Selective partitioning of dietary fatty acids into the VLDL TG pool in the early postprandial period

Richard B. Heath,* Fredrik Karpe,[1,*] Ross W. Milne,[†] Graham C. Burdge,[§] Stephen A. Wootton,[§] and Keith N. Frayn*

Oxford Centre for Diabetes, Endocrinology and Metabolism,* University of Oxford, Oxford, OX3 7LJ, United Kingdom; Lipoprotein and Atherosclerosis Research Group, Department of Pathology,[†] University of Ottawa Heart Institute, Ottawa, Ontario, Canada; and Institute of Human Nutrition,[§] University of Southampton, Southampton, SO16 6YD, United Kingdom

Abstract Circulating triacylglycerol (TG) arises mainly from dietary fat. However, little is known about the entry of dietary fat into the major TG pool, very low-density lipoprotein (VLDL) TG. We used a novel method to study the specific incorporation of dietary fatty acids into postprandial VLDL TG in humans. Eight healthy volunteers (age 25.4 ± 2.2 years, body mass index 22.1 ± 2.3 kg/m^2) were fed a mixed meal containing 30 g fish oil and 600 mg [1-^{13}C]palmitic acid. Chylomicrons and VLDL were separated using immunoaffinity against apolipoprotein B-100. The fatty acid composition of lipoproteins was analyzed by gas chromatography/mass spectrometry. [1-^{13}C]palmitic acid started to appear in VLDL TG 3 h after meal intake, and a similar delay was observed for eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Approximately 20% of dietary fatty acids entered the VLDL TG pool 6 h after meal intake. DHA was clearly overincorporated into this pool compared with [1-^{13}C]palmitic acid and EPA. This seemed to depend on a marked elevation of this fatty acid in the nonesterified fatty acid pool. In summary, the contribution of dietary fatty acids to early postprandial VLDL TG is substantial. The role of DHA in VLDL TG production will require further investigation.—Heath, R. B., F. Karpe, R. W. Milne, G. C. Burdge, S. A. Wootton, and K. N. Frayn. **Selective partitioning of dietary fatty acids into the VLDL TG pool in the early postprandial period. *J. Lipid Res.* 2003.** 44: **2065–2072.**

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Exaggerated postprandial triacylglycerol (TG) concentrations have long been associated with coronary artery disease and atherosclerosis (1). It has been proposed that an accumulation of chylomicron remnants is the major contributor to the atherogenic state (2). However, in the postprandial period, more than 90% of TG-rich lipoproteins (TRL) are very low-density lipoproteins (VLDLs) (3, 4). Prolonged accumulation of VLDL in the postprandial period is seen with diets rich in saturated fatty acids (5). Despite the link between dietary fat and VLDL TG, few attempts have been made to study the incorporation of dietary fatty acids into the VLDL TG pool in vivo in humans.

VLDL and chylomicron remnants are similar in size and composition, and separation of the lipoprotein classes cannot be achieved by conventional methods such as ultracentrifugation. However, the structural protein, apolipoprotein B (apoB), differs between TRL derived from the intestine (apoB-48) and that derived from the liver (apoB-100) (6). ApoB-48 and apoB-100 are encoded by the same gene, but a post-transcriptional modification of apoB-100 occurs in the enterocyte, producing apoB-48 (7). ApoB-48 is homologous to the N-terminal 48% of apoB-100, but shares no homology with the C-terminal end of apoB-100. Monoclonal antibodies, which recognize epitopes in apoB-100 but not in apoB-48, have therefore been used to separate and examine the lipid and apolipoprotein composition of chylomicrons and VLDL, respectively (3, 4, 8–12). However, this technique has not been used to study the dynamic aspects of lipid metabolism, such as the incorporation of dietary fatty acids into TRL in the postprandial state. The first aim of this study was to investigate the appearance of dietary fatty acids in hepatic TRL in the postprandial state in normal healthy subjects.

Studies of postprandial lipid metabolism have shown that fatty acid uptake by adipose tissue is highly regulated and that significant amounts of hydrolysed fatty acids are not taken up by the tissue but are released as nonesteri-

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Abbreviations: apoB, apolipoprotein B; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; RE, retinyl ester; TG, triacylglycerol.

