Postprandial changes of apoB-100 and apoB-48 in TG rich lipoproteins in familial combined hyperlipidemia

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Abstract **Impaired chylomicron (chylo) remnant clearance and small VLDL overproduction are major metabolic abnormalities in familial combined hyperlipidemia (FCHL). Quantitative data on postprandial apolipoprotein B-48 (apoB-48) and apolipoprotein B-100 (apoB-100) in TG rich lipoproteins (TRL) in FCHL have not been reported before. Eight untreated FCHL patients and 10 matched controls underwent a 24 h oral fat load. Fasting apoB-48 and apoB-100 were significantly higher in all TRL in FCHL. Maximal concentrations of chylo-[Svedberg's flotation rate (Sf) >400] apoB-48 and apoB-100 were reached later in FCHL (at $t = 6$ h), in contrast to controls ($t = 4$ h). Maximal VLDL1-(Sf60–400)-apoB-48 occurred at the same time point ($t = 2$ h) in both, whereas VLDL1-apoB-100 was maximal at $t = 4$ h in both, most likely representing delayed VLDL clearance by preferential clearance of chylo and their remnants by competition for the same clearance mechanisms. VLDL2-(Sf20–60)-apoB-48 and VLDL2- apoB-100 decreased in FCHL, in contrast to an increase of apoB-48, and no change of apoB-100 in controls, suggesting impaired conversion of VLDL1-apoB-48 into VLDL2-apoB-48 in FCHL, and partly also of VLDL1-apoB-100. In conclusion, in FCHL clearance of large postprandial Sf >400 apoB-48 and apoB-100 TRL is delayed. ApoB-100 accumulates in the VLDL1 range postprandially in both FCHL and controls, reaching higher levels in FCHL and thereby conferring a higher atherogenic burden in the postprandial situation in FCHL.**—Verseyden, C., S. Meijssen, and M. Castro Cabezas. **Postprandial changes of apoB-100 and apoB-48 in TG rich lipoproteins in familial combined hyperlipidemia.** ***J. Lipid Res.*** **2002.** 43: **274–280.**

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Familial combined hyperlipidemia (FCHL) is the most frequent, dominantly inherited disorder of lipid metabolism leading to increased risk for atherosclerosis (1–5). The diagnosis of FCHL is based on clinical criteria such as the presence of "multiple type hyperlipidemia" (1–6), a positive family history of premature coronary heart disease (CHD), and increased concentrations of plasma apolipoprotein B (apoB), reflecting VLDL overproduction (7, 8). Other metabolic characteristics in FCHL are impaired chylomicron (chylo) remnant clearance (6, 9), a preponderance of small dense LDL (10), the presence of insulin resistance (11, 12), disturbed postprandial free fatty acid (FFA) metabolism (11, 13), and impaired FFA uptake by fibroblasts and adipocytes (14) resulting in enhanced FFA flux to the liver (13, 14). Finally, decreased activity of hormone sensitive lipase has been described in Swedish FCHL patients (15), but not in Finnish patients (16).

In a recent report by Karpe et al., postprandial apolipoprotein B-100 (apoB-100) and apolipoprotein B-48 (apoB-48) changes were described in patients and controls with fasting hypertriglyceridemia (17). In that report, subjects were not selected based on pedigree-analysis, as is necessary for the diagnosis of FCHL. Semi-quantitative data on postprandial chylo remnant clearance in FCHL have been reported by us previously (9). Using the same methods as described by Karpe et al. (17), we have quantitated chylo and their remnants during postprandial lipemia in untreated hypertriglyceridemic FCHL subjects. For this purpose, oral fat loading tests were performed in untreated FCHL subjects, and matched controls and apoB-48 and apoB-100 were quantitated in different TG rich lipoprotein (TRL) fractions isolated by ultracentrifugation.

MATERIALS AND METHODS

Subjects

The Independent Ethics Committee of the University Medical Center Utrecht approved the study protocol, and written informed consent was obtained from each participant. Eight unrelated and untreated FCHL patients fulfilled the following criteria: they were known to have primary hyperlipidemia with varying phenotypic expression and elevated plasma apoB con-

Abbreviations: AUC, absolute area under the curve; CHD, coronary heart disease; chol, cholesterol; chylo, chylomicrons; FCHL, familial combined hyperlipidemia; IAUC, incremental area under the curve; RE, retinyl ester; TRL, TG rich lipoproteins

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centrations, at least one first degree relative had a different hyperlipidemic phenotype, and each index subject had a positive family history of premature CHD defined as myocardial infarction or cerebrovascular disease before the age of 60. In addition, the patients fulfilled the following inclusion criteria: absence of xanthomas, absence of secondary factors associated to hyperlipidemia, body mass index (BMI) <30 kg/m^2, absence of apo E2/E2 genotype, and no use of more than three units of alcohol per day.

Ten normolipidemic, healthy volunteers without a family history of cardiovascular disease, absence of apo E2/E2 genotype, BMI <30 kg/m^2, consumption of no more than three units of alcohol per day, and no use of drugs known to affect lipid metabolism, were recruited by advertisement. Controls were matched to FCHL patients by age, waist to hip ratio (WHR) and BMI.

Oral fat loading test and separation of lipoproteins

Cream was used as a fat source; this is a 40% (w/v) fat emulsion with a P/S ratio of 0.06, which contains 0.001% (w/v) cholesterol (chol), 2.8% (w/v) carbohydrates, 60 g/l dextrose and 480 IU/ml vitamin A. The source of vitamin A was an oil-in-water emulsion, which contained 100,000 IU/ml retinyl palmitate (Bufa BV, Uitgeest, The Netherlands). After an overnight fasting period of 10 h, the subjects ingested cream (50 g/m^2) and were allowed to drink only water and sugar-free tea during the following 24 h. Peripheral blood samples were obtained in sodium EDTA (2 mg/ml) before ($t = 0$ h), and at 2 h intervals up to 8 h, and 24 h after the fat load.

