Differences in apolipoprotein and lipid composition between human chylomicron remnants and very low density lipoproteins isolated from fasting and postprandial plasma

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Abstract **Triglyceride-rich lipoproteins (TRLs) that are modified during alimentary lipemia and their remnants are indicated to play an important role in the development of atherosclerosis. Although recent studies in transgenic and gene knock-out animal models have shed new light on the function of different apolipoproteins (apos) in the metabolism of TRLs and on their respective role in atherogenesis in these models, little is known about the compositional properties of human chylomicron remnants and very low density lipoprotein (VLDL). To address this issue, apos E, C-I, C-II, and C-III and lipids (triglycerides, phospholipids and cholesterol) were measured in Svedberg flotation rate (S_f) 60–400 and S_f 20–60 subfractions of VLDL and chylomicron remnants isolated from fasting and postprandial plasma samples in ten normotriglyceridemic men. VLDL was separated from chylomicron remnants by immunoaffinity chromatography using monoclonal antibodies (4G3 and 5E11) recognizing apoB-100 but not apoB-48 epitopes. The triglyceride, cholesterol and apoC-II contents of large (S_f 60–400) chylomicron remnants were significantly higher compared with large VLDL particles, while the small (S_f 20–60) chylomicron remnants contained significantly more apoC-II molecules but fewer apoC-I molecules than small VLDL. Whereas the apoC-III contents of large chylomicrons decreased, the apoC-III contents of large VLDL increased postprandially. The cholesterol to triglyceride ratio of large VLDL particles increased transiently by 50% in response to the oral fat load, whereas the cholesterol to triglyceride ratio of large chylomicron remnant particles and small TRL remnants increased 50–100% throughout the entire postprandial period. The specific alterations of the apolipoprotein and lipid composition of chylomicron remnants and VLDL particles observed during alimentary lipemia are likely to target these lipoprotein species differently to metabolic routes and to confer both endogenous and exogenous remnant lipoprotein roles in atherogenesis.**—Björkegren, J., F. Karpe, R. W. Milne, and A. Hamsten. **Differences in apolipoprotein and lipid composition between human chylomicron remnants and very low density lipoproteins isolated from fasting and postprandial plasma.** ***J. Lipid Res.*** **1998.** 39: **1412–1420.**

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Alimentary lipemia is a dynamic state persisting for most of the day, and the metabolic fate of triglyceride-rich lipoproteins (TRLs) is determined by a range of postprandial influences. TRLs consist of very low density lipoproteins (VLDL), which are synthesized in the liver and contain apolipoprotein (apo)B-100 as structural protein (1), and chylomicrons and their remnants, which are secreted by the small intestine and have apo B-48 as their structural protein (2). In the bloodstream, TRLs exchange lipids and apolipoproteins with primarily high density lipoproteins (HDL) (3). This exchange determines the apolipoprotein and lipid composition of TRL particles and is of importance for the hydrolysis of TRLs by lipoprotein lipase (LPL) and their subsequent receptor-mediated uptake mainly in the liver (4).

The major change in lipoprotein particle number during alimentary lipemia is the increase of postprandial large (Svedberg flotation rate (S_f) 60–400) VLDL particles (5). Large VLDL particles accumulate during alimentary lipemia secondary to preferential lipolysis of chylomicrons by LPL (6) and account for 90% of the cholesterol increase in the TRL fractions observed during alimentary lipemia (7). Chylomicrons and chylomicron remnants, on the other hand, are fewer and do not exceed 10% of the VLDL particle number. However they are rich in triglycerides and account for 80% of the postprandial increase in plasma triglyceride concentration (8).

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

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Although delayed clearance of postprandial triglycerides and of chylomicron remnants is associated with cardiovascular disease (9–12), other clinical studies suggest a role for VLDL in coronary atherosclerosis (13, 14). Accordingly, the lipoproteins responsible for the association between postprandial triglycerides and atherosclerosis remain to be defined. A wealth of recent studies in transgenic and gene knock-out animal models have shed new light on the function of different apolipoproteins in the metabolism of TRLs and the importance of the apolipoprotein composition of TRL particles for atherogenesis in these models (15–20). Despite these new insights, little is known about the compositional features of human chylomicron remnants and VLDL.

We hypothesized that postprandial lipemia induces alterations of TRL particle composition that differ between the two TRL species. In order to test this hypothesis we have used two monoclonal antibodies directed towards the C-terminus of apoB-100 that do not crossreact with apoB-48 (21, 22) to separate VLDL from chylomicron remnants for subsequent comparison of the apolipoprotein and lipid composition of these TRL species in the fasting and the fed state.

