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Dietary fatty acids make a rapid and substantial contribution to VLDL-triacylglycerol in the fed state

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Heath RB, Karpe F, Milne RW, Burdge GC, Wootton SA, Frayn KN. Dietary fatty acids make a rapid and substantial contribution to VLDL-triacylglycerol in the fed state. *Am J Physiol Endocrinol Metab* 292: E732–E739, 2007. First published November 7, 2006; doi:10.1152/ajpendo.00409.2006.—Exaggerated postprandial lipemia is associated with coronary heart disease and type II diabetes, yet few studies have examined the effect of sequential meals on lipoprotein metabolism. We have used ¹³C-labeled fatty acids to trace the incorporation of fatty acid derived from a meal into apolipoprotein B-100 (apoB-100)-containing lipoproteins and plasma nonesterified fatty acids (NEFA) following two consecutive meals. Healthy volunteers ($n = 8$) were given breakfast labeled with [1-¹³C]palmitic acid, eicosapentaenoic acid, and docosahexaenoic acid, followed 5 h later by lunch containing [1-¹³C]oleic acid. Blood samples were taken over a 9-h period. ApoB-100-containing lipoproteins were isolated by immunoaffinity chromatography. Chylomicron-triacylglycerol (TG) concentrations peaked at 195 min following breakfast but at 75 min following lunch ($P < 0.001$). VLDL-TG concentrations, in contrast, rose to a broad peak after breakfast and then fell steadily after lunch. Breakfast markers followed chylomicron-TG concentrations and appeared in plasma NEFA with a similar profile, whereas [1-¹³C]oleic acid peaked 2 h after lunch in plasma TG and NEFA. Breakfast markers appeared steadily in VLDL, peaking 1–3 h after lunch, whereas [1-¹³C]oleic acid was still accumulating in VLDL at 9 h. Around 17% of VLDL-TG originated from recent dietary fat 5 h after breakfast, and around 40% at the end of the experiment. We conclude that there is rapid flux of fatty acids from the diet into endogenous pools. Further study of these processes may open up new targets for intervention to reduce VLDL-TG concentrations and postprandial lipemia.

postprandial metabolism; chylomicrons; chylomicron remnants; very low-density lipoprotein

EXAGGERATED POSTPRANDIAL TRIACYLGLYCEROL (TG) concentrations have long been associated with coronary artery disease and atherosclerosis (11, 30). It has been proposed that an accumulation of chylomicron remnants is the major contributor to the atherogenic state (33), and many postprandial studies focus on the metabolism of large chylomicrons. However, in the postprandial period, >90% of TG-rich lipoprotein (TRL) particles are VLDL (21, 29).

Few attempts have been made to study VLDL metabolism in the postprandial state. VLDL and chylomicron remnants are similar in size and composition, and the separation of the lipoprotein classes cannot be achieved by conventional meth-

ods such as ultracentrifugation. However, the structural protein apolipoprotein B (apoB) differs between TRL derived from the intestine (apoB-48) and from the liver (apoB-100). ApoB-48 is identical to the NH₂-terminal 48% of apoB-100 but shares no homology with the COOH-terminal end of apoB-100. Therefore, monoclonal antibodies directed against the COOH-terminal portion of apoB-100 have been employed to separate and examine the lipid and apolipoprotein composition of chylomicrons and VLDL from lipoprotein fractions collected in the fasted and postprandial states (2, 6, 14, 29). Using this technology, we showed previously that dietary fatty acids typically appear within 2 h in VLDL-TG following a single meal (14). We also found anomalous behavior of certain dietary fatty acids, notably eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA): whereas EPA was predominant in the test meal used, DHA became more prominent in VLDL-TG. This suggested selective metabolism, possibly at the stage of tissue uptake (14).

These studies have focused on postprandial TG metabolism following a single meal. Few postprandial studies have examined lipoprotein metabolism following sequential meals, although this would be more typical of daily life. Metabolic events following subsequent meals during the day may not reflect those seen after a single meal. For instance, a second meal (“lunch”) may lead to the rapid release from the small intestine of chylomicron particles carrying fat from the earlier meal (9). There are several possible routes by which dietary fatty acids can enter the VLDL-TG pool including delivery to the liver of intact TG in chylomicron remnant particles, and of nonesterified fatty acids (NEFA) produced as “spillover” from the peripheral lipolysis of chylomicron particles (1, 23, 24). These may well differ following sequential, compared with single, meals because of persisting effects of insulin, rapid release of chylomicrons as above, and other factors. Therefore, knowledge regarding the dynamic aspects of lipid metabolism, such as the movement of dietary fatty acids between lipoprotein species, in the context of repeated meal feeding is limited.

We therefore set out to test the hypothesis that the trafficking of fatty acids from diet to endogenous pools would differ between the first and second meals of the day, and in particular to test whether the partitioning of fatty acid delivery to the liver between remnant TG and NEFA would differ following successive meals. We have used [¹³C]palmitic acid and n-3 polyunsaturated fatty acids (PUFA) consumed at breakfast and

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[¹³C]oleic acid consumed at lunch to trace the metabolism of fatty acids consumed in each meal into plasma TG and NEFA fractions. The n-3 PUFA were included to investigate further our previous observation of selective incorporation into VLDL-TG of DHA over EPA. The incorporation of labeled fatty acids into VLDL-TG was used to estimate the contribution of fatty acids derived from the meal to endogenous circulating TG.

