detection limit for apoB-48 and apoB-100 ranged between 0.01 and 0.02 mg/L.

Insulin sensitivity in vivo. Whole body insulin sensitivity was

measured using the euglycemic hyperinsulinemic clamp technique in a subset of 8 carriers and in their 8 matched control subjects as previously described (24, 25). Insulin was infused in a primed continuous fashion for 2 h. The rate of the continuous insulin infusion was 1 mU/kg*min. Normoglycemia was maintained using a 20% glucose infusion. The glucose infusion rate was adjusted based on plasma glucose measurements, which were performed at 5-min intervals from arterialized venous blood (24, 25). Whole-body insulin sensitivity (M value) was calculated from the glucose infusion rate during the second hour of the insulin infusion. The lean body mass and the fat percentage was measured by bioelectrical impedance analysis (Bio-Electrical Impedance Analyzer System, RJL Systems, Detroit, MI).

Lipolytic enzymes. An intravenous bolus injection of heparin (100 IU per kg of body weight) was given to the subjects at a separate visit at least 1 week apart from the oral fat loading test. Blood samples were drawn before and 15 min after the heparin injection into prechilled lithium–heparin tubes. Plasma was separated immediately at 4°C and stored at −80°C. Plasma lipoprotein lipase (LPL) and hepatic lipase activities were measured from preheparin and 15-min postheparin samples with the method of Huttunen et al. (26). Quantification of LPL mass was performed by Commercial Markit-F LPL EIA kit (Dainippon Pharmaceutical Co, Ltd., Osaka, Japan) as described in detail elsewhere (27, 28). The specific activity was calculated by dividing LPL activity by LPL mass.

Analytical methods

Concentrations of retinyl esters, TG, and cholesterol were analyzed in total plasma and in all lipoprotein fractions. TG and cholesterol concentrations were measured by automated enzymatic methods using the Cobas Mira analyzer (Hoffman-La Roche, Basel, Switzerland). Retinyl ester levels were measured with high performance liquid chromatography as described by Ruotolo et al. (29). ApoE phenotyping was performed in serum by using the method of Havekes et al. (30). Concentrations of glucose (31) and free fatty acids (32) were analyzed in fasting samples and during the euglycemic hyperinsulinemic clamp. Insulin and C-peptide were measured by radioimmunoassay (Kabi Pharmacia Diagnostics AB, Uppsala, Sweden and Byk-Sangtec Diagnostica GmbH & Co. KG, Dietzenbach, Germany, kit No 323 161, respectively). Quality of laboratory measurements was controlled with commercial samples for cholesterol (CV = 2.1%), TG (CV = 2.2%), and insulin (CV = 4.7%). CV for the retinyl esters assay was 8.6% for a low control sample and 7.1% for a high control sample.

Statistical analyses

All values are expressed as mean ± standard error (SE) of the mean. We tested whether a dependent variable is influenced by interaction between carrier status and time after the fat load by using the repeated measures analysis of variance (ANOVA). Because of equal group sizes, we used the Greenhouse–Geisser adjustment (33). If adjusted ANOVA suggested overall significance for the dependent variable, the individual time-points were compared with the Kruskal-Wallis test. We also tested the differences for postprandial lipid responses after calculation of area under the curve (AUC) and area under the incremental curve (AUIC) according to Matthews et al. (34). The AUC and AUIC values were computed for TG, retinyl ester, apoB-48, and apoB-100 responses. These and fasting parameters were compared with the Kruskal-Wallis test between the two groups. Logarithmic transformations were performed for variables with skewed distribution (TG, cholesterol, apoB-48, and apoB-100) before testing ANOVA, but not before the non-parametric Kruskal-Wallis test. All calculations were done using the SYSTAT statistical package (SYSTAT Inc., Evanston, IL).

RESULTS

Lipase activities

The postheparin plasma activity of LPL ranged from 78 to 196 mU/mL in carriers and from 158 to 332 mU/mL in control subjects. The postheparin LPL activity was 19% lower in carriers compared to control subjects ($P = 0.082$). LPL mass did not differ significantly between the two groups (188 ± 15 ng/mL in carriers and 227 ± 18 ng/mL in control subjects). By contrast, the specific activity of LPL was decreased in carriers of Asn291Ser variant (range 0.14–0.56 mU/mg) as compared to control subjects (range 0.43–0.53 mU/mg), $P = 0.016$ (**Table 2**). Postheparin hepatic lipase activity (range 216–488 mU/mL in carriers and 77–516 mU/mL in control subjects) was increased by 40% ($P = 0.054$) in carriers compared to values of the control subjects, and the carriers had significantly reduced LPL to hepatic lipase ratio ($P = 0.010$) as shown in Table 2.

Glucose metabolism, euglycemic hyperinsulinemic clamp and free fatty acids

Fasting glucose concentrations were normal in both groups. Fasting serum insulin and C-peptide concentrations and whole body insulin sensitivity were comparable between the groups (Table 1). Fasting free fatty acid concentrations were also similar in the carriers and in the control subjects (747 ± 118 mmol/L and 658 ± 71 mmol/L, respectively, P = n.s.).