[1] To whom correspondence should be addressed.
e-mail: fredrik.karpe@oxlip.ox.ac.uk

fied fatty acids (NEFAs) (13, 14). In particular, postprandial uptake of both docosahexaenoic acid (DHA, C22:6 n-3) and eicosapentaenoic acid (EPA, C20:5 n-3) by adipose tissue is proportionally reduced, compared with saturated and monounsaturated fatty acids. In fact, accumulation of EPA and DHA in the NEFA pool has been observed in the postprandial period (15). Furthermore, it has also been reported that the EPA:DHA ratio is significantly decreased in the NEFA pool compared with that in the plasma TG pool (16–19). This may indicate selective metabolism between these two n-3 fatty acids. Few studies have looked at the EPA:DHA ratio in lipoprotein TG in the postprandial period (16, 20). Therefore, the second aim of this study was to investigate whether there is selective partitioning of dietary EPA and DHA in the postprandial state.

MATERIALS AND METHODS

Subjects

The subjects were three males and five females with a median age of 24.5 years (range: 23–30) and a median body mass index of 22.0 kg/m^2 (range: 18.6–25.7). Subjects had a median fasting total cholesterol concentration of 4.6 mmol/l (range: 3.7–6.4), a median fasting TG concentration of 1.0 mmol/l (range: 0.6–1.8), and a median fasting glucose concentration of 4.8 mmol/l (range: 4.4–6.2). The study was approved by the Oxfordshire Clinical Research Ethics Committee, and all subjects gave informed consent before the study.

Study protocol

To standardize the subjects' nutritional state before the study, all subjects consumed a low-fat (<10 g fat) evening meal. All subjects were instructed to fast overnight and to refrain from alcohol and strenuous exercise. A cannula was inserted into an antecubital vein of the forearm, and blood samples were taken at −20, 0, 30, 60, 90, 120, 150, 180, 240, 270, 300, and 360 min. At 0 min, subjects were given a mixed meal consisting of 40 g Rice Krispies (Kelloggs, Warrington, UK), banana, and a warm chocolate milkshake containing 50 g of fat. The fat consisted of 30 g fish oil (EPAX3000TG; Pronova Biocare, Asslund, Norway), 10 g macadamia nut oil, and 10 g safflower oil (Anglia Oils Ltd., Hull, UK) to provide a range of fatty acids. Six hundred milligrams of [1-^{13}C]palmitic acid (99 atom%; Cambridge Isotopes, Woburn, MA) were added to the test oil. The macronutrient composition and fatty acid composition of the meal are shown in **Table 1** and **Table 2**, respectively. The breakfast was consumed within 10 min, and the subjects remained in the supine position throughout the study.

TABLE 1. Macronutrient composition of test meal[a]

	Carbohydrate	Fat	Protein	Energy
		g		*kJ*
Test oil (50.6 g)	0	50.6	0	1,870
Skimmed milk (250 g)	12.5	0.3	8.3	350
Nesquik (10 g)	8.0	0.3	0.3	152
Rice Krispies (40 g)	35.9	0.3	1.8	472
Banana (100 g)	23.2	0.3	1.2	403
Total	79.4	51.8	11.6	3,247

[a] Determined from food tables and manufacturers' data.

TABLE 2. Composition of test oil[a]

Test Oil	SFA	MUFA	PUFA
		g	
14:0	2.6		
16:0	7.9		
[1-^{13}C] 16:0	0.6		
16:1		5.2	
18:0	2.3		
18:1		11.1	
18:2			7.7
18:3			1.3
20:5			7.1
22:5			0.8
22:6			4.1
Total	13.4	16.3	21

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid.
[a] Determined from food tables and manufacturers' data.

Analyses

Blood samples were collected into heparinized syringes (Sarstedt, Leicester, UK), for analysis of plasma metabolites and lipoprotein analysis. Plasma was separated by centrifugation at 1,700 *g* for 15 min. Samples for plasma TG, NEFA, and cholesterol analysis were stored at −20°C until analyzed.

Plasma glucose, TG, and lipoprotein TG concentrations were measured with kits from Instrumentation Laboratory (Warrington, UK). Cholesterol and NEFA concentrations were measured with kits from Randox Co. (Antrim, UK) and Alpha Laboratories (Eastleigh, UK), respectively. All of the metabolites were batch-analyzed and measured enzymatically with an IL Monarch automated analyzer (Instrumentation Laboratory), exhibiting an intra-assay coefficient of variation of <2.5%.