TG rich lipoproteins were subfractionated by cumulative density gradient ultracentrifugation. This method has been described earlier by Karpe et al. (18) and Mero et al. (19). Briefly, plasma was adjusted to d = 1.10 g/ml with solid KBr. A discontinuous density gradient consisting of 4 ml of d = 1.10 g/ml of plasma, 3 ml of d = 1.063 g/ml, 3 ml of d = 1.019 g/ml, and 2.8 ml d = 1.006 g/ml KBr solutions was formed in Ultraclear tubes (14 × 95 mm; Beckman Instruments, Inc., Palo Alto, CA). Ultracentrifugation was performed at 40,000 rpm at 4°C in a Beckman LE-80 ultracentrifuge. Consecutive runs were carried out to float Svedberg's flotation rate (Sf) >400 (32 min), Sf 60–400 (3 h 28 min) and Sf 20–60 (17 h) lipoprotein fractions were made. These fractions correspond to chylo, VLDL1, and VLDL2 fractions, respectively. After each ultracentrifugation step, the top 1 ml of the gradient containing the respective lipoprotein subclass was aspirated, and 1 ml of d = 1.006 g/ml was used to refill the buckets before the next run. Plasma and lipoprotein fractions were protected from daylight and always kept at 4°C.

Quantification of apoB-48 and apoB-100

The method earlier described by Karpe et al. was used with minor modifications (20). Samples of 500 μl chylo, VLDL1, and VLDL2 fractions were delipidated in a methanol/diethylether solvent system by gently dripping the sample into 4 ml methanol in a 10 ml round bottom glass tube with polystirene stoppers. A volume of 4 ml ice-cold diethylether was added. The delipidation cocktail was mixed and centrifuged for 48 min at 2,500 *g* at 1°C. The supernate was removed by a water suction device and 4 ml of ice-cold diethylether was added. The sample was mixed and again centrifuged for 32 min at 2,500 *g* at 1°C, whereafter the supernate was removed. The sample was dried by vaporization and 50 μl of sample buffer (containing 0.15 M sodium phosphate, 12.5% glycerol, 5% β-mercaptoethanol, 2% SDS, and 0.005% bromophenol blue) was added. The material was dissolved for 30 min at room temperature and then heated at 80°C for 10 min. After denaturation the tubes were centrifuged for 3 min at 2,500 *g* to retain the condensed water in the sample. Aliquots for apoB determination were stored at −80°C, and assayed within 3 months on 3–5% SDS-PAGE. The amount of apoB-100 in the TRL fractions is usually too high to quantitate directly by SDS-PAGE; therefore, each sample was diluted 20 times with sample buffer and then loaded on the gel. For quantitation of apoB-48, each sample was loaded on the gel undiluted. The standard curve was made by delipidated LDL with known absolute amounts of proteins.

In order to assess the equality of chromogenicities of apoB-48 and apoB-100, human chylous ascites, containing significant amounts of apoB-48, was also delipidated and run on each gel. The running time for the gels was 30 min at 40 V, continued by approximately 100 min at 80 V in running buffer (0.19 M glycine, 25 mM tris, and 0.2% SDS, pH 8.5). The proteins were stained with the Colloidal Blue Staining kit from Novex (Invitrogen, Carlsbad, CA), containing Coommassie G-250, and destained by washing the gels at least four times with distilled water. For quantitation of apoB-48 and apoB-100, a PC-based image analysis system was used. A program was developed using the KS400 version 3.0 software package (Carl Zeiss Vision, Oberkochen, Germany). After geometrical calibration, the gels were scanned with a Sony b/w CCD camera type XC-77CE (frame size 640 × 512, 256 gray levels). To delimit the bands, the so-called dynamic discrimination technique was applied. This method operates with a threshold that is dependent on the gray level of the local neighborhood region. In order to verify visually whether the band detection was correct, the delineated bands of interest were displayed in overlay on the monitor over the image and, if necessary, interactively corrected. Since the background of the gels is often not equally gray, background reconstruction was carried out. To determine the integrated optical density of each band, measurements were performed in both the original image and in the background reconstructed image and subtracted from each other.

Quantification of retinyl esters

Retinyl esters (REs) were measured by HPLC (21). In this system, the predominant plasma RE, like retinyl palmitate, oleate, and stearate, co-elute as a single peak in the chromatogram. Different volumes of the TRL were adjusted to 500 μl with saline. Then 200 μl retinyl acetate (0.25 mg/l) was added. Retinyl acetate was used as an internal standard. Methanol (500 μl) and mobile phase buffer (500 μl) were also added. The mobile phase buffer consisted of 90 ml *n*-hexane, 15 ml *n*-butyl-chloride, 5 ml acetonitrile, and 0.01 ml acetic acid. The vials were thoroughly mixed for at least 30 s and centrifuged for 15 min at 350 *g* at 20°C. The standard curve was made of purified retinyl palmitate diluted with mobile phase buffer in the range of 0.20 mg/l to 12.50 mg/l. Quantification was based on the retention time and area response of purified retinyl palmitate.

Analytical methods

Apolipoprotein E (apoE) genotypes were determined as described earlier (22). Blood was placed on ice and centrifuged immediately for 15 min at 800 *g* at 4°C. After centrifugation, a protease inhibitor was added to the plasma (final concentrations: 0.96 mg/ml EDTA, 1.25 mg/ml 6-aminohexanoic acid, 9.63 U/ml aprotinin, 0.05 mg/ml gentamycin, 1 μg/ml sodium azide). Tetrahydrolipstatin (Roche, Basel, Switzerland), to a final concentration of 1 mg/l, was also added to plasma that was used for FFA measurements (23). Plasma samples were stored at −20°C immediately after centrifugation. TG and cholesterol (chol) were measured in duplicate by a commercial colorimetric assay (GPO-PAP and CHOD-PAP, Roche, respectively). HDL-cholesterol (HDL-chol) was determined as described earlier by Burstein et al. using the phosphotungstate/MgCl2 method (24). Total plasma apoB was quantitated by immunoturbidimetry (9). FFA were measured by a commercial enzymatic colorimetric assay (Wako Chemicals GmbH, Neuss, Germany).