MATERIALS AND METHODS

Subjects. Eight healthy male subjects were selected from the Oxford Biobank, a population-based randomized collection of 30- to 50-yr-old men and women in Oxfordshire. All subjects had the ApoE genotype, ApoE3/E3, as verified by the restriction fragment length polymorphism method (15). Their characteristics are shown in Table 1. The study was approved by the Oxfordshire Clinical Research Ethics Committee, and all subjects gave informed consent before the study. Only men were used to reduce intersubject variability, but results of this study can be used to plan more detailed studies of fatty acid metabolism following sequential meals over a 24-h period and compare men and women.

Study protocol. To standardize the subjects' nutritional states before the study, all the subjects consumed a low-fat (<10 g fat) evening meal. Subjects were also requested not to eat fish the day before the study. 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, 210, 240, 270, 300, 330, 360, 390, 420, 450, 480, 510, and 540 min. At time $t = 0$ min, subjects were given breakfast, consisting of 40 g of Rice Krispies (Kelloggs, Warrington, UK), a banana, and a warm chocolate milkshake containing 50 g of fat. The fat consisted of 30 g of fish oil (EPAX3000TG; Pronova Biocare, Asslund, Norway) and 20 g of olive oil (Tesco). [¹⁻¹³C]palmitic acid (99 atom%; Cambridge Isotopes, Woburn, MA) (600 mg) was added to the test oil. The EPA and DHA present in the fish oil and [¹⁻¹³C]palmitic acid acted as tracers for measuring the movement of breakfast-derived fatty acids. The macronutrient and fatty acid composition of the meal are shown in Table 2 and Table 3, respectively. The breakfast was consumed within 10 min. At $t = 300$ min, lunch was given. The fat content of lunch was also 50 g. It consisted of a cheese sandwich and a second warm chocolate milkshake. The fat in this milkshake consisted of 20 g of safflower oil (Anglia Oils, Hull, UK). [¹⁻¹³C]oleic acid (99 atom%; Cambridge Isotopes) (450 mg) was added to the test oil to act as tracer for the movement of lunch-derived fatty acids. The macronutrient and fatty acid composition of the lunch are also shown in Tables 2 and 3, respectively. The fatty acid composition of the meals was chosen to make them distinct, so that the composition could be followed through into plasma lipid fractions as we have done previously (9). During the study, volunteers rested either in the supine position or sitting at a desk doing some light work or reading.

Analyses. Blood samples were collected into heparinized syringes (Sarstedt, Leicester, UK) for plasma metabolite and lipoprotein anal-

Table 1. Fasting characteristics of the subjects

	Mean	Range
Plasma glucose, mmol/l	5.4 ± 0.1	4.8–5.7
Plasma TG, mmol/l	1.0 ± 0.1	0.8–1.5
Plasma cholesterol, mmol/l	5.5 ± 0.2	4.7–6.8
Plasma insulin, mU/l	5.5 ± 0.8	3.1–10.7
Age, yr	46.4 ± 1.4	38–50
BMI, kg/m ²	24.8 ± 0.5	23.1–27.1

TG, triacylglycerol; BMI, body mass index.

Table 2. Macronutrient composition of the breakfast and lunch*

	Carbohydrate, g	Fat, g	Protein, g	Energy, kJ
<i>Breakfast</i>				
Fish oil (30 g)	0	30	0	1,108.8
Olive oil (20 g)	0	20	0	739.2
Skimmed milk (250 g)	12.5	0.3	8.3	350
Nesquik (6 g)	4.8	0.2	0.2	152
Sweetener (1 g)	0	0	0	0
Rice Krispies (40 g)	35.9	0.4	2.4	629.3
Banana (100 g)	23.2	0.3	1.2	403
Label: palmitic acid	0	0.6	0	18.5
Total	76.4	51.9	12.2	3,400.8
<i>Lunch sandwich</i>				
Cheddar cheese (50 g)	0	17.8	12.9	879.5
Pickle (10 g)	3.4	0.1	0.1	57.2
Bread (80 g)	34	3	9.1	920.8
Flora† (10 g)	0.1	8.2	0.1	303.9
Test oil				
Safflower oil (20 g)	0	20	0	739.2
Skimmed milk (50 g)	2.5	0.1	1.7	70
Nesquik (4 g)	3.2	0.1	0.1	60.8
Sweetener (0.4 g)	0	0	0	0
Label: oleic acid	0	0.45	0	18.5
Total	43.4	49.6	24.0	3,049.9

*Determined from food tables and manufacturers' data. †Polyunsaturated fatty acid (PUFA)-rich margarine (Unilever-UK, London, UK).

ysis. Plasma was separated by centrifugation at 1,700 g for 15 min. Samples for plasma TG, NEFA, cholesterol, and insulin 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 (Antrim, UK) and Alpha Laboratories (Eastleigh, UK), respectively. All of the metabolites were batch analyzed and measured enzymatically with an IL Monarch.

TRL 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 S_F 20–400 lipoproteins and then for 16 h to float S_F 20–400 lipoproteins. The top 0.5–1 ml from each tube was aspirated, collected into another preweighed tube, and immediately put on ice. TRL were separated from plasma taken at $t = 0, 60, 120, 180, 240, 300, 360, 420, 480,$ and 540 min.