TRLs were separated by flotation in a density gradient (21). Ultracentrifugation was performed in a SW40Ti swinging bucket rotor at 40,000 rpm at 15°C (XL-70 Ultracentrifuge, Beckman Instruments, Palo Alto, CA). The gradients were run for 32 min to float Svedberg flotation rate (S_f) >400 lipoproteins and for a further 16 h to float S_f 20–400. The top 0.5–1 ml from each tube was aspirated, collected into another preweighed tube, and immediately put on ice. TRLs were separated from plasma taken at −20 and 0 min, and at 90, 180, 270 and 360 min after the mixed meal.

TRLs were further separated by immunoaffinity chromatography, using the specific monoclonal antibodies 3F5, 4G3, and 5E11 against apoB-100, which do not cross-react with apoB-48 (8, 22). Preparation of immunoabsorbants was performed essentially as described by Milne et al. (8). Typically, 4 mg of 3F5, 4 mg of 4G3, and 4 mg of 5E11 were coupled to 1 ml of cyanogen bromide-activated Sepharose. Separation of apoB-48 and B-100 was carried out in 1 ml columns containing the antibody-coupled Sepharose gel dissolved in phosphate-buffered saline (PBS, pH 7.4, 0.02% NaN_3). The column was drained, and 0.5 ml TRL (<0.2 mg TRL protein) was added to the top of the column. The sample was passed through the column 10 times over a 2 h period to ensure optimum binding efficiency. The unbound fraction was collected by draining the column, and further unbound material was washed through with 1.5 ml of PBS. Any nonspecific binding was removed by eluting the column with sodium chloride (1 M NaCl, pH 7.4). The apoB-100-containing TRLs, which were bound to the column, were eluted by successive 1 ml washes with thiocyanate (3 M NaSCN, pH 7.4). Bound material was recovered in 10 ml sodium thiocyanate. Gels were washed with 10 ml PBS and stored at 4°C for later reutilization.

The bound material was concentrated by density gradient ultracentrifugation using a protocol similar to that used before. Briefly, 10 ml of bound sample was pipetted into polyvinyl alcohol-coated Ultra-Clear centrifuge tubes. Three milliliters PBS was added to the top of each tube to create a small gradient. Samples were centrifuged for 16 h as previously described. The bound samples were collected by aspiration into preweighed tubes and immediately put on ice.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (23) showed that the bound fraction was completely devoid of apoB-48. Ninety percent of apoB-100 was found in the bound fraction; this fraction will hereafter be called the VLDL fraction. ApoB in the unbound fraction consisted of 70% apoB-48 and 30% apoB-100 and will be called the "chylomicron remnant fraction," as it is chylomicron remnant rich. The recovery of lipoproteins using the immunoaffinity method was 73.4 ± 17.0% and 77.1 ± 9.3% as judged by recovery of apoB-100 and lipoprotein TG, respectively.

Gas chromatography and mass spectrometry

For analysis of specific fatty acids, lipids were extracted from plasma or from lipoprotein fractions by using chloroform-methanol (2:1; v/v) (24). After separation of the lipid classes by solid-phase extraction, and methylation of fatty acids with methanolic sulfuric acid, gas chromatography was used to analyze the fatty acid composition of plasma NEFA, S_f >400 TG, S_f 20–400 TG, chylomicron remnant TG, and VLDL TG (25, 26). The absolute concentrations of the individual fatty acids were calculated by reference to internal standards added to the plasma during lipid extraction—heptadecanoic acid for NEFA and triheptadecanoyl glycerol for lipoprotein TG. Isotope enrichment was analyzed by gas chromatography-isotope ratio mass spectrometry as described previously by Evans et al. (27). A test meal was homogenized and analyzed using gas chromatography to establish its specific fatty acid composition.

Statistics

Data were analyzed with SPSS software version 10.0 (SPSS UK Ltd., Chertsey, UK). Postprandial metabolite and lipoprotein responses were analyzed by repeated measures ANOVA, using 'time' as a within-subject factor. Differences between remnant and VLDL TG composition were also measured by repeated measures ANOVA, using 'time' and 'lipoprotein' as within-subject factors. $P < 0.05$ was considered statistically significant.

RESULTS

Postprandial responses in plasma TG, NEFA, and glucose

The mean plasma TG rose from 1.04 ± 0.14 mmol/l to peak at 1.95 ± 0.36 mmol/l 3 h after the meal ($P < 0.001$) (**Fig. 1**). Essentially, the average TG concentration returned to baseline at 6 h. Mean plasma NEFA concentrations were suppressed in response to the meal, falling from 550 ± 170 μmol/l to a nadir of 236 ± 41 μmol/l at 3 h, and returning to baseline levels at 6 h ($P < 0.001$). There was also a significant rise in mean plasma glucose ($P < 0.001$), rising from 5.18 ± 0.25 mmol/l in the fasting state, peaking at 150 min at 6.83 ± 0.26 mmol/l, and returning to baseline values by 6 h.