Statistical methods

All values are expressed as mean ± standard error of the mean (SEM). The absolute area under the curve (AUC) was calculated by the trapezoidal rule. Incremental AUC (IAUC) was calculated after correction for fasting concentrations. Mean differences between FCHL subjects and controls were calculated by independent *t*-test. Differences in fasting TG values between FCHL and controls were calculated by Mann-Whitney test. Changes in time were calculated by repeated measures ANOVA. Statistical significance was reached when $P < 0.05$ (two-tailed). Statistical calculations were performed using SPSS 10.0 (SPSS Inc., Chicago, IL). Calculations of AUC were performed with GraphPad Prism version 3.0 (GraphPad Software Inc., San Diego, CA).

RESULTS

FCHL patients and matched healthy controls did not differ in anthropometric characteristics. Fasting plasma concentrations of TG, chol, and apoB were significantly higher in FCHL patients ($P < 0.01$). Fasting plasma FFA and HDL-chol concentrations were similar in both groups (**Table 1**).

Postprandial TG and chol concentrations

In all fractions, fasting TG was significantly higher in FCHL compared with controls ($P < 0.05$). In both FCHL and controls, a significant rise of TG was found after ingestion of the cream in plasma, chylo, and VLDL1. The maximum concentrations were reached at $t = 4$ h in FCHL (plasma: 4.68 ± 0.44 mM; chylo: 1.35 ± 0.34 mM; VLDL1: 1.11 ± 0.17 mM) and in controls (2.52 ± 0.20 mM; 0.44 ± 0.07 mM; 0.62 ± 0.09 mM, respectively). In the VLDL2 fraction, TG concentrations tended to decrease in FCHL from 0.52 ± 0.16 mM at $t = 0$ h to 0.34 ± 0.04 mM at $t = 24$ h, whereas no change was seen in controls (0.15 ± 0.01 mM at $t = 0$ h).

Plasma chol did not change in any of the groups. Chol in the chylo fraction increased to a maximum at $t = 6$ h in FCHL and controls. VLDL1-chol rose in FCHL and controls to a maximum at $t = 4$ h. In VLDL2, a decrease of chol was seen in FCHL ($P < 0.05$), whereas no change was observed in controls. Chol concentrations in VLDL1 and VLDL2 fractions paralleled the TG changes in the same fractions in contrast to chol changes in plasma and in the chylo fraction (**Figs. 1** and **2**)

TABLE 1. The anthropometric characteristics and fasting values of metabolic parameters of eight FCHL subjects and 10 matched healthy volunteers

	FCHL	Controls	*P*
N	8 (5M/3F)	10 (5M/5F)	
Age (years)	45.4 ± 3.1	44.7 ± 1.7	NS
WHR	0.87 ± 0.03	0.86 ± 0.03	NS
BMI (kg/m^2)	26.3 ± 1.0	25.1 ± 0.9	NS
Plasma-TG (mM)	2.85 ± 0.43	1.27 ± 0.10	<0.01
Plasma-chol (mM)	7.12 ± 1.00	4.31 ± 0.31	<0.01
Plasma-apoB (g/l)	1.40 ± 0.16	0.89 ± 0.06	<0.01
HDL-chol (mM)	0.76 ± 0.10	0.85 ± 0.05	NS
FFA (mM)	0.37 ± 0.04	0.38 ± 0.05	NS

Data are given as mean ± SEM. FCHL, familial combined hyperlipidemia; BMI, body mass index; Chol, cholesterol; WHR, waist-to-hip ratio.

Postprandial apoB-48 and apoB-100; RE concentrations

In FCHL, fasting apoB-48 and apoB-100 in all fractions were higher than in controls. In FCHL, fasting chylo-apoB-48 (0.32 ± 0.14 mg/l) increased postprandially, reaching maximal concentrations at $t = 6$ h (1.11 ± 0.40 mg/l, $P < 0.05$). A similar response was found for chylo-apoB-100 (from 5.37 ± 2.19 to 12.30 ± 5.21 mg/l). Fasting VLDL1-apoB-48 reached its maximum at $t = 2$ h (2.09 ± 0.40 mg/l, $P < 0.05$) and was followed by a later peak for apoB-100 at $t = 4$ h (65.38 ± 14.83 mg/l). A similar pattern of apoB-48 was found in controls, albeit reaching lower concentrations in the chylo fraction (postprandial rise from 0.03 ± 0.01 mg/l to 0.27 ± 0.06 mg/l) and VLDL1 (from 0.41 ± 0.10 mg/l to 1.09 ± 0.15 mg/l). ApoB-100 in chylo and in VLDL1 in controls behaved more or less similarly to the apoB-48 changes. In FCHL, VLDL2-apoB-48 (fasting concentration 1.88 ± 0.65 mg/l) and apoB-100 (49.29 ± 12.03 mg/l) decreased after ingestion of the oral fat load, reaching a nadir at $t = 24$ h (0.90 ± 0.24 mg/l) for apoB-48 and at $t = 6$ h (36.59 ± 9.23 mg/l) for apoB-100. In controls, VLDL2-apoB-48 (fasting 0.34 ± 0.07 mg/l) showed a similar increase as in the VLDL1 fraction (rise to 0.69 ± 0.09 mg/l) and VLDL2-apoB-100 did not change significantly (fasting concentration 12.09 ± 2.25 mg/l) (**Fig. 3** and **Table 2**).

Overall, absolute AUC for apoB-48 and apoB-100 in FCHL were significantly higher than in controls (data not shown). Statistical significance between both groups was not reached after correction for fasting values.

Figure 4 shows the changes for RE in plasma and TRL fractions. The plasma, chylo, VLDL1, and VLDL2 responses calculated as absolute AUC or IAUC did not differ significantly between both groups (data not shown).