MATERIALS AND METHODS

Subjects

Lipoproteins from 10 healthy normolipidemic men were studied. The basic characteristics of the plasma donors are shown in **Table 1**. No dietary restraints were imposed on participants prior to the study. They were asked to fast during the 12 h before the oral fat load during which period they were also asked to refrain from smoking. In addition, participants were asked to abstain from alcohol intake during the preceding 3 days. Major fasting plasma lipoprotein lipids were determined by a combination of preparative ultracentrifugation and precipitation of apoB-containing lipoproteins followed by lipid analysis (23). All participants had an apoE3/3 or apoE3/4 genotype as determined by restriction isotyping (24). All subjects gave oral informed consent to the study, which was approved by the ethics committee of the Karolinska Hospital.

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 (25). 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% as protein. 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 content of the meal was 1000 kCal with 60.2 E% from fat, 13.3E% 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.

Lipoprotein separation

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 into $S_f > 400$, S_f 60–400, and S_f 20–60 fractions (25). The isolated S_f 60–400 and S_f 20–60 TRL fractions were subjected to immunoaffinity chromatography, using the specific monoclonal antibodies 4G3 and 5E11 against the C-terminal of apoB-100. Typically, 20 mg of 4G3 and 20 mg of 5E11 antibodies were bound to 5 ml CNBr-activated Sepharose 4B according to the manufacturer's description (Pharmacia Fine Chemicals, Inc., Uppsala, Sweden). 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 as described (25). Briefly, 1–2 ml (<1 mg of total protein) of S_f 60–400 and S_f 20–60 TRL fractions were added to ten 5-ml tubes, each containing 2 ml of Sepharose gel dissolved in phosphate-buffered saline (PBS, pH 7.4, 0.02% NaN_3). TRLs bound to the Sepharose gel, i.e., TRLs containing apoB-100 (VLDL and VLDL remnants), were dissociated by addition of 2 ml of thiocyanate (3 m NaSCn, pH 7.4) to each tube. To

TABLE 1. Apolipoprotein E genotype, age, body mass index, and fasting plasma and lipoprotein lipid concentrations of the 10 study subjects

Subject #	ApoE Genotype	Age	BMI	Plasma		VLDL		LDL		HDL	
				Chol	TG	Chol	TG	Chol	TG	Chol	TG
		yr	*kg/m²*	*mmol/l*		*mmol/l*		*mmol/l*		*mmol/l*	
1	3/3	45	22	4.32	1.29	0.25	0.50	2.37	0.53	1.29	0.26
2	3/3	47	21	4.22	1.05	0.25	0.58	2.97	0.35	1.27	0.13
3	3/4	48	26	5.79	2.14	0.81	1.54	4.22	0.41	1.21	0.20
4	3/4	48	24	6.29	1.44	0.39	1.04	4.24	0.31	1.15	0.12
5	3/3	48	23	6.03	1.44	0.42	0.99	4.32	0.33	0.97	0.13
6	3/3	48	23	6.32	1.49	0.47	1.05	4.49	0.34	1.36	0.10
7	3/3	47	32	4.88	1.43	0.29	1.02	3.49	0.35	1.06	0.06
8	3/3	43	26	5.27	1.71	0.50	1.24	4.32	0.38	0.80	0.09
9	3/3	50	19	5.99	1.20	0.32	0.67	4.23	0.39	1.44	0.11
10	3/3	45	23	4.90	1.07	0.23	0.64	3.34	0.31	1.39	0.11
Mean ± SD		47 ± 2.3	24 ± 4	5.40 ± 0.79	1.43 ± 0.35	0.39 ± 0.17	0.93 ± 0.33	3.80 ± 0.72	0.37 ± 0.07	1.19 ± 0.20	0.13 ± 0.06

Apo, apolipoprotein; BMI, body mass index; VLDL, d < 1.006 kg/l lipoproteins; LDL, d 1.006–1.063 kg/l lipoproteins; HDL, d >1.063 kg/l lipoproteins, as determined by preparative ultracentrifugation and precipitation of apoB-containing lipoproteins followed by lipid analysis; Chol, cholesterol, TG, triglycerides.

further purify the unbound fraction, i.e. the chylomicron remnants, from VLDL contamination, this fraction was gently (0.5 ml/min) passed over two parallel coupled columns containing 5 ml of Sepharose to which 4G3 and 5E11 antibodies had been coupled at room temperature. A total of 8–10 ml of the unbound fractions was collected with the use of an UV detector (UV-1, Pharmacia) and a fraction collector (Frac-200, Pharmacia). The protein contents of each TRL fraction were determined using the method of Lowry et al. (26) with sodium dodecyl sulfate (SDS) added to clear turbidity. The recovery of S_f 60–400 and S_f 20–60 TRLs added to the affinity gel was 89 ± 10% and 91 ± 5.9% (mean ± SD, n = 10), respectively, as estimated by measuring the sum of recovered protein from the bound and unbound fractions. SDS polyacrylamide gel electrophoresis (SDS-PAGE) showed that 91 ± 5.4% (S_f 60–400) and 95 ± 3.6% (S_f 20–60) (mean ± SD, n = 10) of the total apoB-100 was found in the recovered bound fraction. There was no contamination by apoB-48 in the bound fraction as estimated on SDS-PAGE. ApoB-48 represented 73% of total apoB contents in the unbound fraction of both the S_f 60–400 (73 ± 11%, mean ± SD, n = 10) and the S_f 20–60 (73 ± 12%, mean ± SD, n = 10) TRLs. TRL particles within the unretained fractions are, despite the apoB-100 contamination, from here on referred to as chylomicron remnant particles. The terms large and small TRLs, and S_f 60–400 and S_f 20–60 lipoprotein fractions, respectively, are hereafter interchangeable.