The S_F 20–400 was further separated by immunoaffinity chromatography, using specific monoclonal antibodies 3F5, 4G3, and 5E11 against apoB-100, which do not cross-react with apoB-48. The methods were described in detail previously (14). This resulted in a bound fraction that was completely devoid of apoB-48, which will hereafter be called the VLDL fraction. The unbound fraction contained all the apoB-48 in the S_F 20–400 fraction together with some apoB-100 (14) and will be called the "chylomicron remnant fraction," as it is chylomicron remnant rich. The mean recovery of apoB-100 was 73.4% and that of TG was 77%. This recovery did not change systematically with nutritional state, so the patterns of response shown for the isolated lipoprotein fractions should not be affected.

Gas chromatography and mass spectrometry. For analysis of specific fatty acids, lipids were extracted from plasma or lipoprotein fractions and separated and analyzed as described previously (14). A sample of each test meal was homogenized and analyzed using gas chromatography (GC) to establish specific fatty acid composition.

Stable isotope enrichment was determined by GC-combustion-isotope ratio mass spectrometry essentially as described earlier (5).

Table 3. Fatty acid content (in g) of the breakfast and lunch*

Fatty Acid (g)	14:0	16:0	16:1 n-7	18:0	18:1 n-9	18:2 n-6	20:5 n-3	22:6 n-3
<i>Breakfast</i>								
Fish oil (30 g)	2.5	5.3	2.8	1.0	3.9	0.3	5.6	3.8
Olive oil (20 g)	0	2.0	0.1	0.6	14.4	1.5	0	0
Skimmed milk (250 g)	0	0	0	0	0	0	0	0
Nesquik (10 g)	0	0	0	0	0	0	0	0
Sweetener (2 g)	0	0	0	0	0	0	0	0
Rice Krispies (40 g)	0	0	0	0	0	0	0	0
Banana (100 g)	0	0	0	0	0	0	0	0
Label: palmitic acid	0	0.6	0	0	0	0	0	0
Total	2.5	7.9	2.9	1.6	18.3	1.8	5.6	3.8
<i>Lunch sandwich</i>								
Cheddar cheese (50 g)	1.8	4.1	0.4	2.0	4.4	0.2	0	0
Pickle (10 g)	0	0	0	0	0	0	0	0
Bread (80 g)	0	0.4	0	0.1	0.3	0.8	0	0
Flora (10 g)	0.1	1.1	0	0.7	1.5	4.1	0	0
Banana (100 g)	0	0	0	0	0	0	0	0
Safflower oil (20 g)	0	1.3	0	0.5	2.3	14.8	0	0
Skimmed milk (50 g)	0	0	0	0	0	0	0	0
Nesquik (4 g)	0	0	0	0	0	0	0	0
Sweetener (0.4 g)	0	0	0	0	0	0	0	0
Label: oleic acid	0	0	0	0	0.5	0	0	0
Total	1.9	6.9	0.4	3.3	9.0	19.9	0	0

*Determined from food tables and manufacturers' data. Minor fatty acids including shorter-chain fatty acids in the cheese account for the differences from Table 2.

Fatty acid methyl esters (FAME) were resolved on a 50 m × 0.25 mm × 0.32 mm BPX-70 fused silica capillary column (SGE Europe, Milton Keynes, UK) using an HP6890 GC (Hewlett Packard, Wokingham, Berks, UK). FAME were converted to CO₂ by heating to 860°C in the presence of Pt/CuO using an Orchid IRMS interface (PDZ-Europa, Crewe, Cheshire, UK) and ¹³CO₂:¹²CO₂ was determined by a 20/20 Stable Isotope Analyser (PDZ-Europa). Tricosanoic acid methyl ester was used as isotopic enrichment standard (1.135 atom%). The concentrations of labeled fatty acids were calculated from the total concentration of each fatty acid in plasma and the fractional enrichment.

Calculations and statistics. The contribution of dietary fatty acids to VLDL-TG fatty acids was calculated as follows. The molar proportion of [1-¹³C]palmitic acid in dietary fatty acids in the breakfast was calculated, allowing for the fact that the fat content is TG and converting to fatty acids (1.3 mol%). The contribution of dietary fatty acids to VLDL-TG was then calculated from the tracer concentration in the VLDL fraction assuming that there was uniform metabolic handling of all dietary fatty acids. Since this assumption is clearly not absolutely true, the calculations were also performed treating EPA in the breakfast meal as a tracer (12.6 mol%). Similar calculations were made for fatty acids derived from lunch, using [1-¹³C]oleic acid as a tracer (0.92 mol% of lunch fatty acids).

Data were analyzed with SPSS software version 10.0 (SPSS-UK, 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. *P* <

0.05 was considered statistically significant. All data are presented as means ± SE or medians.

RESULTS

Postprandial responses in plasma TG, NEFA, and glucose. A biphasic pattern in plasma TG concentration (Fig. 1A) was seen following sequential meals, with mean peak TG concentrations at *t* = 180 min and *t* = 360 min. The peak TG concentration following lunch was significantly earlier (79 ± 11 min to reach peak) than the peak TG concentration following breakfast (200 ± 17 min to reach peak, *P* < 0.001).

Suppression of plasma NEFA (Fig. 1B) was seen following both breakfast and lunch. However, NEFA concentrations tended to rise after lunch (mean 426 μmol/l at 330 min vs. 377 μmol/l at 300 min, *P* < 0.01) before decreasing more slowly (time to nadir postbreakfast 101 ± 18 min, postlunch 189 ± 16 min, *P* < 0.001). The degree of suppression of plasma NEFA was considerably less following the second meal (nadir 290 ± 43 μmol/l vs. 230 ± 42 μmol/l, *P* = 0.011).