DISCUSSION

The present paper provides for the first time quantitative data on apoB-48 and apoB-100 in the postprandial situation in untreated patients with FCHL. The most striking finding is that, besides the well-known delayed clearance of postprandial Sf >400 TRL and the elevated fasting VLDL concentrations, FCHL subjects also showed a postprandial accumulation of VLDL1-apoB-100-containing particles, which occurred later than for VLDL1-apoB-48. These data suggested delayed clearance of VLDL by influx of chylo in the postprandial situation in both FCHL and controls. However, due to higher fasting levels of apoB-48- and apoB-100-containing particles, the diurnal levels of the TRL were higher in FCHL, resulting in a higher total load of potentially atherogenic particles. It is well known that these large TG rich particles are easily taken up by macrophages (25), hence this postprandial exaggerated accumulation of VLDL1 may be directly related to the development of atherosclerosis in FCHL. This is of importance because, despite the fact that fasting lipids are not

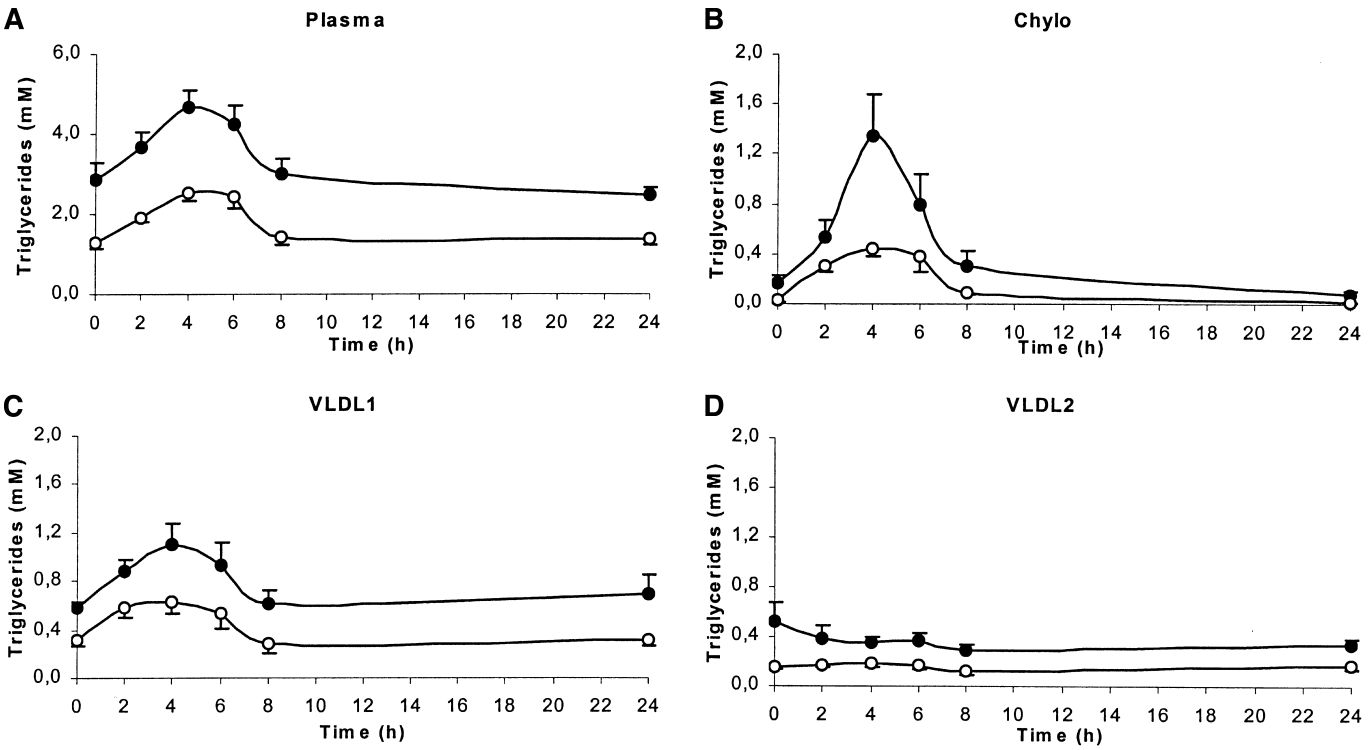

Fig. 1. Mean postprandial changes of TG in plasma (A), chylomicron- (chylo) (B), VLDL1- (C), and VLDL2- (D) fraction in eight untreated familial combined hyperlipidemia (FCHL) subjects (closed dots) and ten healthy matched volunteers (open dots) during an oral fat load. Data are given as mean ± SEM. Note: the Y-scale of plasma is three times higher than the Y-scale of the TG rich lipoproteins (TRL) fractions.