Determination of TRL lipids and apolipoproteins

Triglycerides and phospholipids in the bound and unbound 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 (27), the latter if very low concentrations were expected such as in the unbound fractions. ApoB and E were quantified by SDS-PAGE (28), whereas C apos were determined by urea gel electrophoresis (25). A laser densitometer (Ultroscan XL, LKB Pharmacia, Bromma, Sweden) was used to scan the gels and to subsequently evaluate the contents of apoCs, apoE, apoB-100, and apoB-48 on the respective gels (Gelscan XL 2400 Software). The relative chromogenicity of insulin in comparison to that of apoC-I, C-II, and C-III and the relative chromogenicity of apoB-100 in comparison to that of apoE was determined by amino acid analysis (25).

Calculations and statistical methods

The number of apolipoprotein and lipid molecules per particle was calculated by dividing apolipoprotein and lipid concentrations in density fractions by their respective molecular mass (apoB-100 = 549 kD, apoB-48 = 256 kD, apoE = 36.5 kD, apoC-I = 6.5 kD, apoC-II = 8.9 kD, and apoC-III = 8.9 kD). The fraction molarity of apolipoproteins and lipids was then divided by the corresponding molarity of apo B.

Conventional methods were used for calculating means and standard deviations. The statistical significance of compositional differences between chylomicron remnants and VLDL from the same plasma donor was tested by paired two-tailed *t*-test. Associations between lipoprotein parameters were determined by calculation of Pearson correlation coefficients.

RESULTS

Postprandial responses of apoB-48 and apoB-100

Fasting and postprandial plasma concentrations of apoB-48 and apoB-100 in the $S_f > 400$, S_f 60–400, and S_f 20–60 fractions are shown in **Table 2**. The apoB-100 concentrations in the S_f 60–400 and S_f 20–60 fractions were approximately 10 times higher than the corresponding levels of apoB-48. ApoB-100 seemed to peak slightly later than apoB-48 in the S_f 60–400 fraction. The apoB-100 concentration in the S_f 20–60 fraction appeared to be largely unaffected by the oral fat load whereas the apoB-48 concentration in this fraction increased transiently. The responses of apoB-48 and apoB-100 concentrations in the $S_f >$ 400, S_f 60–400, and S_f 20–60 fractions to the mixed meal in individuals with an apoE3/4 genotype (n = 2) did not differ from the corresponding responses in the remaining individuals with an apoE3/3 genotype (29).

Apolipoprotein composition of fasting and postprandial S_f 60–400 and S_f 20–60 VLDL and chylomicron remnant particles

The apolipoprotein contents of large and small VLDL and chylomicron remnant particles isolated from fasting plasma and 2, 4, 6, and 8 h after ingestion of the mixed meal are shown in **Fig. 1**. A transient increase of the apoE contents of large VLDL and of large chylomicron remnant particles of around 100% and 25%, respectively, was observed in response to the test meal. A similar pattern was observed for the two small TRL species, which both

TABLE 2. Concentrations of plasma triglycerides and of apoB-100 and apoB-48 in $S_f > 400$, S_f 60–400 and S_f 20–60 fractions of triglyceride-rich lipoproteins in fasting and postprandial plasma samples

		$S_f > 400$		S_f 60–400		S_f 20–60	
Time	Plasma Triglycerides	ApoB-48	ApoB-100	ApoB-48	ApoB-100	ApoB-48	ApoB-100
h	*mmol/l*	*mg/l*		*mg/l*		*mg/l*	
0	1.3 ± 0.3	T	T	1.1 ± 0.5	24 ± 10	1.6 ± 0.6	38 ± 14
2	2.4 ± 0.9[a]	0.3 ± 0.2	0.2 ± 0.1	2.3 ± 0.7[a]	34 ± 13[a]	2.2 ± 0.5[a]	37 ± 13[c]
4	2.8 ± 1.1[a]	0.5 ± 0.3	0.4 ± 0.4	2.2 ± 0.8[a]	37 ± 15[a]	2.2 ± 0.8[b]	45 ± 18[c]
6	1.5 ± 0.4[c]	0.2 ± 0.2	0.3 ± 0.3	1.1 ± 0.5[c]	23 ± 15[c]	2.0 ± 1.3[a]	45 ± 17[c]
8	1.0 ± 0.2[b]	T	T	0.7 ± 0.3[a]	11 ± 5[a]	1.5 ± 0.7[c]	35 ± 13[c]

Values are mean ± SD, n = 10. T, Trace amount. The statistical significance of differences between the fasting and the 2, 4 , 6, and 8 postprandial h determinations was calculated by Student's paired *t*-test and are indicated below postprandial time points.