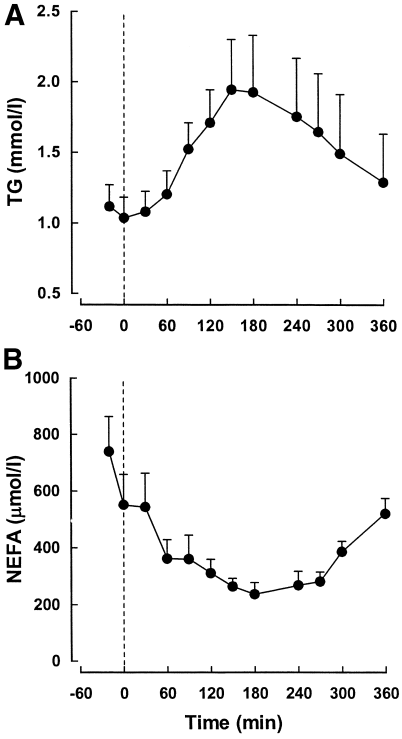

Fig. 1. Plasma triacylglycerol (TG) (A) and plasma nonesterified fatty acid (NEFA) (B) concentrations before and after the mixed meal. Mean ± SEM, n = 8.

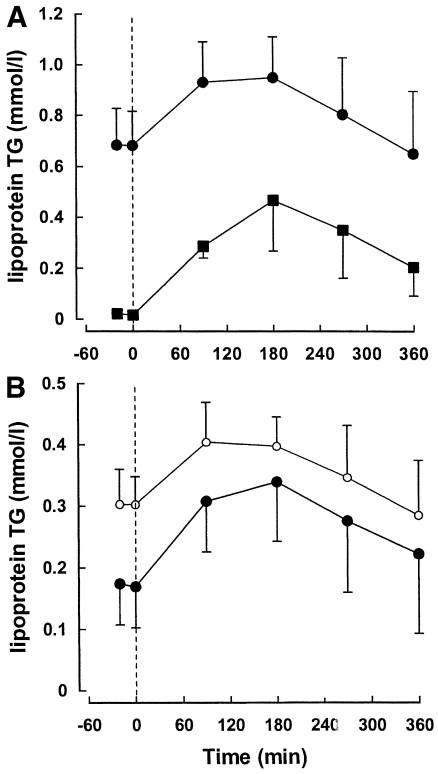

Fig. 2. A: Svedberg flotation rate (S_f) >400 TG (closed squares) and S_f 20–400 TG (closed circles) concentrations before and after the mixed meal. B: S_f 20–400 TG is divided in the lower panel into exogenous TG, the chylomicron remnant TG (closed circles), and endogenous TG, VLDL TG (open circles). Mean ± SEM, n = 8.

TG concentrations of S_f >400 and S_f 20–400 lipoproteins in the postprandial period

Fasting and postprandial TG concentrations of S_f >400 and S_f 20–400 lipoproteins are shown in **Fig. 2**. Both S_f >400 TG and S_f 20–400 TG significantly increased in response to the test meal ($P < 0.004$). The peaks in TG of lipoproteins in both S_f >400 and S_f 20–400 coincided with the peak in plasma TG. S_f 20–400 TG returned to baseline values at 6 h, whereas S_f >400 TG remained higher than baseline at 6 h. TG concentrations of both chylomicron remnants and VLDL rose after the meal. There was a significant rise in chylomicron remnant TG ($P < 0.001$), and the postprandial change in chylomicron remnant TG followed a pattern similar to that in S_f >400 TG, whereas the postprandial rise in VLDL TG was not significant ($P = 0.159$).