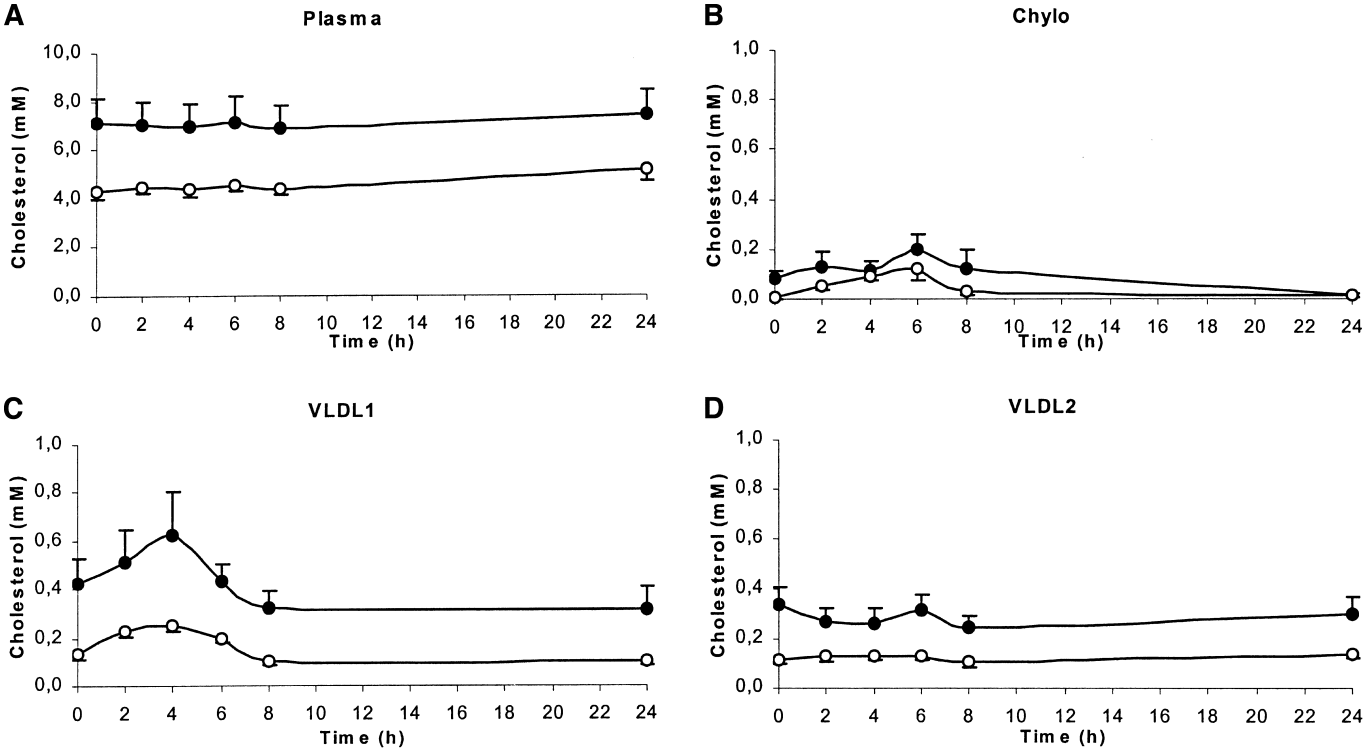

Fig. 2. Postprandial changes of cholesterol (chol) in plasma (A), chylo- (B), VLDL1- (C), and VLDL2- (D) fraction in untreated FCHL subjects (closed dots) and matched volunteers (open dots) during an oral fat load. Data are given as mean ± SEM. Note: the Y-scale of plasma is ten times higher than the Y-scale of the TRL fractions.

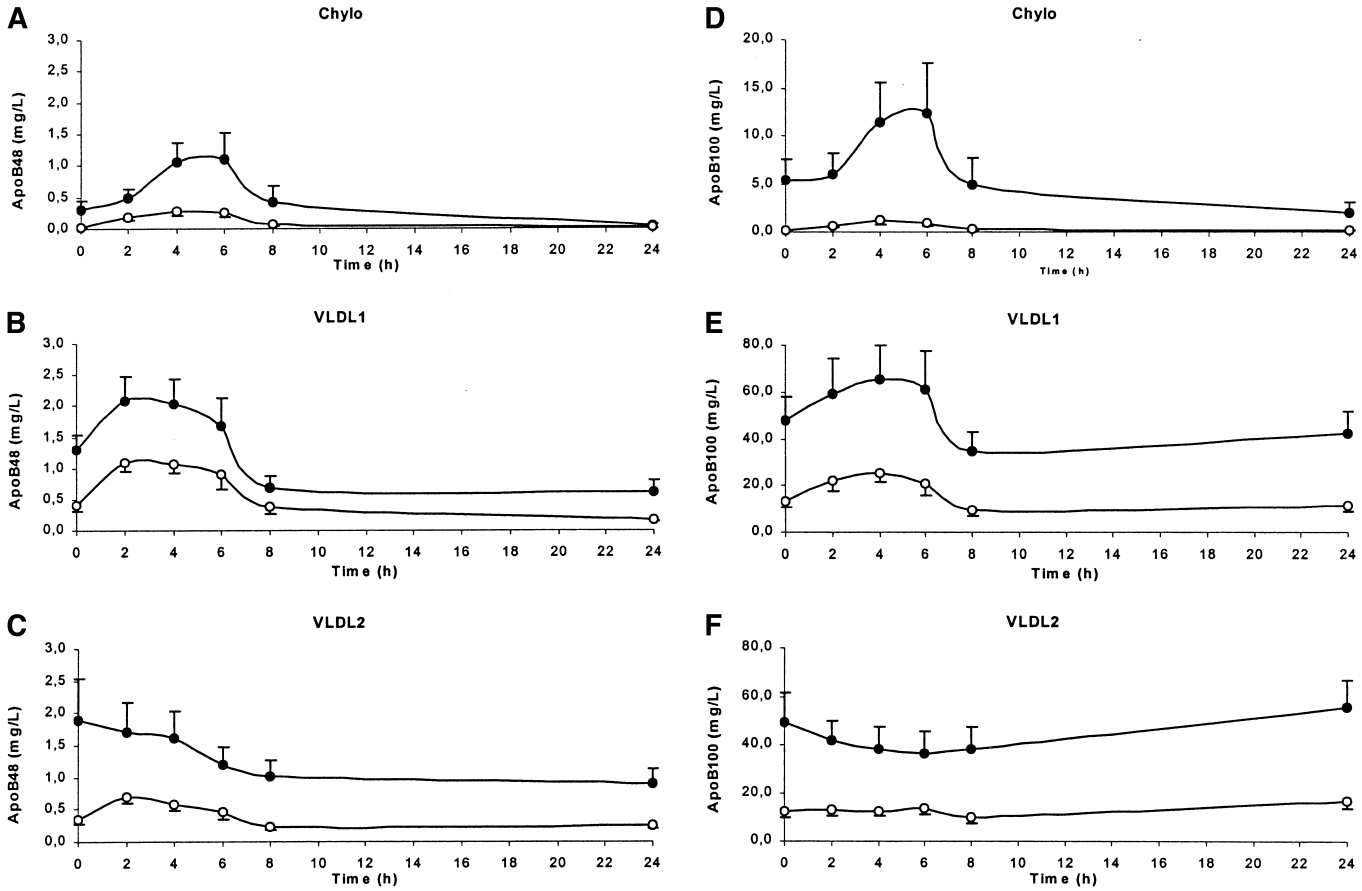

Fig. 3. Mean postprandial changes of apolipoprotein B-48 (apoB-48) in chylo- (A), VLDL1- (B), VLDL2-fraction (C), and apoB-100 in chylo- (D), VLDL1- (E), and VLDL2- (F) fraction in untreated FCHL subjects (closed dots) and matched controls (open dots) during an oral fat load. Data are given as mean ± SEM. Note: the Y-scales are different in apolipoprotein B-100 (apoB-100) curves.

dramatically disturbed in FCHL, premature atherosclerosis is closely linked to this disorder (1–4). We hypothesize that decreasing postprandial chylo production in FCHL may lead to improved postprandial metabolism of VLDL in this disorder due to less pressure on the common metabolic pathway (26, 27).