[a] $P < 0.005$.

[b] $P < 0.05$.

[c] NS, not significant.

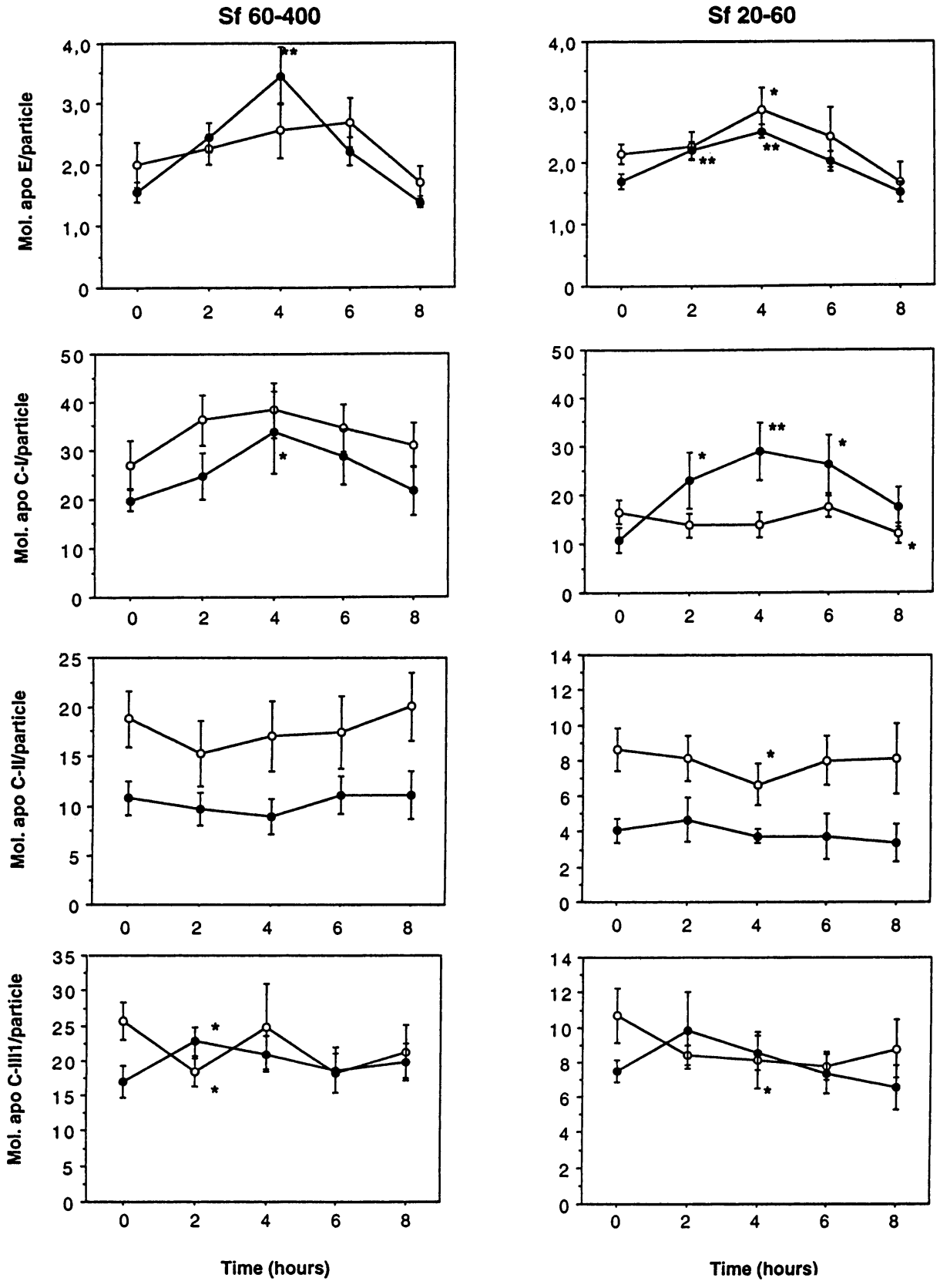

Fig. 1. Line plots of changes in VLDL and chylomicron remnant apolipoprotein contents (number of apolipoprotein molecules per particle) in S_f 60–400 and S_f 20–60 fractions in response to ingestion of the mixed meal in 10 normotriglyceridemic men. The left panel shows line plots of the apolipoprotein contents of VLDL (–●–) and chylomicron remnants (–○–) in the S_f 60–400 fraction and the right panel shows line plots of the apolipoprotein contents of VLDL (–●–) and chylomicron remnants (–○–) in the S_f 20–60 fraction. Values are mean ± SEM with SEM indicated by bars. *$P < 0.05$ and **$P < 0.005$ compared with the fasting sample.

exhibited a transient increase of their apoE contents of approximately 25%.