Postprandial responses of triglycerides

Table 2 presents the fasting TG concentrations, which ranged from 0.79 to 1.43 mmol/L in carriers and from 0.70 to 1.53 mmol/L in control subjects. Although fasting plasma and VLDL TG concentrations were slightly higher in carriers, the difference was not significant. **Figure 1** illustrates the postprandial responses of TG in plasma, chylomicron, VLDL1, and VLDL2 fractions. Postprandial plasma and VLDL1 TG concentrations were higher in the carriers than in control subjects, and the difference was greatest 9 h after the fat load ($P = 0.014$ for plasma and $P = 0.013$ for VLDL1 fraction, Kruskal-Wallis), and repeated measures ANOVA suggested that the response patterns indeed differed more than would be expected to occur by chance (Fig. 1). Plasma TG AUC was higher in

TABLE 2. Fasting lipoproteins and lipase activities

	Carriers	Controls	*P*
P-TG, mmol/L	1.35 ± 0.13	1.11 ± 0.12	n.s.
VLDL-TG, mmol/L	0.61 ± 0.07	0.43 ± 0.04	0.088
P-Chol, mmol/L	4.75 ± 0.28	5.04 ± 0.31	n.s.
LDL-Chol, mmol/L	2.60 ± 0.23	2.63 ± 0.22	n.s.
HDL-Chol, mmol/L	1.25 ± 0.05	1.53 ± 0.06	0.005
Ph-LPL, mU/mL	188 ± 15	227 ± 18	0.082
LPL sp act, mU/mg	0.43 ± 0.03	0.49 ± 0.01	0.016
Ph-HL, mU/mL	340 ± 32	242 ± 47	0.054
LPL/HL	0.60 ± 0.09	1.24 ± 0.20	0.010

Carriers, heterozygote for the Asn→Ser variant in the LPL gene; Chol, cholesterol; TG, triglyceride; LPL, lipoprotein lipase; Sp act, Specific activity; HL, hepatic lipase; Ph, postheparin. Data are means ± SE. Differences are calculated with Kruskal-Wallis test.

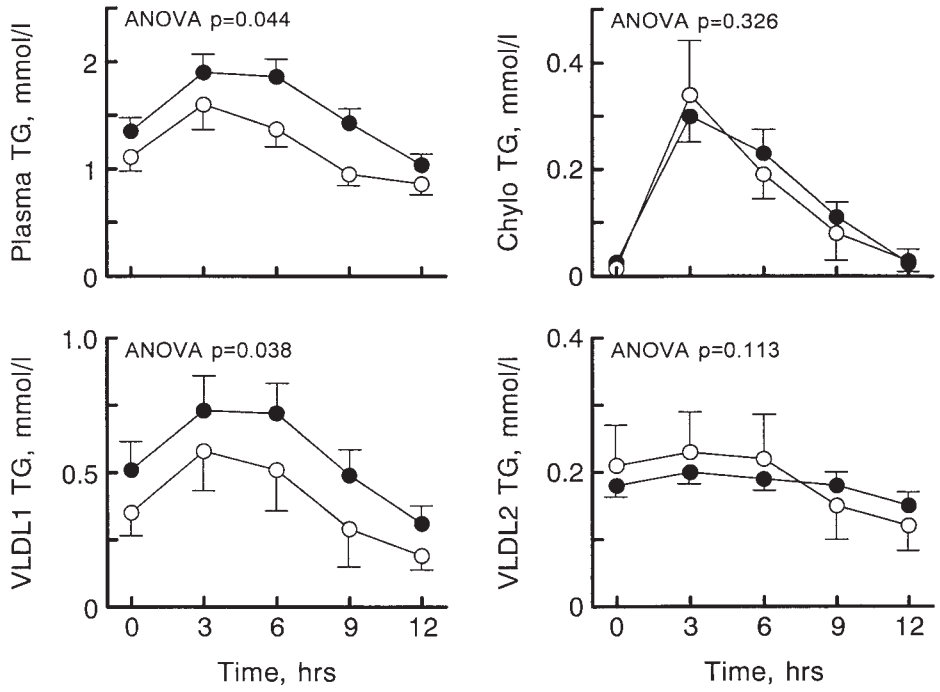

Fig. 1. Line plots show the postprandial responses of triglycerides in plasma, chylomicrons ($S_f > 400$), VLDL1 (S_f 60–400), and in VLDL2 (S_f 20–60) in carriers of Asn291Ser variant in the LPL gene (●) and in control subjects (○). Plotted on the y-axis is the concentration and on the x-axis time in hours after the lipid-rich meal. Data points are mean; error bars indicate SE. The difference between the two postprandial concentration curves is indicated as *P* value within each panel when significant. Calculations are repeated measures ANOVA.

carriers (12.5 ± 1.0 mmol/L*h vs. 9.7 ± 1.1 mmol/L*h, $P = 0.038$, Kruskal-Wallis) than in control subjects. The incremental area for TG concentrations in plasma and TRL fractions did not differ between the two groups. In the HDL fraction, the concentration of TG increased postprandially in carriers compared with the fasting value ($P = 0.048$, repeated measures ANOVA). In contrast, HDL triglycerides tended to decrease after the fat load in control subjects compared to the fasting concentration ($P = 0.063$, repeated measures ANOVA). Consequently, carriers and control subjects had clearly different HDL TG curves postprandially by repeated measures ANOVA ($P = 0.017$).