A biphasic pattern in plasma glucose (Fig. 1C) was seen following breakfast, whereas a single peak in plasma glucose concentration was observed following lunch. This pattern was similar to that of plasma insulin (Fig. 1D), which also showed a biphasic pattern following breakfast but a single peak following lunch.

TG concentrations of S_f >400 and S_f 20–400 lipoproteins in the postprandial period. A biphasic pattern was observed in S_f >400 TG concentrations (Fig. 2A) following the sequential meals, with peak concentrations at *t* = 180 min and *t* = 360 min. The peak in S_f >400 TG concentration following lunch was significantly earlier (time to peak = 75.0 ± 9.8 min) than that observed following breakfast (time to peak = 195 ± 15 min, *P* < 0.001), mirroring the pattern of the total plasma TG concentrations.

S_f 20–400 TG concentrations (Fig. 2B) did not exhibit a biphasic pattern, instead exhibiting a broad peak over 5 h and declining quickly thereafter, reaching baseline values by *t* = 540 min. The S_f 20–400 TG concentrations represent both small chylomicron-TG and VLDL-TG. S_f 20–400 lipoproteins were separated using immunoaffinity chromatography to produce a pure VLDL fraction and a chylomicron remnant fraction. The majority of the rise in S_f 20–400 TG was accounted for by the chylomicron remnant fraction (Fig. 2C). VLDL-TG concentrations, on the other hand, only rose slightly during the first postprandial period and fell steadily in the second postprandial period.

Incorporation of dietary fatty acids into the plasma TG and NEFA. The three breakfast-derived tracers, [1-¹³C]palmitic acid (Fig. 3A), EPA, and DHA (Fig. 3C), were incorporated into the plasma TG within 60 min of the breakfast. In the TG pool, the concentrations of [1-¹³C]palmitic acid, EPA, and DHA had reached their postbreakfast peaks and begun to plateau by *t* = 300 min. Following lunch, however, there was a distinct second peak in all three tracers at *t* = 360 min. The postlunch peaks in all three tracers were earlier (each *P* < 0.001) and higher in absolute concentrations (EPA, *P* = 0.013; DHA, *P* = 0.009) than those exhibited after breakfast. The appearance of all three fatty acids in the plasma NEFA pool (Fig. 3, B and D) was similar to that in the plasma TG pool, although DHA was the predominant n-3 PUFA in the plasma

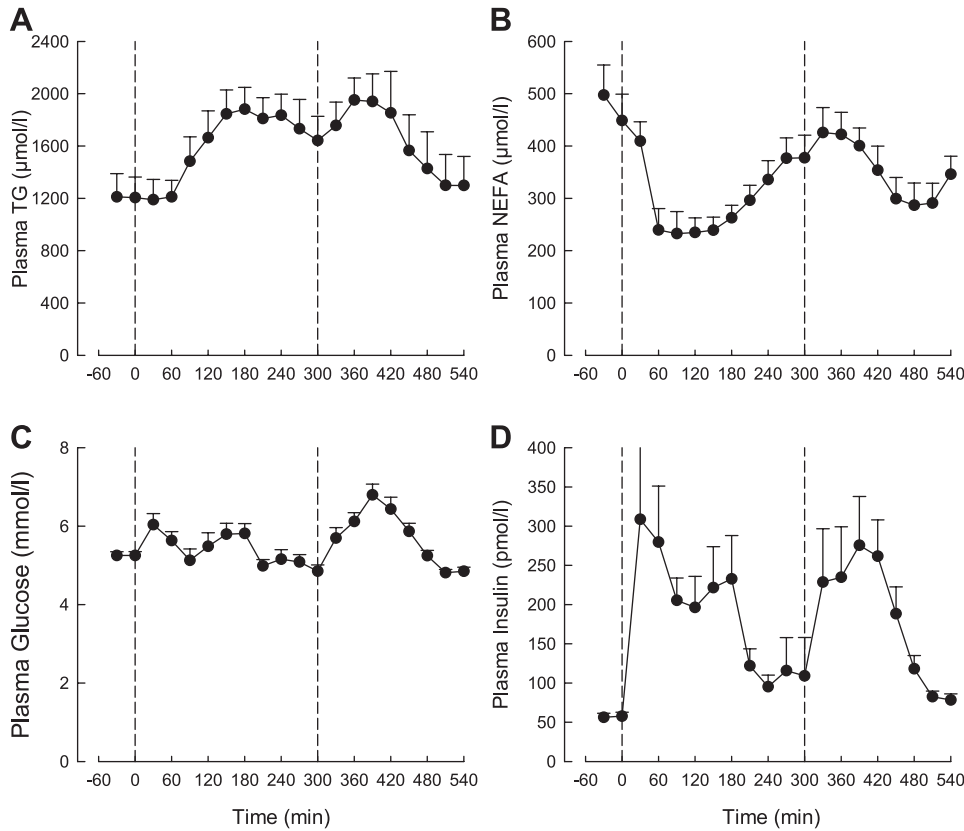


Fig. 1. Plasma triacylglycerol (TG; A), non-esterified fatty acids (NEFA; B), glucose (C), and insulin (D) concentrations following sequential meals. Dotted lines represent times at which meals were given. Values are means \pm SE; $n = 8$.