The apoC-I contents of the two large TRL species exhibited similar patterns in response to the oral fat load, with an average transient increase of the number of apoC-I molecules per particle of 75–150%. In contrast, while the small VLDL exhibited a transient postprandial increase of the number of apoC-I molecules per particle, the opposite was observed for small chylomicron remnants. This resulted in a statistically significant difference in apoC-I contents between the two small TRL species at 4 h after intake of the test meal ($P < 0.05$, n = 10).

Whereas the postprandial apoC-II pattern was similar for the two TRL species (exhibiting a transient decrease of the number of apoC-II molecules per large and small TRL particle of around 25%), the apoC-II content of chylomicron remnants exceeded that of VLDL by 50–100% at all time points ($P < 0.05$, at 0, 4, and 8 h, n = 10).

The apoC-III_1 content of large chylomicron remnant particles isolated from fasting plasma was shown to be approximately 50% higher compared with large VLDL ($P < 0.05$, n = 10). Interestingly, while the apoC-III_1 content of large VLDL particles increased in the early postprandial period ($P < 0.05$, n = 10), the apoC-III content of the chylomicron remnant decreased ($P < 0.05$, n = 10). The postprandial pattern of the apoC-III_2 contained in the larger TRLs was similar to that of apoC-III_1, but the relative contents were lower (data not shown). The apoC-III_1 and apoC-III_2 contents of small chylomicron remnant particles decreased by approximately 25% in response to the oral fat load, whereas the small VLDL apoC-III contents seemed to be largely unaffected.

Lipid composition of fasting and postprandial S_f 60–400 and S_f 20–60 VLDL and chylomicron remnant particles

The lipid contents of large and small VLDL and chylomicron remnant particles isolated from fasting plasma and 2, 4, 6, and 8 h after ingestion of the mixed meal are shown in **Fig. 2**. The triglyceride contents differed significantly between the two large TRL species in the fasting state and during the first six postprandial h ($P < 0.05$, n = 10). Large chylomicron remnant particles carried approximately 70% more triglyceride molecules than large VLDL in the fasting state. However, this difference decreased during the postprandial period, partly due to a transient 10% increase of the triglyceride contents of large VLDL particles ($P < 0.005$ at 2 h, n = 10), but also due to a late decrease of the number of triglyceride molecules per large chylomicron remnant particle. The triglyceride content of the two small TRL species was a third of that of large TRLs as a reflection of the smaller particle volume. The small chylomicron remnant triglyceride content decreased during the postprandial period ($P < 0.05$, n = 10), whereas the small VLDL triglyceride content was largely unaffected. Although the cholesterol contents of large chylomicron remnant particles were largely unaffected by the oral fat load, the large chylomicron remnant particles contained 70% more cholesterol molecules compared with large VLDL particles isolated in the fasting state and after 6 h into the postprandial period ($P < 0.05$, n = 10 at 6 h). In contrast to the cholesterol contents of large chylomicron particles, the cholesterol contents of large VLDL particles increased transiently by 50% in response to the oral fat load ($P < 0.05$, n = 10). Small VLDL and small chylomicron remnant particles contained a similar amount of cholesterol, and the cholesterol contents responded similarly to the test meal, with a late 20% increase in the number of cholesterol molecules. The cholesterol to triglycerides ratio of the small TRLs increased considerably (50% for small VLDL and 100% for small chylomicron remnants) during the later half of the test (**Fig. 3**). The increase in the cholesterol to triglyceride ratio was shown to be highly significant for both TRL species between the fourth and the sixth postprandial h ($P < 0.005$, n = 10). The cholesterol to triglyceride ratio of large chylomicron remnants seemed to increase throughout the postprandial period (by 30% at 8 h), whereas the corresponding ratio of large VLDL particles increased by 20% during the first four postprandial h ($P < 0.005$, n = 10) and thereafter returned to baseline (Fig. 3).

The phospholipid contents of both large and small chylomicron remnants exceeded that of large and small VLDL by 60–170% (Fig. 2). For the small TRLs this profound difference was surprising considering their similar triglyceride contents.

DISCUSSION

To the best of our knowledge this is the first detailed account of the compositional features of human postprandial chylomicron remnants. Monoclonal antibodies directed against apoB-100 epitopes have previously been used to isolate postprandial lipoproteins by immunoaffinity purification from a crude fraction of TRLs in normolipidemic individuals by us (25) and previously by others (7, 8). The rationale for separate characterizations of chylomicron remnant and VLDL particles of different size was primarily the fact that these lipoprotein species are metabolized differently during alimentary lipemia (5) and that chylomicron remnants are believed to be implicated in atherogenesis (9–12). Whether the association of chylomicron remnants with the presence or progression of atherosclerosis in fact implicates chylomicron remnants or VLDL, or both, is unknown.