TABLE 2. Concentrations of apoB-48 and apoB-100, fasting and at time of peak or nadir in FCHL and controls

	FCHL	Time	Controls	Time
		h		*h*
ApoB-48 (mg/l)				
Chylo	0.32 ± 0.14	0	0.03 ± 0.01	0
	1.11 ± 0.40[a]	6	0.27 ± 0.06[a]	4
VLDL1	1.29 ± 0.25	0	0.41 ± 0.10	0
	2.09 ± 0.40[a]	2	1.09 ± 0.15[a]	2
VLDL2	1.88 ± 0.65	0	0.34 ± 0.07	0
			0.69 ± 0.09[a]	2
	0.90 ± 0.24	24	0.24 ± 0.07	8
ApoB-100 (mg/l)				
Chylo	5.37 ± 2.19	0	0.19 ± 0.08	0
	12.30 ± 5.21	6	1.16 ± 0.43[a]	4
VLDL1	48.03 ± 9.93	0	13.37 ± 2.62	0
	65.38 ± 14.83	4	25.09 ± 3.62[a]	4
VLDL2	49.29 ± 12.03	0	12.09 ± 2.25	0
	36.59 ± 9.23	6	12.28 ± 1.74	4
	55.35 ± 11.25	24	16.64 ± 2.93	24

FCHL and controls differ significantly at all shown values, except fasting chylo-apoB-48. Data are mean ± SEM. Chylo, chylomicron.
[a] $P < 0.05$ vs. $t = 0$.

Delayed clearance of chylo remnants in FCHL has been shown previously using different markers (6, 9, 28). ApoB-48 and apoB-100 were measured in three different fractions of TRLs isolated by ultracentrifugation. In all fractions, both apoB-48 and apoB-100 were elevated already in fasting conditions in untreated FCHL (Fig. 3). Postprandially, an accumulation of apoB-100 was observed in the chylo and VLDL1 fractions, which could be due to the formation of apoB-48-containing chylo and competition with VLDL for the same clearance mechanisms (26, 29). Alternatively, a rapid postprandial production of intestinal TRL containing apoB-100 as structural protein may have occurred in FCHL subjects. However, the latter is less likely since it has been shown that the human intestine produces only small amounts of apoB-100-containing lipoproteins (30).

Interestingly, apoB-100 in VLDL1 showed a similar accumulation in FCHL and controls, although higher concentrations were present in FCHL at all time points. Since apoB-48 in VLDL1 showed an earlier increase (at $t = 2$ h), our data are consistent with the view that increase of chylo in the postprandial phase results in delayed clearance of large VLDL1 particles (27). This physiological concept

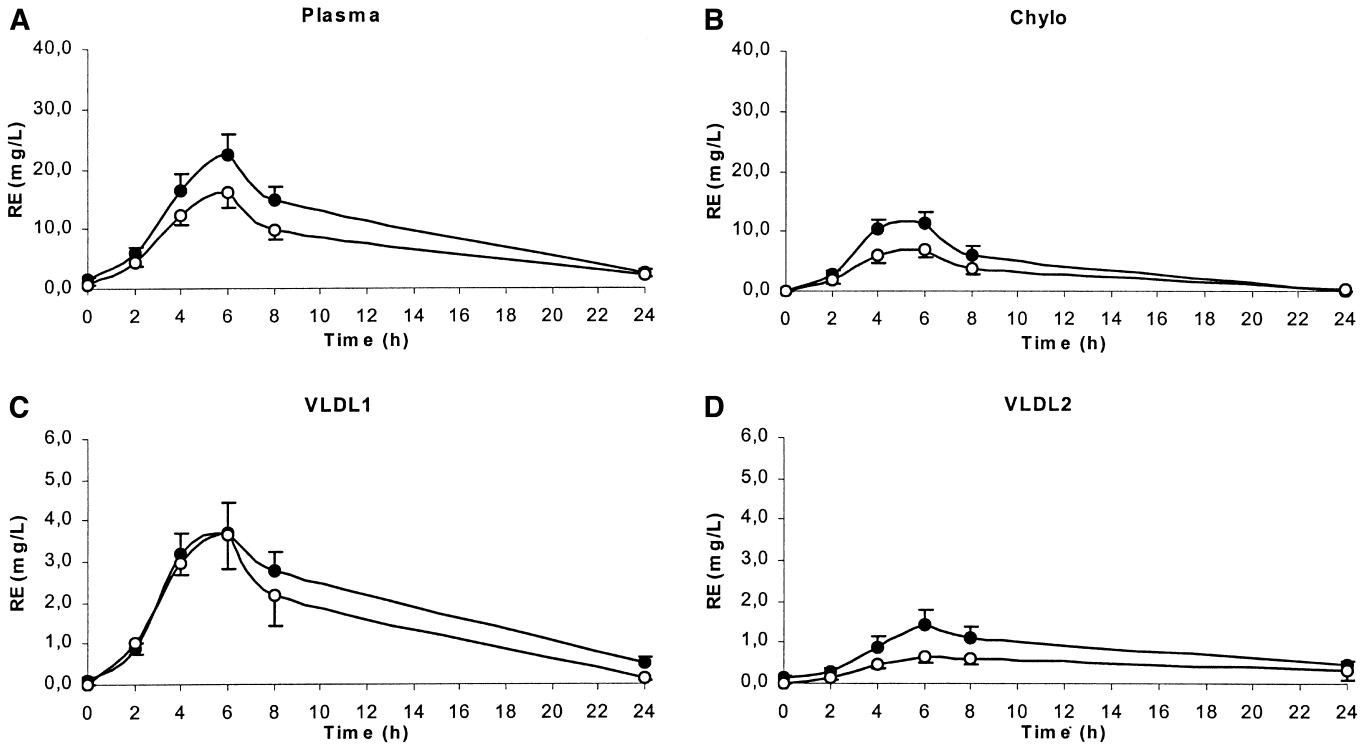

Fig. 4. Mean postprandial changes of retinyl esters (REs) in plasma (A), chylo- (B), VLDL1- (C), and VLDL2- (D) fraction in untreated FCHL patients (closed dots) and healthy matched controls (open dots) during an oral fat load. Data are given as mean ± SEM. Note: the Y-scales of plasma and the chylo-fraction are different than the Y-scales of the VLDL fractions.

seems to apply also for FCHL patients and results in large concentrations of VLDL1 particles accumulating in the plasma.