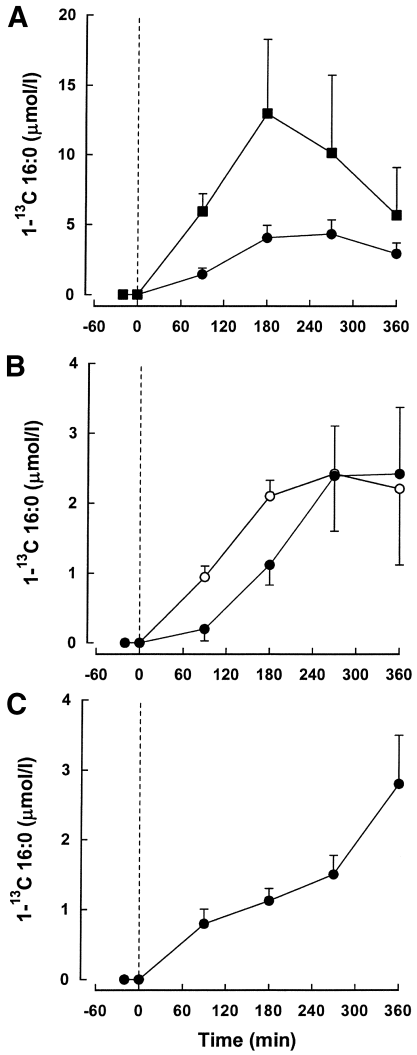

Fig. 3. A: Dietary [1-^{13}C]palmitic acid incorporation into S_f >400 TG (closed squares) and S_f 20–400 TG (closed circles) concentrations after the mixed meal. B: The appearance of the labeled fatty acid in both chylomicron remnants (open circles) and VLDL (closed circles) TG is shown in S_f 20–400 lipoproteins. C: The appearance of [1-^{13}C]palmitic acid in the NEFA pool. Mean ± SEM, n = 8.

Incorporation of dietary palmitic acid into postprandial lipoprotein TG pools

The postprandial appearance of [1-^{13}C]palmitic acid in NEFA and TRL TG is shown in **Fig. 3**. As expected, [1-^{13}C]palmitic acid was rapidly incorporated into both S_f >400 and S_f 20–400 TG ($P < 0.003$), and the curve shapes were similar to that of plasma TG concentration. The concentration peaked at 3 h in S_f >400 TG and remained high 6 h postprandially. In the S_f 20–400 fraction, the peak concentration was 90 min later than in S_f >400 TG and remained high at 6 h after the meal. There was also a rapid accumulation of [1-^{13}C]palmitic acid in the NEFA pool.

[1-^{13}C]palmitic acid was rapidly incorporated into both VLDL and chylomicron remnant TG ($P < 0.001$). However, the time courses were different for palmitic acid incorporation into VLDL TG and chylomicron remnant TG. The [1-^{13}C]palmitic acid incorporation into the chylomicron remnants mirrored that of plasma TG, while there was at least a 90 min delay before the label appeared in the VLDL TG pool. The peak concentration was attained later in VLDL TG compared with remnant TG.

Incorporation of EPA and DHA into postprandial lipoprotein TG pools

The postprandial appearance of EPA and DHA in S_f >400 and S_f 20–400 TRL TG is shown in **Fig. 4**. In S_f >400, there was a rapid rise in both EPA and DHA in lipoprotein TG ($P < 0.001$) and both remained at high relative concentrations 6 h after the meal. The relative increase of EPA to DHA in the S_f >400 mirrored the composition of the test meal (EPA:DHA ratio 1.73). Both EPA and DHA were rapidly incorporated into both VLDL and chylomicron remnant TG pools ($P < 0.001$) and appeared at the same time as labeled palmitic acid in both pools. However, there was a significant difference in the relative concentrations of n-3 PUFA in these two TG pools: in chylomicron remnant TG, EPA concentration peaked at 55.4 ± 12.0 μmol/l at 270 min, whereas DHA concentration peaked at 31.3 ± 7.6 μmol/l at 270 min; in VLDL TG, EPA concentration was 27.7 ± 8.1 μmol/l at 360 min, whereas DHA concentration was 31.4 ± 7.7 μmol/l at this time. The EPA:DHA ratio in remnant TG was 1.90 ± 0.16, yet the EPA:DHA ratio was significantly lower in the VLDL TG pool at 0.77 ± 0.16 ($P < 0.003$).