The immunoaffinity procedure used in the present study yielded a retained fraction completely devoid of apoB-48-containing TRL particles. However, the unretained fraction was found to contain a mixture of apoB-48 containing particles (70%) and apoB-100 containing particles (30%). The failure of the monoclonal antibodies 4G3 and 5E11 to recognize their epitopes in the LDL receptor-binding region of apoB-100 of some VLDL particles is interesting, as it indicates that a subpopulation of VLDL particles (5–15%) has remnant characteristics. Indeed, plasma samples subjected to a mixture of monoclonal antibodies against apoA-I and apoB-100 epitopes, recognizing 97% of LDL and HDL and the majority of

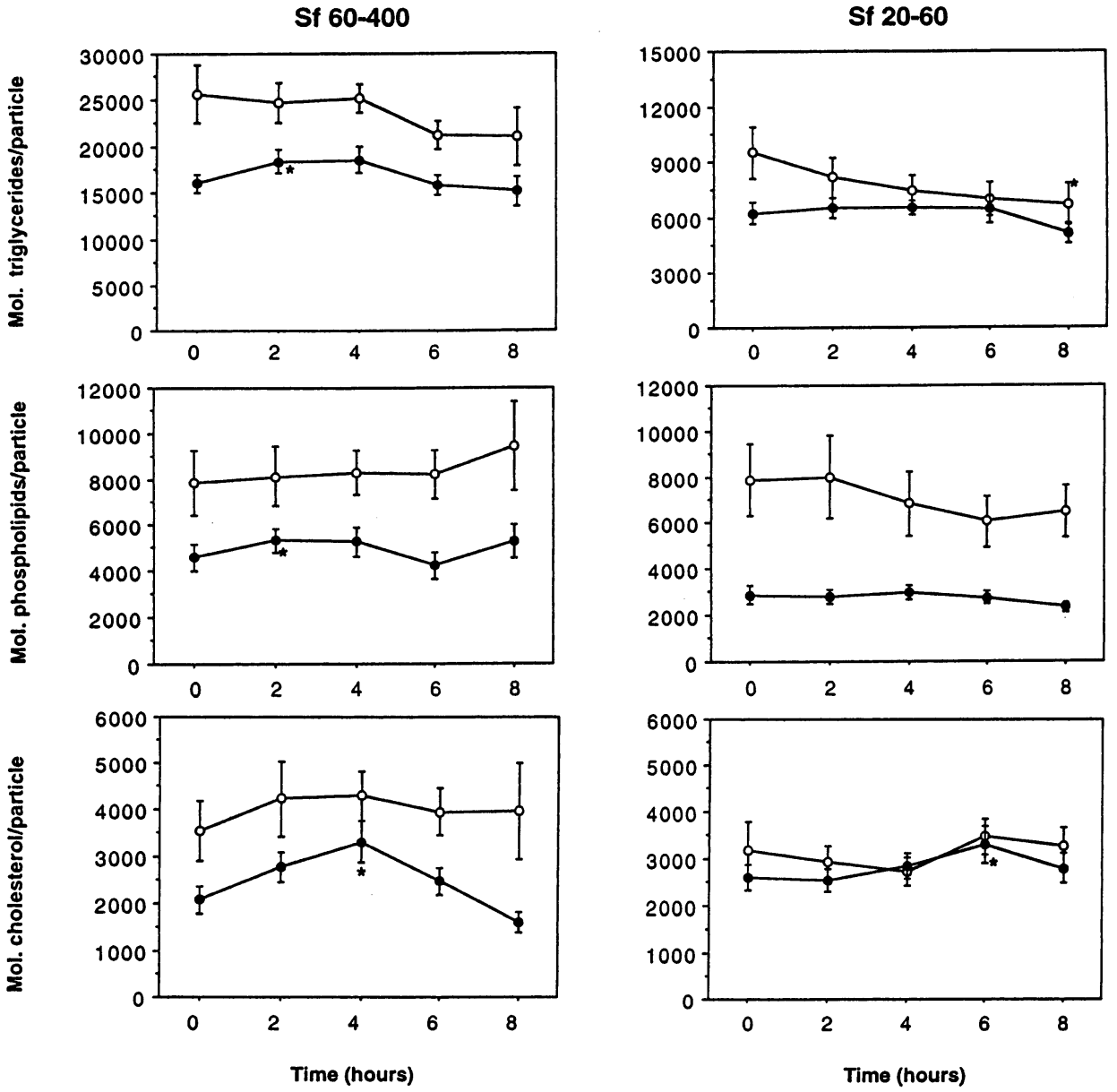

Fig 2. Line plots of changes in VLDL and chylomicron remnant lipid contents (number of lipid molecules per particle) in S_f 60–400 and S_f 20–60 lipoprotein fractions in response to ingestion of the mixed meal in 10 normotriglyceridemic men. The left panel shows line plots of the lipid contents of VLDL (–●–) and chylomicron remnants (–○–) in the S_f 60–400 fraction and the right panel shows line plots of the lipid contents of VLDL (–●–) and chylomicron remnants (–○–) in the S_f 20–60 fraction. Values are mean ± SEM with SEM indicated by bars. *$P < 0.05$.