Postprandial responses of apoB-48

Fasting concentrations of apoB-48 in chylomicrons, VLDL1, and VLDL2 fractions were comparable. The postprandial responses are shown in **Fig. 2**. The early postprandial increases between 3 to 6 h in apoB-48 concentrations were comparable between the two groups in each TRL fraction. The AUC responses of apoB-48 are presented in **Table 3**.

Postprandial responses of apoB-100

Fasting concentrations of apoB-100 in chylomicron ($P = 0.022$, Kruskal-Wallis) and VLDL1 ($P = 0.01$, Kruskal-Wallis) fractions were higher in carriers than in control subjects. The postprandial concentration profiles of apoB-100 also differed in these two fractions between the carriers and the control subjects (**Fig. 3**). The peak concentration of apoB-100 was observed later in the chylomicron fraction in the carriers (6 h) than in the control subjects (3 h). In carriers, the 6- and 9-h concentrations of apoB-100 in the chylomicron fraction ($P = 0.049$ and $P = 0.028$, respectively, Kruskal-Wallis) and the 9-h concentration in the VLDL1 fraction ($P = 0.003$, Kruskal-Wallis) were clearly higher than in the control subjects. The AUC responses of apoB-100 are presented in Table 3.

Postprandial responses of retinyl esters

Plasma retinyl ester concentrations were similar during the fat load in the two groups (data not shown). In chylomicron and VLDL1 fractions, the peak of postprandial retinyl ester concentration occurred at 6 h after the fat intake in both groups, but at the later postprandial timepoints the retinyl ester concentration remained elevated in carriers. Likewise, in the VLDL2 fraction, retinyl ester concentration persisted elevated up to 12 h after the fat load in carriers. In fact, retinyl ester concentrations in chylomicron, VLDL1, and VLDL2 fractions at 9 and 12 h were significantly higher in the carriers than in the control subjects (data not shown).

Postprandial responses of cholesterol

Fasting cholesterol concentrations are given in Table 2. In the whole plasma and the chylomicron fraction, the postprandial responses of cholesterol were comparable (data not shown). Postprandial cholesterol concentrations

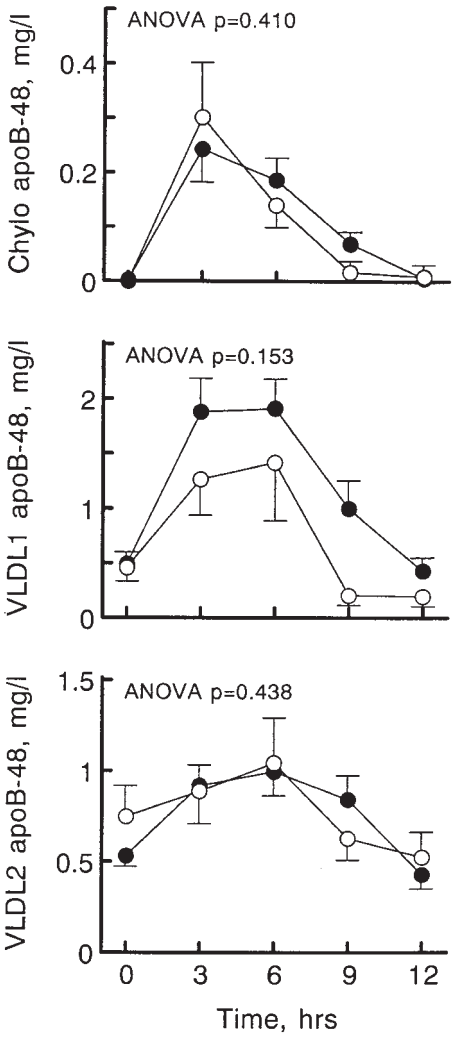

Fig. 2. Line plots show the postprandial responses of apolipoprotein B-48 in chylomicrons ($S_f > 400$), VLDL1 (S_f 60–400), and VLDL2 (S_f 20–60) in carriers of Asn291Ser variant in the LPL gene (●) and in control subjects (○). For other explanations, see legend to Fig. 1.

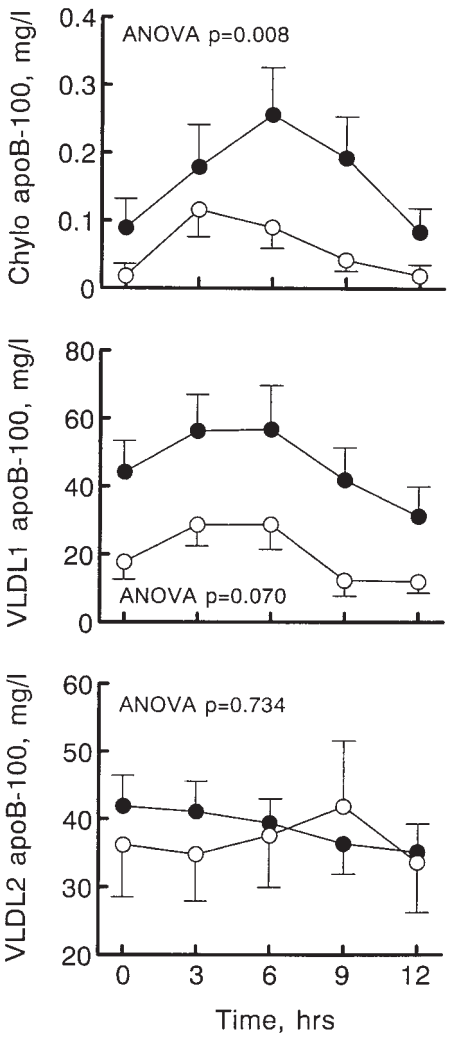

Fig. 3. Line plots show the postprandial responses of apolipoprotein B-100 in chylomicrons ($S_f > 400$), VLDL1 (S_f 60–400), and VLDL2 (S_f 20–60) in carriers of Asn291Ser variant in the LPL gene (●) and in control subjects (○). For other explanations, see legend to Fig. 1.