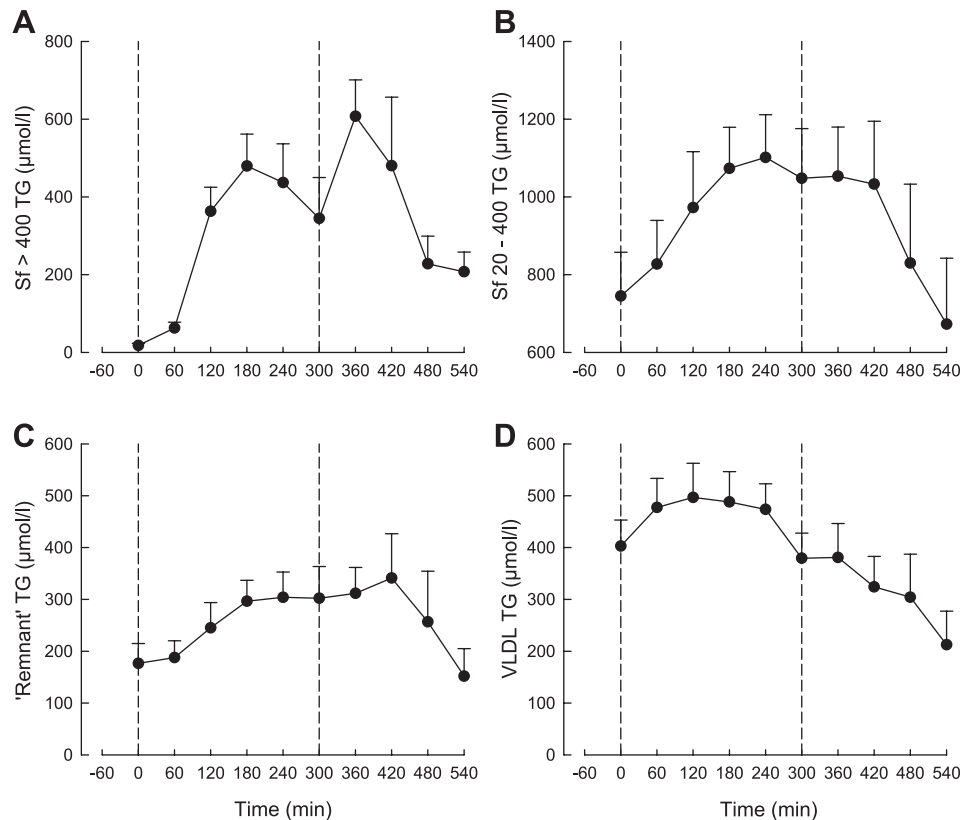


Fig. 2. Plasma Svedberg flotation rate (S_f) >400 TG (A) and S_f 20–400 TG (B) concentrations following sequential meals. S_f 20–400 TG was separated into remnant TG (C), and endogenous TG (VLDL-TG; D). Dotted lines represent times at which meals were given. Values are means \pm SE; $n = 8$.

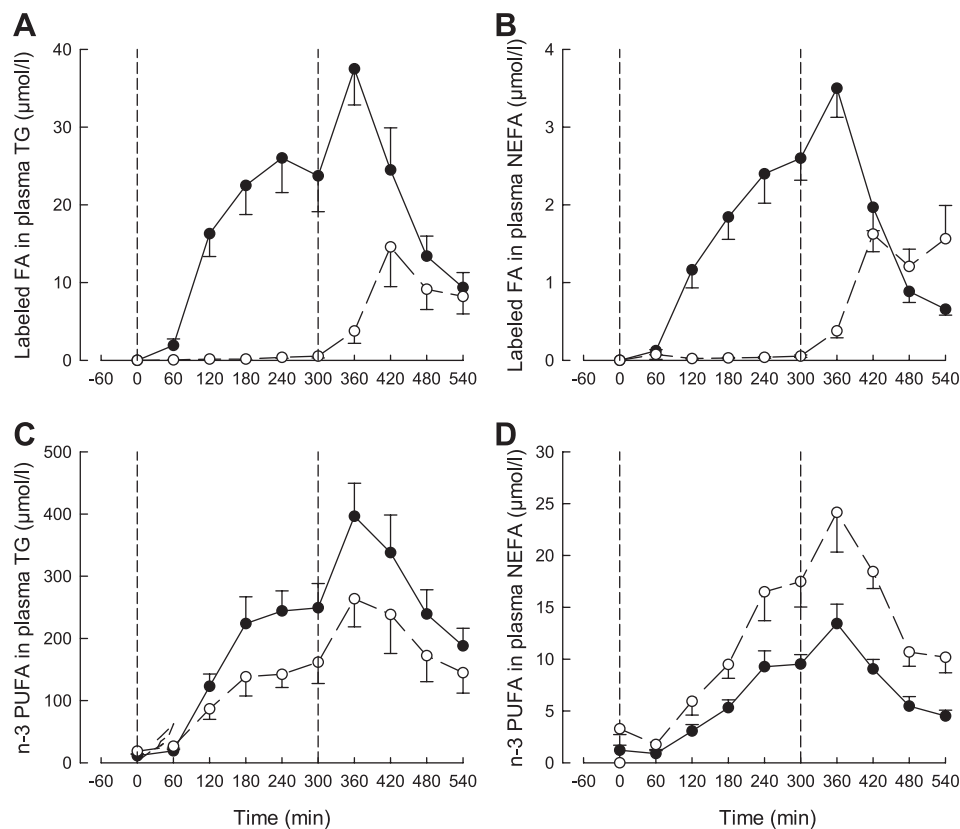


Fig. 3. Dietary [$1-^{13}\text{C}$]palmitic acid (solid circles) and [$1-^{13}\text{C}$]oleic acid (open circles) incorporation into plasma TG (A) and plasma NEFA (B) following sequential meals. The appearance of eicosapentaenoic acid (EPA; solid circles) and docosahexaenoic acid (DHA; open circles) in the plasma TG (C) and plasma NEFA (D) following sequential meals. FA, fatty acids; PUFA, polyunsaturated fatty acids. Dotted lines represent times at which meals were given. Values are means \pm SE; $n = 8$.