VLDL particles, uniformly contain a population of particles termed remnant-like particles (RLP), containing either apoB-48 or apoB-100 (30). Plasma levels of these RLP are elevated in individuals with diabetes, hypertriglyceridemia, and coronary heart disease (30).

Both large and small chylomicron remnants were found to contain significantly more apoC-II and phospholipid molecules per particle than VLDL in the fasting state and throughout the entire postprandial period. Furthermore, the triglyceride and cholesterol contents of large chylomicron remnant particles were significantly higher compared with large VLDL. In contrast, the postprandial apoC-I contents of small chylomicron remnants was significantly lower than the corresponding apoC-I contents of small VLDL. The cholesterol to triglyceride ratio of large VLDL particles increased transiently in response to the oral fat load whereas the cholesterol to triglyceride ratio of large chylomicron remnant particles and small TRL remnants increased throughout the entire postprandial period.

The apoE contents of chylomicron remnants and VLDL particles did not differ consistently, i.e., both TRL particles exhibited a transient increase in apoE during the postprandial period. The postprandial enrichment of TRL particles with apoE, which has previously been dem-

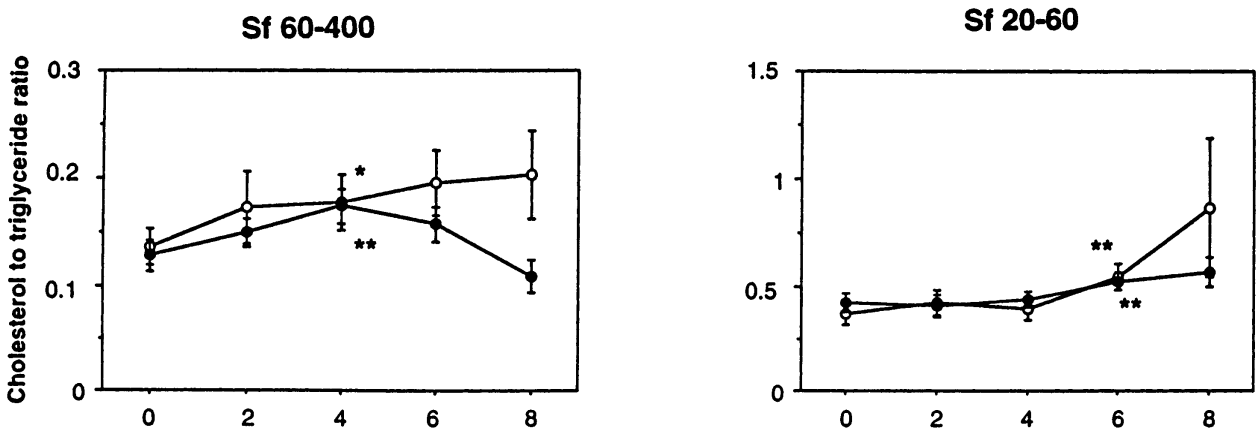

Fig 3. Line plots of the cholesterol to triglyceride ratio in S_f 60–400 and S_f 20–60 fractions in response to ingestion of the mixed meal in 10 normotriglyceridemic men. The left panel shows line plots of the cholesterol to triglyceride ratio of VLDL (–●–) and chylomicron remnants (–○–) in the S_f 60–400 fraction and the right panel shows line plots of the cholesterol to triglyceride ratio of VLDL (–●–) and chylomicron remnants (–○–) in the S_f 20–60 fraction. Values are mean ± SEM with SEM indicated by bars. *$P < 0.05$ and **$P < 0.005$ compared with the fasting sample.

onstrated for VLDL particles (25), would facilitate clearance of these particles from the bloodstream through LDL receptor (31–33) or LRP pathways (34), potentially through increased binding of TRLs to heparan sulfate proteoglycans (HSPG) (35).

The concomitant apoC-I enrichment and apoC-II depletion of postprandial TRLs observed in the present study may also affect the receptor-mediated clearance of these particles. Sehayek and Eisenberg (36) have shown that the facilitated uptake of apoE-enriched TRLs in vitro could be inhibited by addition of exogenous apoC, of which apoC-I had the strongest inhibitory effect. However, in a recent study by Shachter and co-workers (19), TRLs isolated from transgenic mice overexpressing human apoC-I, in contrast to TRLs from transgenic mice overexpressing apoC-II or apoC-III, seemed to bind HSPG normally, suggesting a difference between the apoE-opposing effect of apoC-I and that of other apoCs. In support of this difference, we have recently shown that postprandial VLDL particles isolated from normolipidemic individuals are enriched with apoE and C-I but depleted of apoC-II compared with VLDL particles isolated in the fasting state (25). Despite the apoC-I enrichment, VLDL isolated during transient triglyceridemia compete more favorably with LDL for binding to the LDL receptor on fibroblasts in vitro than do VLDL particles isolated from fasting plasma. However, the increased binding to fibroblasts in vitro seem to have little or no effect on the LDL receptor-mediated uptake of the postprandial VLDL particles (33). Although apoC-I has limited effect on TRL binding to HSPG, the difference in apoC-I contents between small chylomicron remnants and small VLDL observed in the present study could be interpreted to favor the receptor-mediated uptake of small chylomicron remnants.