The ratios of EPA and DHA to [1-^{13}C]palmitic acid in lipoprotein TG and NEFA pools are shown in **Fig. 5**. Both the DHA:[1-^{13}C]palmitic acid ratio and the EPA:[1-^{13}C]palmitic acid ratio in S_f >400 TG were similar to those in the meal. In the VLDL TG pool, there was a 4-fold increase in the number of moles of DHA molecules per mole of [1-^{13}C]palmitic acid, whereas the EPA:[1-^{13}C]palmitic acid ratio remained the same as in the meal. Interestingly, the DHA:[1-^{13}C]palmitic acid ratio was significantly higher than the EPA:[1-^{13}C]palmitic acid ratio in the NEFA pool, and twice the ratio of DHA:[1-^{13}C]palmitic acid seen in either S_f >400 or the test meal. In summary, EPA incorporation into VLDL TG is stoichiometrically sim-

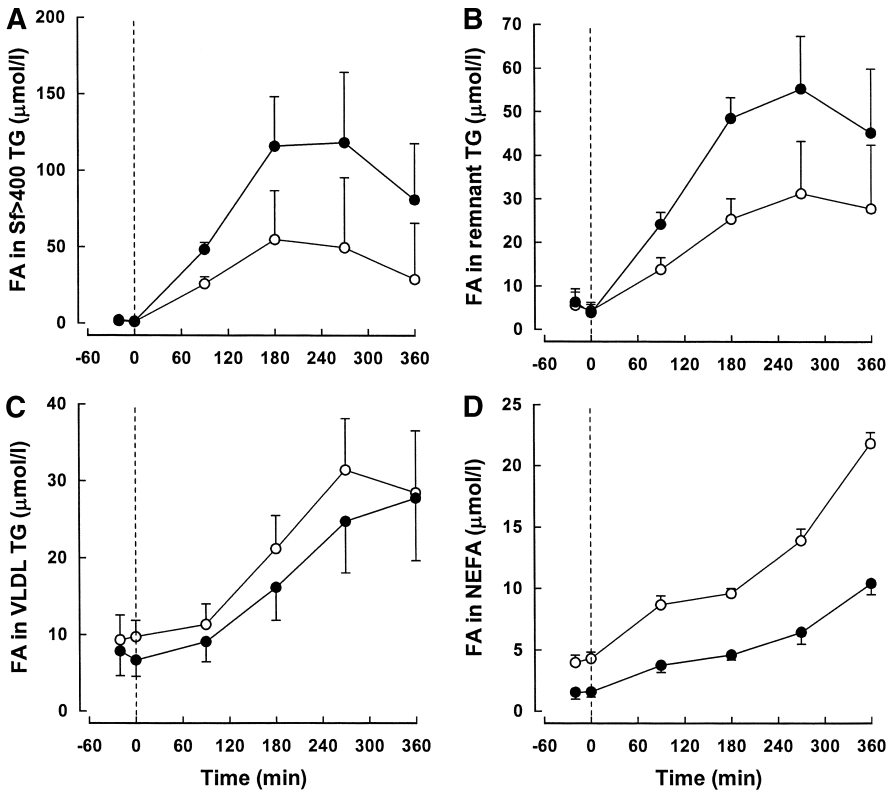

Fig. 4. The incorporation of eicosapentaenoic acid (EPA) (closed circles) and docosahexaenoic acid (DHA) (open circles) into S_f >400 TG (A), chylomicron remnant TG (B), VLDL TG (C), and NEFA pool (D). Mean ± SEM, n = 8.

ilar to that of [1-^{13}C]palmitic acid, whereas the VLDL-DHA incorporation appears to be much higher.

DISCUSSION

The present study demonstrates a novel method for tracing dietary fatty acids into both the intestinal and hepatic TG pools in vivo. VLDL and chylomicron remnants have similar densities, and it is therefore impossible to separate them by conventional ultracentrifugation. Using preparatory immunoaffinity chromatography, we isolated a pure VLDL fraction, as verified by SDS-PAGE. The unbound fraction contained some apoB-100-containing lipoproteins. This agrees with the previous observations (9), and it has been suggested that the unbound VLDL particles are remnant-like particles (28). This is the first time that this technique has been used to trace dietary fatty acids through both the exogenous and endogenous pathways in the postprandial period.

Dietary fatty acids were rapidly incorporated into plasma TRL shortly after meal intake. Different incorporation was observed when S_f 20–400 lipoproteins were separated into chylomicron remnants and VLDL. Labeled palmitic acid was rapidly incorporated into the chylomicron remnant TG pool, yet after a 90 min delay, the incorporation into the VLDL TG pool was substantial. Perfused liver studies have shown a lag time of 45 min before incorporation of labeled oleic acid into newly secreted VLDL TG (29). In the current study, EPA and DHA were incorporated into the VLDL TG pool at the same time as labeled palmitic acid, a saturated fatty acid. Therefore, the consistent appearance of three different exogenous tracers reinforces the conclusion that dietary fatty acids are recirculated into newly synthesized VLDL from an early stage in the postprandial period.