NEFA pool, whereas EPA was the predominant n-3 PUFA in the plasma TG pool. The lack of immediate suppression of NEFA after the second meal is therefore largely explained by appearance of NEFA derived from chylomicrons containing the fatty acids from the first meal.

[$1-^{13}\text{C}$]oleic acid, the marker of the lunch-derived fatty acids, peaked at $t = 420$ min in the plasma TG pool, but the overall appearance of this fatty acid in the circulation was noticeably blunted compared with the appearance of [$1-^{13}\text{C}$]palmitic acid, which was still appearing in the circulation 6 h following breakfast. This diminished response of oleic acid was clearly seen in both the plasma TG and NEFA pools. Although the amount of [$1-^{13}\text{C}$]oleic acid (450 mg) was lower than the amount of [$1-^{13}\text{C}$]palmitic acid (600 mg) used in the study, if the observed concentrations of oleic acid were adjusted to compensate for this, the difference was still apparent and must reflect differences in the metabolism of fatty acids in these two different postprandial periods.

Incorporation of dietary fatty acids into TRL fractions. All three fatty acid tracers given in the breakfast {[$1-^{13}\text{C}$]palmitic acid (Fig. 4A), EPA, and DHA (Fig. 4D)} were incorporated into the $S_f > 400$ TG fraction. The appearance of fatty acids in this fraction was similar to their appearance in whole plasma TG. In the chylomicron remnant fraction TG, dietary fatty acids {[$1-^{13}\text{C}$]palmitic acid (Fig. 4B), EPA, and DHA (Fig. 4E)} appeared within 60 min of the breakfast. The concentration of all three tracers increased at a fairly consistent rate and continued to rise even after the second meal had been consumed. The concentration of [$1-^{13}\text{C}$]palmitic acid in this fraction peaked at $t = 360$ min, whereas the concentrations of EPA and DHA peaked later at $t = 420$ min. The incorporation of the

three tracers into VLDL-TG was noticeably delayed, with dietary fatty acids entering this fraction after 60 min.

[$1-^{13}\text{C}$]oleic acid was quickly incorporated into chylomicron remnant TG (Fig. 4B) following lunch. In VLDL-TG (Fig. 4C), oleic acid was incorporated later than in the chylomicron remnant TG and appeared to rise at a constant rate and was still rising at $t = 540$ min. In both fractions, the appearance of this fatty acid was blunted compared with the earlier appearance of palmitic acid.

The contribution of breakfast-derived fatty acids to VLDL-TG fatty acids was calculated assuming [$1-^{13}\text{C}$]palmitic acid to be a tracer of breakfast fat (see MATERIALS AND METHODS). This calculation showed that 17 mol% (median) of VLDL-TG fatty acids arose from breakfast fat at 300 min, rising to 29 mol% at 540 min. With the use of EPA as a tracer, the equivalent figures were 9 mol% (median) at 300 min and 25 mol% at 540 min. Fatty acids from lunch, calculated using [$1-^{13}\text{C}$]oleic acid as tracer, contributed 13 mol% (median) of VLDL-TG at 540 min. The combined contribution from breakfast and lunch fats, calculated using [$1-^{13}\text{C}$]palmitic acid and [$1-^{13}\text{C}$]oleic acid tracers, was 43 mol% (median) at 540 min.

DISCUSSION

Humans spend much of the 24 h of each day in a postprandial state. However, studies of VLDL metabolism are often confined to the postabsorptive state (10), both to overcome problems of non-steady state that occur after meals and to avoid the methodological problems of separating VLDL from chylomicron particles. Postprandial VLDL metabolism is likely to be important, however. VLDL particles far outnumber