TABLE 3. Postprandial responses (AUC) of triglycerides (TG, mmol/l*h), retinyl esters (RE, μmol/l*h), apoB-48 (mg/l*h), and apoB-100 (mg/l*h) in chylomicron, VLDL1, and VLDL2 fractions

	Carriers	Controls	*P*
Chylo TG	1.22 ± 0.19	1.17 ± 0.37	n.s.
Chylo RE	4.61 ± 0.76	3.98 ± 1.06	n.s.
Chylo B-48	0.91 ± 0.18	0.8 ± 0.27	n.s.
Chylo B-100	1.34 ± 0.38	0.50 ± 0.17	0.096
VLDL1 TG	4.60 ± 0.77	3.21 ± 0.97	0.096
VLDL1 RE	4.35 ± 0.75	2.61 ± 0.26	0.023
VLDL1 B-48	9.80 ± 1.48	5.95 ± 1.62	0.070
VLDL1 B-100	380 ± 78	164 ± 39	0.016
VLDL2 TG	1.47 ± 0.12	1.51 ± 0.44	n.s.
VLDL2 RE	1.08 ± 0.10	0.97 ± 0.09	n.s.
VLDL2 B-48	6.23 ± 0.58	6.24 ± 1.28	n.s.
VLDL2 B-100	312 ± 24	298 ± 60	n.s.

Carriers, heterozygote for the Asn→Ser variant in the LPL gene. Data are means ± SE. Differences are calculated with Kruskal-Wallis test.

were higher in carriers than in control subjects in VLDL1 ($P = 0.004$, repeated measures ANOVA) and in VLDL2 fractions ($P = 0.012$, repeated measures ANOVA), differences being most marked at 9 and 12 h after the fat load (each *P* value from 0.002 to 0.02, Kruskal-Wallis).

HDL cholesterol concentration decreased during alimentary lipemia compared with fasting values in both groups ($P = 0.001$ for carriers and $P = 0.002$ for control subjects, repeated measures ANOVA). As responses of HDL triglycerides were different, we calculated the TG/cholesterol ratio for the HDL fraction (**Fig. 4**). In the fasting state the ratio was similar (carriers 0.22 ± 0.02 and control subjects 0.23 ± 0.03, *P* = n.s.).

DISCUSSION

The present study shows that carriers of LPL Asn291Ser gene variant with normal concentrations of fasting plasma

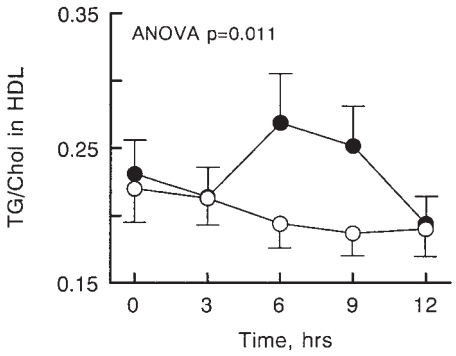

Fig. 4. Line plots show the postprandial ratio of triglycerides to cholesterol in HDL fraction in carriers of Asn291Ser variant in the LPL gene (●) and in control subjects (○). Plotted on the y-axis is the ratio and on the x-axis time in hours after the lipid-rich meal. For other explanations, see legend to Fig. 1.

TG values exhibit an abnormal response in the VLDL1 fraction to a lipid-rich meal. In addition, the carriers had a lower concentration of fasting HDL cholesterol than the control subjects, which is consistent with previous population-based studies (7–9). After the meal, the early postprandial rise in TRL particles was similar in the two groups, suggesting normal formation and secretion of chylomicrons in the intestine. In the late postprandial hours, 9 and 12 h after the meal, the concentrations of apoB-48- and apoB-100-containing particles in carriers were higher than in control subjects, suggesting impaired clearance of TRL particles. These differences in postprandial lipid metabolism were observed despite similar BMI, age, gender, apoE phenotype, and insulin sensitivity.

The carriers had 19% lower postheparin plasma LPL activity than non-carriers. Our results regarding the effect of LPL Asn291Ser heterozygosity on LPL activity agree well with the findings of previous studies (9, 10). There was significant overlapping in the LPL activity as well as the LPL mass between the groups. Therefore, we confirm the previous observations that carrier status cannot be distinguished by measuring postheparin LPL activity or LPL protein concentration. The calculated specific activity of LPL was reduced in the carriers ($P = 0.016$). However, the range of individual values for specific activity was too wide to discriminate carriers from non-carriers. This finding is in line with the results of Reymer et al. (9), who found, in addition to reduced LPL specific activity, a decrease in proportion of dimeric to monomeric enzyme as a result of more rapid dissociation into an inactive LPL monomer in the carriers.