Postprandial VLDL2-apoB-100 showed a sequential decrease in FCHL, reaching a nadir at $t = 6$ h. In controls, the VLDL2-apoB-100 did not change postprandially. These data may be explained by direct clearance of apoB-100 from the VLDL1 density, especially in FCHL subjects, in line with previous reports that large TRL are cleared preferentially (20, 31). Direct clearance from the VLDL1 density in FCHL may also take place for apoB-48-containing particles since a similar pattern was seen for VLDL2-apoB-48 as for VLDL2-apoB-100. In contrast, controls had a similar VLDL2-apoB-48 curve as the VLDL1-apoB-48, suggesting that in non-FCHL subjects the conversion of large into small remnants occurs adequately. Alternatively, in controls an early postprandial production of VLD2-apoB-48 may also explain the small increase of apoB-48 found in this fraction.

Our study bears similarities to the study by Karpe et al. (17), although the groups of patients included in the two studies differed. For example, our patients did not have clinical evidence of coronary sclerosis. The apoB-48 curves in our FCHL group resembled those described by Karpe et al. in hypertriglyceridemic CHD patients (17), except for the VLDL1-apoB-48 which were lower in our FCHL subjects compared with the hypertriglyceridemic patients of Karpe's paper, and resembled more the concentrations in the hypertriglyceridemic controls. Fasting Sf >400-apoB-100 was higher in our FCHL, but fasting VLDL2-apoB-100 was lower than in Karpe's patients. Postprandial VLDL1-apoB-100 in our FCHL subjects resembled the hypertriglyceridemic controls (17). We cannot rule out that some of the differences may have been caused by the different composition of the study meals in both studies, but the differences in the fasting condition suggest a different group of patients in both studies.

In line with other studies, we have shown that REs do not reflect exactly the metabolism of postprandial chylo and their remnants (32–34). In fact, analysis of RE areas under the curve did not result in statistically significant differences in FCHL compared to controls, probably due to the relatively small numbers of subjects studied. Thus RE measurements seem to be less sensitive to detect differences in postprandial chylo remnant clearance than apoB-48 measurements. Measurements of apoB-48 and apoB-100 in different TRL fractions provide a better insight into postprandial lipoprotein metabolism and clearly illustrate the differences between hyperlipidemic and control subjects. In the case of VLDL2, contrasting findings were found between RE measurements and the other lipoprotein-associated markers like apoB-48, apoB-100, and lipids.

In conclusion, in FCHL subjects, postprandial accumulation of apoB-100-containing TRL, especially in the VLDL1 density range, may be part of the dyslipidemic phenotype, ultimately leading to atherosclerosis. Modulation of intesti-

nal synthesis of chylo may be a novel target to improve postprandial VLDL metabolism in FCHL.