The higher apoC-II contents of both small and large chylomicron remnants in comparison with VLDL would make these particles a better substrate for LPL than the corresponding VLDL particles, but would simultaneously delay the clearance of chylomicron remnants by receptor-mediated mechanisms compared with VLDL. However, considering the fact that the lipolytic conversion from the $S_f > 400$ to the S_f 60–400 range for intestinal lipoproteins is very limited, that the further conversion to S_f 20–60 is negligible (37), and that an excess of apoC-II on TRL particles in apoC-II transgenic mice leads to an impaired lipolysis and hypertriglyceridemia rather than to increased lipolysis (38), we believe that these lipoproteins are true remnants that despite the higher triglyceride and apoC-II contents, have difficulties in undergoing further lipolysis and receptor-mediated uptake.

The importance of the TRL apoC-III contents for the clearance of these particles, both in the fasting state in vitro (39) and in vivo (17) as well as in the postprandial state in vivo (18), is well established. Our observation that large VLDL exhibit an early increase of the apoC-III_1 and apoC-III_2 constituents, whereas chylomicron remnants simultaneously exhibit a decrease of the apoC-III_1 and apoC-III_2 contents, is therefore of interest. The interpretation could be that apoC-III has higher affinity for VLDL particles than for chylomicron remnant particles or, alternatively, is co-secreted with VLDL particles but not with chylomicron particles, as apoC-III is mainly synthesized in the liver and only in minor quantities by the intestine (40). The metabolic consequence would be a delayed clearance of postprandial VLDL compared with chylomicrons and fasting VLDL both via receptor-mediated pathways (16) and even more so through LPL-mediated hydrolysis (20, 41), both contributing to the postprandial accumulation of VLDL (6). Theoretically, the differences in apoC-III contents between the two TRL species could explain their difference in plasma levels and turnover rates in man.

The VLDL concentration is rate limiting for cholesteryl ester transfer to TRL particles by cholesteryl ester transfer protein (CETP) in normolipidemic plasma, i.e. in the fasting state in normolipidemic individuals. However, the CETP mass concentration becomes rate limiting for the

increased cholesteryl ester transfer in hypertriglyceridemic individuals and presumably also in the postprandial state of normolipidemic individuals (42). Furthermore, the major cholesteryl ester acceptor among TRLs in the postprandial state in normolipidemic subjects is, according to a recent study, large TRL remnants (S_f 60–400 particles), rather than chylomicrons ($S_f > 400$ particles) or smaller TRL remnants ($S_f < 60$ particles) (43). The present observations, that the cholesterol contents of large VLDL particles increases transiently as a response to the oral fat load together with the transient postprandial increase of S_f 60–400 apoB-100, confirm and extend the similar observation made earlier independently by us (5) and by Schneeman et al. (7), namely that the postprandial TRL accumulation of cholesterol is mainly accounted for by large VLDL.

Small remnants of either particle species share with LDL the potential for causing lipid accumulation in the arterial intima in humans (44), and small cholesteryl ester-rich TRLs have been shown to be related to global severity and rate of progression of coronary atherosclerosis (13, 14). The striking increase of the cholesterol to triglyceride ratio of small TRL remnants during alimentary lipemia, indicating formation of smaller and cholesterol-enriched particles, is of particular interest in this context.

The precise metabolic pathways underlying the specific alterations of the apolipoprotein and lipid composition of chylomicron remnants and VLDL remnants during alimentary lipemia and the consequences for atherogenesis cannot be defined with the present study design. This will require a combination of experimental and clinical studies. However, it is evident from the present work that future studies of chylomicron remnants and VLDL in relation to human atherosclerosis need to take the apolipoprotein and lipid composition of these particles into account.

Currently, the postprandial alterations of the apolipoprotein and lipid composition of VLDL particles along with the low number of chylomicron remnant particles compared with VLDL particles in plasma, both in the fasted and fed state, challenge the original atherogenic chylomicron remnant hypothesis of Zilversmit (45) and suggest a role for postprandial VLDL in atherogenesis.

In summary, the present study has revealed specific alterations of the apolipoprotein and lipid composition of chylomicron remnant and VLDL particles during alimentary lipemia that are likely to target these lipoprotein species differently to metabolic routes.

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