Most significant postprandial differences were observed in the VLDL1 (S_f 60–400) fraction. Increases of apoB-48 and retinyl ester concentrations in VLDL1 fraction suggest delayed clearance of exogenous remnant particles. Although the number of apoB-48-containing particles in the postprandial state is approximately 10% of the total apoB concentration, the apoB-48 fraction nevertheless carries a significant TG load in the postprandial state (35). However, apoB-100 concentration in both chylomicron and VLDL1 fractions was also significantly elevated especially in the late postprandial hours. It is widely recognized that chylomicrons and VLDL particles compete for the same catabolic processes *1*) lipolysis by LPL and *2*) receptor-mediated uptake in the liver (36, 37). Karpe and Hultin (38) have suggested preferential clearance of exogenous particles when the lipolytic capacity is limited. Recently, van Beek et al. (39) reported preferential clearance of apoB-48-containing particles at low levels (<120 mU/mL) of LPL activity. However, when abundant LPL was released (>140 mU/mL) comparable percentage reductions of apoB-48 and apoB-100 were seen. In this study, both apoB-100-containing and apoB-48-containing VLDL1 particles accumulated in the circulation. It is therefore possible that if LPL concentration is subnormal both intestinal and hepatic TRLs are cleared inefficiently. Previous studies in type I hyperlipidemia have shown elevation of VLDL1 particles due to reduced direct catabolism and poorer conversion into VLDL2 particles (40, 41). Decreased conversion of VLDL1 to VLDL2 might be another factor responsible for higher concentration of apoB-100-containing VLDL1 particles in both fasting and postprandial states in carriers.

Insulin sensitivity is a key determinant of VLDL1 metabolism. Malmström et al. (42) have recently shown that acute hyperinsulinemia suppresses hepatic VLDL1 apoB production in healthy men, but not in subjects with type 2 diabetes (43). The two groups displayed similar rate of glucose utilization during insulin infusion, which suggests that the carriers do not overproduce large VLDL particles due to insulin insensitivity.

A study using perfused rat liver has shown that LPL binds to very low density lipoprotein particles and augments the hepatic removal of TRL (44). Previous in vitro studies suggest that this function is not impaired in Asn291Ser gene variant (12). The postprandial concentrations of TRL particles in VLDL2 (S_f 20–60) and IDL (S_f 12–20) fractions were similar between the two groups, which suggests that the hepatic clearance of TRL remnants is not impaired.

There are two previous studies reporting the response to oral fat load in Asn291Ser carriers (8, 17), as well as postprandial studies on carriers of other mutations of the LPL gene (8, 45). The results have shown delayed clearance of postprandial lipemia in subjects with decreased lipolytic activity (45, 46). In EARS study by Gerdes et al. (8) a fat load was given to a large group of carriers and control subjects. Although only plasma TG was measured, the authors found increased postprandial response of TG in carriers. Recently Pimstone et al. (17) studied three heterozygotes with LPL Asn291Ser polymorphism after a fat load with vitamin A administration and found increased chylomicron retinyl palmitate and triglyceride responses. This study did not, however, match the cases and control subjects for apoE phenotype or fasting triglycerides, which are strong determinants for postprandial lipemia (47–49). A novel finding of our study is that carriers with the LPL Asn291Ser variant exhibit impaired removal of VLDL1 particles of both intestinal and hepatic origin, and this results in accumulation of TRL particles in circulation.

In the postprandial state, metabolism of TRL particles influences the concentrations of HDL cholesterol and apolipoprotein composition, and thus may modify reverse cholesterol transport (50, 51). There was an increase in TG content of HDL fraction in carriers, but no significant changes in control subjects were detected. High TG concentration predisposes HDL particles to the hydrolysis by hepatic lipase, resulting in increased proportion of small, dense HDL particles (52) and decreased HDL cholesterol concentration (53). In the carriers, delayed postprandial clearance of TRL remnants was therefore reflected in a low fasting HDL cholesterol concentration.

The clinical significance of LPL Asn291Ser gene variant as a risk factor for hypertriglyceridemia and low HDL cholesterol may be more important if secondary factors such as obesity (11), insulin resistance (10), apoE-4 allele (12), familial hyperlipidemia (54), combined hyperlipidemia (9), or postmenopausal state (7) are superimposed. The present study shows that even normolipidemic and insulin-sensitive carriers of LPL Asn291Ser gene variant have defective lipolytic capacity and impaired clearance of alimentary TRL particles. In carriers, low concentrations of fasting HDL cholesterol as a result of abnormal postprandial TRL metabolism were observed. Altogether, the changes in VLDL1 and HDL metabolism may constitute an atherogenic risk factor for carriers of this common polymorphism.