Using several lines of reasoning, it may be possible to estimate the contribution of dietary fat to the postprandial VLDL TG pool under the present experimental conditions. First, the turnover rates of the VLDL and chylomicron remnants are similar (30), due to the similarities in composition of these two particles (10). Second, chylomicron turnover studies have demonstrated that ~20% of core lipid is transferred from S_f >400 chylomicrons to S_f 20–400 chylomicron remnants (31). Third, here the rate of appearance of labeled fatty acids in both the VLDL TG pool and the chylomicron remnant TG pool was similar, and therefore it can be assumed that the entry of dietary fat into both pools occurs at the same rate. Following this line of reasoning, it may be concluded that as much as 20% of dietary fat is recycled as VLDL TG within the postprandial period following a single meal.

It could be argued that the early appearance of dietary fat in the VLDL TG pool reflects the transfer of TG and cholesteryl ester between VLDL and chylomicrons in cir-

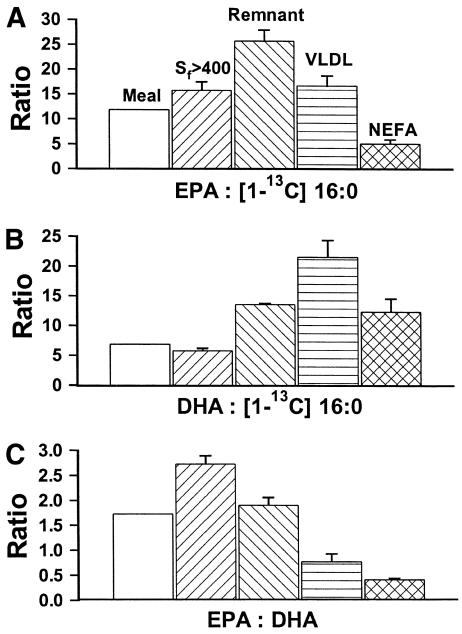

Fig. 5. The ratios of EPA, DHA, and [1-^{13}C]palmitic acid incorporation into different lipid classes. EPA:[1-^{13}C]palmitic acid (A), DHA:[1-^{13}C]palmitic acid (B), and EPA:DHA ratios (C) in the meal, S_f >400 TG, chylomicron remnant TG, VLDL TG, and NEFA. For all but the meal, these ratios are averaged over the postprandial period. Repeated measures ANOVA shows that the DHA:[1-^{13}C]palmitic acid ratio in the VLDL TG is statistically higher than that of both S_f >400 chylomicron ($P < 0.001$) and chylomicron remnant TG pools ($P = 0.021$), but is not statistically different from that of the NEFA pool ($P = 0.247$). EPA:[1-^{13}C]palmitic acid ratios are statistically different among all lipid classes, except between S_f >400 TG and VLDL TG pools ($P = 0.692$). The EPA:DHA ratio in chylomicron remnant TG is statistically higher than that seen in the VLDL TG pool ($P < 0.001$) and the NEFA pool ($P < 0.001$).

culation by the cholesteryl ester transfer protein (CETP). Previous studies have examined the transfer of lipids between VLDL and chylomicrons by using retinyl palmitate-labeled chylomicrons and chylomicron remnants. Retinyl esters (REs) are produced in the intestine after ingestion of vitamin A, and are packaged into chylomicrons along with dietary fat. REs in chylomicron remnants are taken up by the liver, and either stored or released as unesterified retinol bound to retinyl binding protein. The results from these studies are mixed; in vitro studies show that very little RE is transferred from chylomicrons to VLDL (32, 33); however, in vivo studies suggest that this transfer is much greater. In vivo, and using techniques comparable to ours, Cohn et al. (3) separated apoB-100-containing lipoproteins from TRL collected following an oral fat load labeled with vitamin A, and found that 25% of RE was recovered in the apoB-100 TRL at 6 h after meal intake. The substantial appearance of RE in apoB-100 TRL in vivo may even suggest that RE can be secreted in VLDL particles. We believe that the high and early incorporation of dietary fatty acids into the VLDL pool cannot be explained to any substantial degree by CETP-mediated transfer. The examination of the TG fatty acid composition in the VLDL pool argues against CETP-mediated transfer. If the main entry of dietary fat into the VLDL TG pool were by transfer between VLDL and chylomicrons, it would be expected that the relative composition of dietary fatty acid tracers would be similar in these TG pools. We show that the n-3 PUFA compositions of VLDL TG and chylomicron TG pools were remarkably different: chylomicrons and their remnants contained TG enriched with DHA and EPA in concentrations corresponding to the meal, whereas VLDL TG was enriched more with DHA than with EPA. In fact, the EPA:DHA ratio in the VLDL TG pool was half that seen in the remnant TG pool, suggesting under-incorporation of EPA into VLDL TG. The different ratios support the idea that dietary fat is rapidly and substantially incorporated into the VLDL TG pool through hepatic secretion.