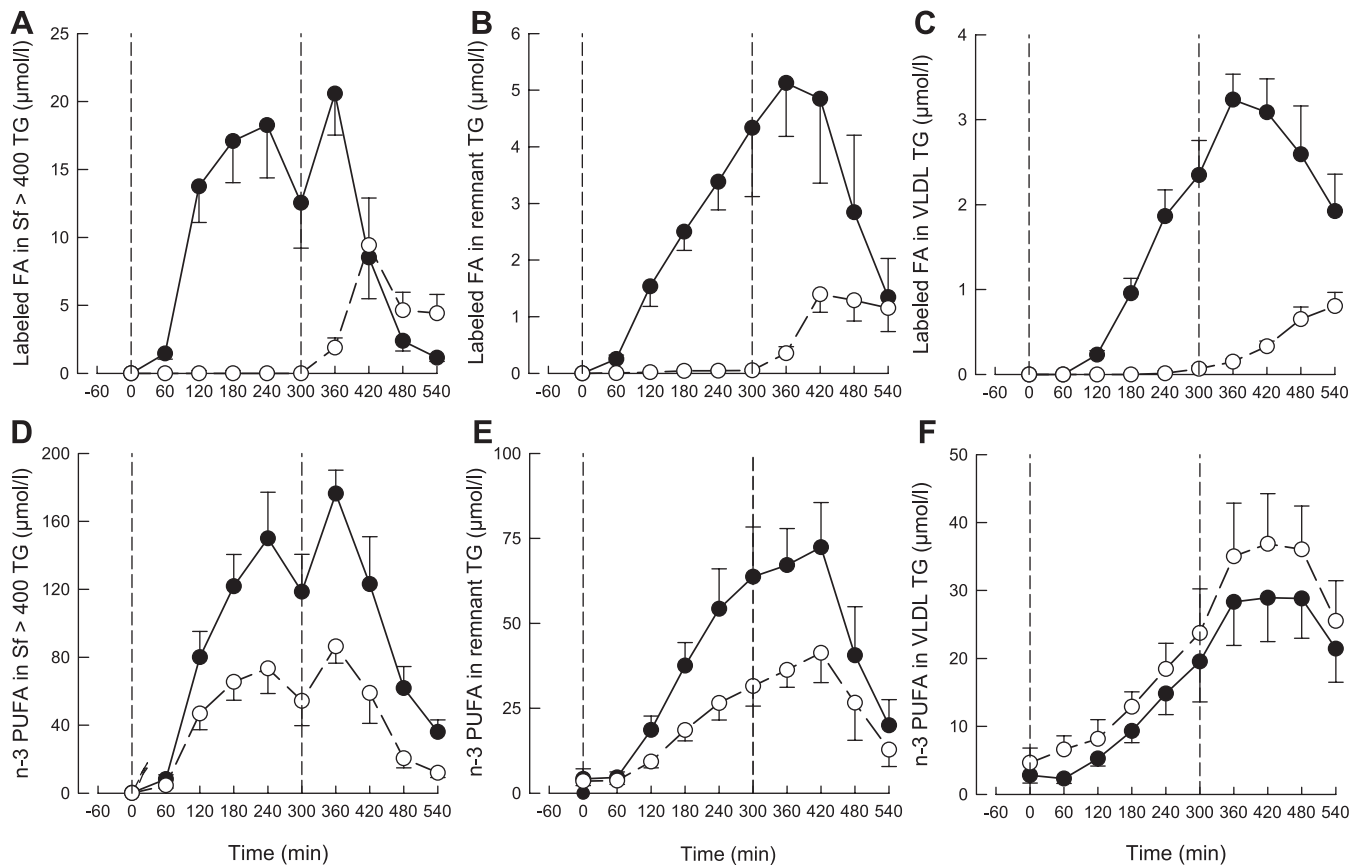


Fig. 4. Dietary [¹⁻¹³C]palmitic acid (solid circles) and [¹⁻¹³C]oleic acid (open circles) incorporation into the S_f >400 TG fraction (A), remnant TG fraction (B), and VLDL-TG fraction (C) following sequential meals. The appearance of EPA (solid circles) and DHA (open circles) in the S_f >400 TG fraction (D), remnant TG fraction (E), and VLDL-TG fraction (F) following sequential meals. Dotted lines represent times at which meals were given. Values are means ± SE; n = 8.

chylomicron particles even during postprandial lipemia (7), VLDL particles compete with chylomicrons for clearance by lipoprotein lipase (4), and postprandial lipemia, to which VLDL-TG contributes significantly (6, 29), is a well-established risk factor for cardiovascular disease.

Immunoaffinity separation of VLDL and chylomicron remnants has been used previously to study aspects of VLDL and chylomicron metabolism following a single meal (2, 6, 14, 29). However, this is the first study to use this technique to examine the effect of a second meal on VLDL metabolism. A novel finding was that the changes in VLDL-TG concentrations following sequential meals were different from those of chylomicron and chylomicron remnant TG. VLDL-TG concentrations returned to baseline following lunch and then continued to decrease, in contrast to the increase in chylomicron and chylomicron remnant TG soon after lunch. This might suggest that the decrease in VLDL-TG concentration is due to a decrease in VLDL production. However, the sustained incorporation of dietary fatty acids into the VLDL-TG pool is direct evidence of the secretion of newly synthesized VLDL particles. It has been shown previously that dietary fats are incorporated into the VLDL-TG pool within 90 min of food intake (14). In the current study, the breakfast-derived fatty acids (EPA, DHA, and [¹⁻¹³C]palmitic acid) were rapidly incorporated into the VLDL-TG pool in the postbreakfast period, as shown previously. The surprising and novel observation was that lunch-derived fatty acids were also incorporated into the

VLDL-TG pool within 2 h, clearly demonstrating the secretion of new VLDL particles in both postprandial periods. Therefore, it seems likely that the postlunch decrease in VLDL-TG was caused by increased catabolism of VLDL-TG. There are two pieces of evidence in the current study to support this idea. First, the concentrations of both chylomicron-TG and chylomicron remnant TG also fell rapidly in the later postlunch period. Second, the rapid appearance of dietary fatty acids in the NEFA pool in the postlunch period is an indicator of increased generation of NEFA directly from chylomicron lipolysis.

Our results emphasize that responses following a single test meal cannot be extrapolated to the normal daily fed situation. An early peak in plasma TG concentrations is often, but not always (5), seen following a second meal. This peak is associated with an influx of chylomicron particles (9, 18, 25). This “second-meal phenomenon” was clearly observed in the current study, with an exaggerated early peak seen after lunch in both plasma TG and chylomicron-TG. As in the study of Fielding et al. (9), the early postlunch increase in chylomicron-TG concentration was associated with a rapid increase in breakfast-derived fat in this pool. In this case, peak concentrations of breakfast-derived fatty acid markers, EPA, DHA, and [¹⁻¹³C]palmitic acid, coincided with the early peak in chylomicron-TG concentration, whereas the peak concentration of [¹⁻¹³C]oleic acid, the lunch-derived fatty acid marker, was much later. This second-meal effect reflects storage of dietary