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REFERENCES

1. Cooper, A. D. 1997. Hepatic uptake of chylomicron remnants. *J. Lipid Res.* **38:** 2173–2192.
2. Olivecrona, G., and T. Olivecrona. 1995. Triglyceride lipases and atherosclerosis. *Curr. Opin. Lipidol.* **6:** 291–305.
3. Gordon, D. J., J. L. Probstfield, R. J. Garrison, J. D. Neaton, W. P. Castelli, J. D. Knoke, D. R. J. Jacobs, S. Bangdiwala, and H. A. Tyroler. 1989. High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. *Circulation.* **79:** 8–15.
4. Austin, M. A., J. E. Hokanson, and K. L. Edwards. 1998. Hypertriglyceridemia as a cardiovascular risk factor. *Am. J. Cardiol.* **81:** 7B–12B.
5. Brunzell, J. D. 1995. Familial lipoprotein lipase deficiency and other causes of the chylomicronemia syndrome. *In* The Metabolic and Molecular Basis of Inherited Disease. 7th edition. C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle, editors. McGraw-Hill, New York. 1913–1932.
6. Murthy, V., P. Julien, and C. Gagne. 1996. Molecular pathobiology of the human lipoprotein lipase gene. *Pharmacol. Ther.* **70:** 101–135.
7. Wittrup, H. H., A. Tybjaerg-Hansen, S. Abildgaard, R. Steffensen, P. Schnohr, and B. G. Nordestgaard. 1997. A common substitution (Asn291Ser) in lipoprotein lipase is associated with increased risk of ischemic heart disease. *J. Clin. Invest.* **99:** 1606–1613.
8. Gerdes, C., R. M. Fisher, V. Nicaud, J. Boer, S. E. Humphries, P. J. Talmud, and O. Faergeman. 1997. Lipoprotein lipase variants D9N and N291S are associated with increased plasma triglyceride and lower high-density lipoprotein cholesterol concentrations. Studies in the fasting and postprandial states; the European Atherosclerosis Research Studies. *Circulation.* **96:** 733–740.
9. Reymer, P. W. A., E. Gagne, B. E. Groenemeyer, H. Zhang, I. Forsyth, H. Jansen, J. C. Seidell, D. Kromhout, J. Kastelein, and M. R. Hayden. 1995. A lipoprotein lipase mutation (Asn291 Ser) is associated with reduced HDL cholesterol levels in premature atherosclerosis. *Nature Genet.* **10:** 28–34.
10. Syvänne, M., M. Antikainen, S. Ehnholm, H. Tenkanen, S. Lahdenperä, C. Ehnholm, and M-R. Taskinen. 1996. Heterozygosity for Asn^{291}→Ser mutation in the lipoprotein lipase gene in two Finnish pedigrees: effect of hyperinsulinemia on the expression of hypertriglyceridemia. *J. Lipid Res.* **37:** 727–738.
11. Fisher, R. M., F. Mailly, R. E. Peacock, A. Hamsten, M. Seed, J. S. Yudkin, U. Beisiegel, G. Feussner, G. Miller, S. E. Humpries, and P. J. Talmud. 1995. Interaction of the lipoprotein lipase asparagine 291→serine mutation with body mass index determines elevated plasma triacylglycerol concentrations: a study in hyperlipidemic subjects, myocardial infarction survivors, and healthy adults. *J. Lipid Res.* **36:** 2104–2112.
12. Zhang, H., P. W. A. Reymer, M. Liu, I. J. Forsythe, B. E. Groenemeyer, J. Frochlich, J. D. Brunzell, J. J. P. Kastelein, M. R. Hayden, and Y. Ma. 1995. Patients with apoE3 deficiency (E2/2, E2/3 and E4/4) who manifest with hyperlipidemia have increased frequency of Asn 291→Ser mutation in the human LPL gene. *Arterioscler. Thromb. Vasc. Biol.* **15:** 1695–1703.
13. Jemaa, R., F. Fumeron, O. Poirier, L. Lecerf, A. Evans, D. Arveiler, G. Luc, J-P. Cambou, J-M. Bard, and J-C. Fruchart. 1995. Lipoprotein lipase gene polymorphisms: associations with myocardial infarction and lipoprotein levels, the ECTIM study. *J. Lipid Res.* **36:** 2141–2146.
14. Goldberg, I. J. 1996. Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogeneis. *J. Lipid Res.* **37:** 693–707.
15. Peacock, R. E., A. Hamsten, P. Nilsson-Ehle, and S. Humphries. 1992. Associations between lipoprotein lipase gene polymorhisms and plasma correlations of lipids, lipoproteins and lipase activities in young myocardial infarction survivors and age-matched healthy individuals from Sweden. *Atherosclerosis.* **97:** 171–185.
16. Benlian, P., J. L. De Gennes, L. Foubert, H. Zhang, S. E. Gagne, and M. Hayden. 1996. Premature atherosclerosis in patients with familial chylomicronemia caused by mutations in the lipoprotein lipase gene. *N. Engl. J. Med.* **335:** 848–854.
17. Pimstone, S. N., S. M. Clee, S. E. Gagne, L. Miao, H. Zhang, E. A. Stein, and M. R. Hayden. 1996. A frequently occurring mutation in the lipoprotein lipase gene (Asn291Ser) results in altered postprandial chylomicron triglyceride and retinyl palmitate response in normolipidemic carriers. *J. Lipid Res.* **37:** 1675–1684.
18. Knudsen, P., S. Murtomaki, M. Antikainen, S. Ehnholm, S. Lahdenperä, C. Ehnholm, and M. R. Taskinen. 1997. The Asn-291→Ser and Ser-477→Stop mutations of the lipoprotein lipase gene and their significance for lipid metabolism in patients with hypertriglyceridaemia. *Eur. J. Clin. Invest.* **27:** 928–935.
19. Syvänen, A-C., A. Sajantila, and M. Lukka. 1993. Identification of individuals by analysis of biallelic DNA markers, using PCR and solid-phase minisequencing. *Am. J. Hum. Genet.* **52:** 46–59.
20. Mullis, K. B., and F. A. Faloona. 1987. Specific synthesis of DNA in vitro via polymerase-catalyzed chain reaction. *Methods Enzymol.* **155:** 335–350.
21. Mero, N., M. Syvänne, M. Rosseneu, C. Labeur, H. Hilden, and M-R. Taskinen. 1998. Comparison of three fatty meals in healthy normolipidemic men: high post-prandial retinyl ester response to soybean oil. *Eur. J. Clin. Invest.* **28:** 407–415.
22. Karpe, F., and A. Hamsten. 1994. Determination of apolipoproteins B-48 and B-100 in triglyceride-rich lipoproteins by analytical SDS-PAGE. *J. Lipid Res.* **35:** 1311–1317.
23. Karpe, F., M. Bell, J. Björkegren, and A. Hamsten. 1995. Quantification of postprandial triglyceride-rich lipoproteins in healthy men by retinyl ester labeling and simultaneous measurement of apolipoproteins B-48 and B-100. *Arterioscler. Thromb. Vasc. Biol.* **15:** 199–207.
24. DeFronzo, R. A., J. D. Tobin, and R. Andres. 1979. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am. J. Physiol.* **237:** E214–E223.
25. Yki-Järvinen, H., A. Consoli, N. Nurjhan, A. Young, and J. E. Gerich. 1989. Mechanism for underestimation of isotopically determined glucose disposal. *Diabetes.* **38:** 744–751.