The relative incorporation of the three exogenous tracers, EPA, DHA, and [1-^{13}C]palmitic acid into VLDL TG and chylomicron TG pools may also provide an insight into the hepatic partitioning of dietary fatty acid pools into VLDL TG production. By comparing the moles of either EPA or DHA per mole of labeled palmitic acid, it is clear that there is a difference between the different pools. Both the EPA:[1-^{13}C]palmitic acid and DHA:[1-^{13}C]palmitic acid ratios in the S_f >400 reflect those seen in the meal, yet the DHA:[1-^{13}C]palmitic acid ratio increases by 3-fold in the VLDL TG pool, while the EPA:[1-^{13}C]palmitic acid ratio remains constant. This demonstrates clearly that DHA is over-incorporated into VLDL TG, compared with both EPA and [1-^{13}C]palmitic acid.

There are two pools of dietary fat being delivered to the liver in the postprandial period. The first pool contains dietary fatty acids that have been released by lipoprotein lipase but not taken up by skeletal muscle or adipose tissue. The second pool contains dietary fat contained in chylomicron remnants, which are taken up directly by the liver. It is estimated that as much as 50% of dietary fat is taken up as remnant TG by the liver, whereas a proportion of dietary fat escapes tissue uptake and is delivered to the liver as NEFA bound to albumin (34). In addition, it is well recognized that elevated NEFA concentration is associated with increased VLDL production, suggesting that NEFA is another major source of fatty acids for the pre-VLDL TG pool. We have found that the EPA:[1-^{13}C]palmitic acid ratio in the NEFA pool was half that seen in the meal, yet the chylomicron remnants were enriched in EPA. If the chylomicron remnants were the main source of TG, it would be expected that VLDL TG would have a higher enrichment of EPA than observed. On the other hand, the DHA:[1-^{13}C]palmitic acid ratio in the NEFA pool was twice that seen in the meal, and the VLDL TG pool showed signs of over-incorporation of DHA, suggesting that NEFA is a major precursor for VLDL TG. DHA enrichment in both the NEFA pool and fasting VLDL TG have been previously demonstrated (15, 16, 20). In particular, Summers et al. (15) noted that the EPA:DHA ratio in plasma NEFA was significantly less than that in the meal, despite similar composition of chylomicron TG and meal fatty acids. In a long-term dietary study, Sadou et al. (20) studied the incorporation of EPA and DHA into choles-

teryl esters, phospholipids, and TGs of two lipoprotein fractions: a combined VLDL and LDL fraction and an HDL fraction. Meal EPA:DHA ratios were similar to those of Summers et al., yet the observed EPA:DHA ratios in (VLDL + LDL) TG were similar to the NEFA ratios observed by Summers et al., suggesting that the composition of the VLDL TG pool may be directly influenced by the NEFA pool, as demonstrated by the current study. The over-incorporation of DHA into both the VLDL TG and NEFA pools could be due to differential lipolysis of n-3 PUFA-containing TG (35, 36), as it has been shown that DHA-containing TGs are preferentially lipolysed, compared with EPA-containing TGs. In addition, it is possible that short-term differential tissue uptake of n-3 PUFA occurs in the postprandial state, further exaggerating the enrichment of DHA in the NEFA pool.

In conclusion, we have demonstrated a new method for tracing dietary fatty acids into TRL TG. This is the first study to show that dietary fatty acids are rapidly incorporated into the VLDL TG pool, enabling efficient recycling of dietary fatty acids for further uptake by extrahepatic tissues. There is also significant partitioning of fatty acids into different lipid pools within the liver before hepatic TG synthesis, as seen by differential incorporation of n-3 PUFA into the VLDL TG pool. In particular, dietary DHA appears to be preferentially recirculated into apoB-100 TRL particles.

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