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REFERENCES

1. Goldstein, J. L., H. G. Schrott, W. R. Hazzard, E. L. Bierman, and A. G. Motulsky. 1973. Hyperlipidemia in coronary heart disease. II. Genetic analysis of lipid levels in 176 families and delineation of a new inherited disorder, combined hyperlipidemia. *J. Clin. Invest.* **52:** 1544–1568.
2. Nikkila, E. A., and A. Aro. 1973. Family study of serum lipids and lipoproteins in coronary heart-disease. *Lancet.* **1:** 954–959.
3. Rose, H. G., P. Kranz, M. Weinstock, J. Juliano, and J. I. Haft. 1973. Inheritance of combined hyperlipoproteinemia: evidence for a new lipoprotein phenotype. *Am. J. Med.* **54:** 148–160.
4. Pitkanen, O. P., P. Nuutila, O. T. Raitakari, K. Porkka, H. Iida, I. Nuotio, T. Rönnemaa, J. Viikari, M. R. Taskinen, C. Ehnholm, and J. Knuuti. 1999. Coronary flow reserve in young men with familial combined hyperlipidemia. *Circulation.* **99:** 1678–1684.
5. Castro Cabezas, M., T. W. de Bruin, and D. W. Erkelens. 1992. Familial combined hyperlipidaemia: 1973–1991. *Neth. J. Med.* **40:** 83–95.
6. Castro Cabezas, M., T. W. de Bruin, H. Jansen, L. A. Kock, W. Kortlandt, and D. W. Erkelens. 1993. Impaired chylomicron remnant clearance in familial combined hyperlipidemia. *Arterioscler. Thromb.* **13:** 804–814.
7. Chait, A., J. J. Albers, and J. D. Brunzell. 1980. Very low density lipoprotein overproduction in genetic forms of hypertriglyceridaemia. *Eur. J. Clin. Invest.* **10:** 17–22.
8. Janus, E. D., A. M. Nicoll, P. R. Turner, P. Magill, and B. Lewis. 1980. Kinetic bases of the primary hyperlipidaemias: studies of apolipoprotein B turnover in genetically defined subjects. *Eur. J. Clin. Invest.* **10:** 161–172.
9. Castro Cabezas, M., D. W. Erkelens, L. A. W. Kock, and T. W. A. de Bruin. 1994. Postprandial apolipoprotein B-100 and B-48 metabolism in familial combined hyperlipidaemia before and after reduction of fasting plasma triglycerides. *Eur. J. Clin. Invest.* **24:** 669–678.
10. Austin, M. A., J. D. Brunzell, W. L. Fitch, and R. M. Krauss. 1990. Inheritance of low density lipoprotein subclass patterns in familial combined hyperlipidemia. *Arteriosclerosis.* **10:** 520–530.
11. Castro Cabezas, M., T. W. A. De Bruin, H. W. De Valk, C. C. Shoulders, H. Jansen, and D. W. Erkelens. 1993. Impaired fatty acid metabolism in familial combined hyperlipidemia. A mechanism associating hepatic apolipoprotein B overproduction and insulin resistance. *J. Clin. Invest.* **92:** 160–168.
12. Ascaso, J. F., A. Merchante, R. I. Lorente, J. T. Real, J. Martinez-Valls, and R. Carmena. 1998. A study of insulin resistance using the minimal model in nondiabetic familial combined hyperlipidemic patients. *Metabolism.* **47:** 508–513.
13. Meijssen, S., M. Castro Cabezas, Th. B. Twickler, H. Jansen, and D. W. Erkelens. 2000. In vivo evidence of defective postprandial and postabsorptive free fatty acid metabolism in familial combined hyperlipidemia. *J. Lipid Res.* **41:** 1096–1102.
14. Sniderman, A., K. Cianflone, P. Arner, L. K. Summers, and K. N. Frayn. 1998. The adipocyte, fatty acid trapping, and atherogenesis. *Arterioscler. Thromb. Vasc. Biol.* **18:** 147–151.
15. Reynisdottir, S., M. Eriksson, B. Angelin, and P. Arner. 1995. Impaired activation of adipocyte lipolysis in familial combined hyperlipidemia. *J. Clin. Invest.* **95:** 2161–2169.
16. Ylitalo, K., V. Large, P. Pajukanta, S. Reynisdottir, K. V. Porkka, J. Vakkilainen, I. Nuotio, M. R. Taskinen, and P. Arner. 2000. Reduced hormone-sensitive lipase activity is not a major metabolic defect in Finnish FCHL families. *Atherosclerosis.* **153:** 373–381.
17. Karpe, F., M. L. Hellénius, and A. Hamsten. 1999. Differences in postprandial concentrations of very-low-density lipoprotein and chylomicron remnants between normotriglyceridemic and hypertriglyceridemic men with and without coronary heart disease. *Metabolism.* **48:** 301–307.
18. Karpe, F., T. Olivecrona, A. Hamsten, and M. Hultin. 1997. Chylomicron/chylomicron remnant turnover in humans: evidence for margination of chylomicrons and poor conversion of larger to smaller chylomicron remnants. *J. Lipid Res.* **38:** 949–961.
19. Mero, N., M. Syvanne, B. Eliasson, U. Smith, and M. R. Taskinen. 1997. Postprandial elevation of apoB-48-containing triglyceride-rich particles and retinyl esters in normolipemic males who smoke. *Arterioscler. Thromb. Vasc. Biol.* **17:** 2096–2102.
20. 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.
21. Ruotolo, G., H. Zhang, V. Bentsianov, and N. A. Le. 1992. Protocol for the study of the metabolism of retinyl esters in plasma lipoproteins during postprandial lipemia. *J. Lipid Res.* **33:** 1541–1549.
22. Dallinga-Thie, G. M., M. van Linde-Sibenius Trip, L. A. Kock, and T. W. de Bruin. 1995. Apolipoprotein E2/E3/E4 genotyping with agarose gels. *Clin. Chem.* **41:** 73–75.
23. Krebs, M., H. Stingl, P. Nowotny, D. Weghuber, M. Bischof, W. Waldhausl, and M. Roden. 2000. Prevention of *in vitro* lipolysis by tetrahydrolipstatin. *Clin. Chem.* **23:** 950–954.
24. Burstein, M., H. R. Scholnick, and R. Morfin. 1970. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. *J. Lipid Res.* **11:** 583–595.
25. Gianturco, S. H., W. A. Bradley, S. Nozaki, G. L. Vega, and S. M. Grundy. 1993. Effects of lovastatin on the levels, structure, and atherogenicity of VLDL in patients with moderate hypertriglyceridemia. *Arterioscl. Thromb.* **13:** 472–481.
26. Björkegren, J., C. J. Packard, A. Hamsten, D. Bedford, M. Caslake, L. Foster, J. Shepherd, P. Stewart, and F. Karpe. 1996. Accumulation of large very low density lipoprotein in plasma during intravenous infusion of a chylomicron-like triglyceride emulsion reflects competition for a common lipolytic pathway. *J. Lipid Res.* **37:** 76–86.
27. Karpe, F., G. Steiner, T. Olivecrona, L. A. Carlson, and A. Hamsten. 1993. Metabolism of triglyceride-rich lipoproteins during alimentary lipemia. *J. Clin. Invest.* **91:** 748–758.
28. Genest, J., A. Sniderman, K. Cianflone, B. Teng, S. Wacholder, Y. Marcel, and P. Kwiterovich, Jr. 1986. Hyperapobetalipoproteinemia. Plasma lipoprotein responses to oral fat load. *Arteriosclerosis.* **6:** 297–304.
29. 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.
30. Hoeg, J. M., D. D. Sviridov, G. E. Tennyson, S. J. Demosky, Jr., M. S. Meng, D. Bojanovski, I. G. Safonova, V. S. Repin, M. B. Kuberger, V. N. Smirnov, K. Higuchi, R. E. Gregg, and H. B. Brewer, Jr. 1990. Both apolipoproteins B-48 and B-100 are synthesized and secreted by the human intestine. *J. Lipid Res.* **31:** 1761–1769.
31. Hultin, M., R. Savonen, and T. Olivecrona. 1996. Chylomicron metabolism in rats: lipolysis, recirculation of triglyceride-derived fatty acids in plasma FFA, and fate of core lipids as analyzed by compartmental modelling. *J. Lipid Res.* **37:** 1022–1036.
32. Cohn, J. S., E. J. Johnson, J. S. Millar, S. D. Cohn, R. W. Milne, Y. L. Marcel, R. M. Russel, and E. J. Schaefer. 1993. Contribution of apoB-48 and apoB-100 triglyceride-rich lipoproteins (TRL) to postprandial increases in the plasma concentrations of TRL triglycerides and retinyl esters. *J. Lipid Res.* **34:** 2033–2040.
33. 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.
34. Krasinski, S. D., J. S. Cohn, R. M. Russell, and E. J. Schaefer. 1990. Postprandial plasma vitamin A metabolism in humans: a reassessment of the use of plasma retinyl esters as markers for intestinally derived chylomicrons and their remnants. *Metabolism.* **39:** 357–365.