26. Huttunen, J. K., C. Ehnholm, P. K. J. Kinnunen, and E. A. Nikkilä. 1975. An immunochemical method for selective measurement of two triglyceride lipases in human postheparin plasma. *Clin. Chim. Acta.* **63:** 335–347.
27. Ikeda, Y., A. Takagi, Y. Ohkaru, K. Nogi, T. Iwanaga, S. Kurooka, and A. Yamamoto. 1990. A sandwich-enzyme immunoassay for quantification of lipoprotein lipase and hepatic triglyceride lipase in human postheparin plasma using monoclonal antibodies to the corresponding enzymes. *J. Lipid Res.* **31:** 1911–1924.
28. Antikainen, M., L. Suurinkeroinen, M. Jauhiainen, C. Ehnholm, and M.-R. Taskinen. 1996. Development and evaluation of an ELISA method for determination of lipoprotein lipase mass concentration—comparison with a commercial, one-step enzyme immunoassay. *Eur. J. Clin. Chem. Clin. Biochem.* **34:** 547–553.
29. Ruotolo, G., Z. Huizhen, V. Bentsianov, and L. Ngoc-Anh. 1992. Protocol for the study of the metabolism of retinyl esters in plasma lipoproteins during postprandial lipemia. *J. Lipid Res.* **33:** 1541–1549.
30. Havekes, L. M., P. de Knijff, U. Beisiegel, J. Havinga, M. Smith, and E. Klasen. 1987. A rapid micromethod for apolipoprotein E phenotyping directly in serum. *J. Lipid Res.* **28:** 455–463.
31. Kadish, A. H., R. L. Litle, and J. C. Sternberg. 1968. A new and rapid method for the determination of glucose by measurement of rate of oxygen consumption. *Clin. Chem.* **14:** 116–131.
32. Miles, J., R. Glasscock, J. Aikens, J. Gerich, and M. Haymond. 1983. A microfluorometric method for the determination of free fatty acids in plasma. *J. Lipid Res.* **24:** 96–99.
33. Ludbrook, J. 1994. Repeated measurements and multiple comparisons in cardiovascular research. *Cardiovasc. Res.* **28:** 303–311.
34. Matthews, J., D. Altman, M. Campbell, and P. Royston. 1990. Analysis of serial measurements in medical research. *Brit. Med. J.* **300:** 230–235.
35. Schneeman, B. O., L. Kotite, K. M. Todd, and R. J. Havel. 1993. Relationships between the responses of triglyceride-rich lipoproteins in blood plasma containing apolipoproteins B-48 and B-100 to a fat-containing meal in normolipidemic humans. *Proc. Natl. Acad. Sci. USA.* **90:** 2069–2073.
36. Brunzell, J. D., W. R. Hazzard, D. Porte, Jr., and E. L. Bierman. 1973. Evidence for a common, saturable, triglyceride removal mechanism for chylomicrons and very low density lipoproteins in man. *J. Clin. Invest.* **52:** 1578–1585.
37. Beisiegel, U., W. Weber, and G. Bengtsson-Olivecrona. 1991. Lipoprotein lipase enhances the binding of chylomicrons to low density lipoprotein receptor-related protein. *Proc. Natl. Acad. Sci. USA.* **88:** 8342–8346.
38. Karpe, F., and M. Hultin. 1995. Endogenous triglyceride-rich lipoproteins accumulate in rat plasma when competing with a chylomicron-like triglyceride emulsion for a common lipolytic pathway. *J. Lipid Res.* **36:** 1557–1566.
39. Van Beek, A. P., H. H. J. J. van Barlingen, F. C. de Ruijter-Heijstek, H. Jansen, D. W. Erkelens, G. M. Dallinga-Thie, and T. W. A. de Bruin. 1998. Preferential clearance of apoB-48-containing lipoproteins after heparin-induced lipolysis is modulated by lipoprotein lipase activity. *J. Lipid Res.* **39:** 322–332.
40. Stalenhoef, A. F., M. J. Malloy, J. P. Kane, and R. J. Havel. 1984. Metabolism of apolipoproteins B-48 and B-100 of triglyceride-rich lipoproteins in normal and lipoprotein lipase-deficient humans. *Proc. Natl. Acad. Sci. USA.* **81:** 1839–1843.
41. Demant, T., A. Gaw, G. F. Watts, P. Durrington, B. Buckley, C. W. Imrie, C. Wilson, C. J. Packard, and J. Shepherd. 1993. Metabolism of apoB-100-containing lipoproteins in familial hyperchylomicronemia. *J. Lipid Res.* **34:** 147–156.
42. Malmström, R., C. J. Packard, T. D. G. Watson, S. Rannikko, M. Caslake, M. Bedford, P. Stewart, H. Yki-Järvinen, J. Shepherd, and M-R. Taskinen. 1997. Metabolic basis of hypertriglyceridemic effects of insulin in normal men. *Arterioscler. Thromb. Vasc. Biol.* **17:** 1454–1464.
43. Malmström, R., C. J. Packard, M. Caslake, D. Bedford, P. Stewart, H. Yki- Järvinen, J. Shepherd, and M.-R. Taskinen. 1997. Defective regulation of triglyceride metabolism by insulin in the liver in NIDDM. *Diabetologia.* **40:** 454–462.
44. Skottova, N., R. Savonen, A. Lookene, M. Hultin, and G. Olivecrona. 1995. Lipoprotein lipase enhances removal of chylomicrons and chylomicron remnants by the perfused rat liver. *J. Lipid Res.* **36:** 1334–1344.
45. Miesenböck, G., B. Hölzl, B. Föger, E. Brandstätter, B. Paulweber, F. Sandhofer, and J. R. Patsch. 1993. Heterozygous lipoprotein lipase deficiency due to a missense mutation as the cause of impaired triglyceride tolerance with multiple lipoprotein abnormalities. *J. Clin. Invest.* **91:** 448–455.
46. Sprecher, D. L., S. L. Knauer, D. M. Black, L. A. Kaplan, A. A. Akeson, M. Dusing, D. Lattier, E. A. Stein, M. Rymaszewski, and D. A. Wiginton. 1991. Chylomicron-retinyl palmitate clearance in type I hyperlipidemic families. *J. Clin. Invest.* **88:** 985–994.
47. Weintraub, M. S., S. Eisenberg, and J. L. Breslow. 1987. Dietary fat clearance in normal subjects is regulated by genetic variation in apolipoprotein E. *J. Clin. Invest.* **80:** 1571–1577.
48. Cohn, J. S., J. R. McNamara, S. D. Cohn, J. M. Ordovas, and E. J. Schaefer. 1988. Postprandial plasma lipoprotein changes in human subjects of different ages. *J. Lipid Res.* **29:** 469–479.
49. O'Meara, N. M., G. F. Lewis, V. G. Cabana, P. H. Iverius, G. S. Getz, and K. S. Polonsky. 1992. Role of basal triglyceride and high density lipoprotein in determination of postprandial lipid and lipoprotein responses. *J. Clin. Endocrinol. Metab.* **75:** 465–471.
50. Castro, G. R., and C. J. Fielding. 1985. Effects of postprandial lipemia on plasma cholesterol metabolism. *J. Clin. Invest.* **75:** 874–882.
51. Tall, A., D. Sammett, and E. Granot. 1986. Mechanisms of enhanced cholesteryl ester transfer from high density lipoproteins to apolipoprotein B-containing lipoproteins during alimentary lipemia. *J. Clin. Invest.* **77:** 1163–1172.
52. Fan, J., J. Wang, A. Bensadoun, S. J. Lauer, Q. Dang, R. W. Mahley, and J. M. Taylor. 1994. Overexpression of hepatic lipase in transgenic rabbits leads to a marked reduction of plasma high density lipoproteins and intermediate density density lipoproteins. *Proc. Natl. Acad. Sci. USA.* **91:** 8724–8728.
53. Patsch, J., G. Miesenböck, T. Hopferwieser, V. Muhlberger, E. Knapp, J. Dunn, A. Gotto, Jr., and W. Patsch. 1992. Relation of triglyceride metabolism and coronary artery disease. Studies in the postprandial state. *Arterioscler. Thromb.* **12:** 1336–1345.
54. Pimstone, S. N., S. E. Gagne, C. Gagne, P. J. Lupien, D. Gaudet, R. R. Williams, M. Kotze, P. W. A. Reymer, J. C. Defesche, J. J. P. Kastelein, S. Moorjani, and M. R. Hayden. 1995. Mutations in the gene for lipoprotein lipase: a cause for low HDL cholesterol levels in individuals heterozygous for familial hypercholesterolemia. *Arterioscler. Thromb. Vasc. Biol.* **15:** 1704–1712.