fat in enterocytes and perhaps intestinal lymphatics, with release following consumption of a second meal (26). Previous studies in which TRL fractions have been investigated after sequential meals have shown that most of the increase in TG is present in the S_f 60–400 flotation range and very little in the S_f 20–60 range (smaller VLDL) (17). But it is that larger fraction, S_f 60–400, in which the “contamination” by chylomicrons, evidenced by the presence of apoB-48, is most prominent; in fact, there is typically more apoB-48 in that fraction than in the S_f >400 fraction (17). The early appearance of retinyl palmitate, administered with lunch, in the S_f 20–400 fraction also shows clearly the difficulty with the assumption that this fraction represents hepatic VLDL (20). It was of interest that dietary fatty acids were still present in the NEFA pool throughout the experiment. Labeled fatty acids administered with a meal accumulate rapidly in adipose tissue (27), such that by 24 h after the test meal, almost all the tracer fatty acid administered can be accounted for in adipose depots provided that omental fat is included (19). The implication of our findings is that passage through VLDL-TG is but one pathway by which these dietary fatty acids reach their destination in fat cells.

We noted previously that there are differences in the metabolic handling of dietary EPA and DHA (14). This was seen again in this study: while EPA predominated in the meal and in chylomicron-TG, this was reversed in plasma NEFA and in VLDL-TG. The difference was rather more marked following the second meal. We speculated previously that there is selective lipolysis of DHA, or a reduced uptake in adipose tissue of newly lipolysed DHA, from the chylomicron-TG. The marked appearance of DHA in plasma NEFA during the early peak of lipemia following lunch adds some weight to this idea. The inclusion of such a large amount of n-3 PUFA in the test meal is unlikely to have had a major influence on postprandial events. In studies in which n-3 PUFA have been fed as part of a single, acute test meal, no differences in plasma TG responses have been found when compared with other test oils (13, 18). Effects of n-3 PUFA on VLDL assembly and secretion have been shown in cellular systems (3, 22, 31). However, we have shown in preliminary experiments more recently (16) that similar rapid incorporation of dietary fatty acids into VLDL-TG is seen following a breakfast without fish oil. We used different fatty acid tracers, [$1-^{13}C$]palmitic acid and [$1-^{13}C$]oleic acid, to mark breakfast and lunch fat, respectively. When these tracers were given as part of a liquid feed, greater proportional oxidation of oleate was seen (28); this was also true of a study of their relative oxidation during exercise (32). However, the plasma turnover of these two fatty acids is similar (12), as is their handling in adipose tissue (8). Therefore, some caution must be exercised in comparing directly the behavior of the fatty acid tracers after breakfast and lunch, but differences in their metabolism are unlikely to explain completely the different time course of their incorporation into VLDL-TG.

Little is understood about the sources of VLDL-TG production in the postprandial period. Our data show dietary fatty acids making a rapid and substantial contribution to VLDL-TG production. The calculations of the contribution of dietary fatty acids to VLDL-TG are necessarily simplifications of a complex process: interpretation of the data is complex because of the many variables inherent in such a study. It is likely that, by 540

min, fatty acids have been recycled through VLDL, NEFA (or intermediate-density lipoprotein), and back through the liver for further VLDL-TG synthesis. Nevertheless, our data show that there is rapid flux of fatty acids from the diet into endogenous pools. It may be that further study of the details of these processes will open up new targets for intervention to reduce VLDL-TG concentrations and postprandial lipemia.

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GRANTS

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REFERENCES

1. Barrows BR, Parks EJ. Contributions of different fatty acid sources to very low-density lipoprotein-triacylglycerol in the fasted and fed states. *J Clin Endocrinol Metab* 91: 1446–1452, 2006.
2. Björkegren J, Hamsten A, Milne RW, Karpe F. Alterations of VLDL composition during alimentary lipemia. *J Lipid Res* 38: 301–314, 1997.
3. Brown AM, Castle J, Hebbachi AM, Gibbons GF. Administration of n-3 fatty acids in the diets of rats or directly to hepatocyte cultures results in different effects on hepatocellular ApoB metabolism and secretion. *Arterioscler Thromb Vasc Biol* 19: 106–114, 1999.
4. Brunzell JD, Hazzard WR, Porte DJ, Bierman EL. Evidence for a common, saturable, triglyceride removal mechanism for chylomicrons and very low density lipoproteins in man. *J Clin Invest* 52: 1578–1585, 1973.
5. Burdge GC, Jones AE, Frye SM, Goodson L, Wootton SA. Effect of meal sequence on postprandial lipid, glucose and insulin responses in young men. *Eur J Clin Nutr* 57: 1536–1544, 2003.
6. Cohn JS, Johnson EJ, Millar JS, Cohn SD, Milne RW, Marcel YL, Russell RM, Schaefer EJ. Contribution of apoB-48 and apoB-100 triglyceride-rich lipoproteins (TRL) to postprandial increases in the plasma concentration of TRL triglycerides and retinyl esters. *J Lipid Res* 34: 2033–2040, 1993.
7. Cohn JS, McNamara JR, Krasinski SD, Russell RM, Schaefer EJ. Role of triglyceride-rich lipoproteins from the liver and intestine in the etiology of postprandial peaks in plasma triglyceride concentration. *Metabolism* 38: 484–490, 1989.
8. Evans K, Burdge GC, Wootton SA, Clark ML, Frayn KN. Regulation of dietary fatty acid entrapment in subcutaneous adipose tissue and skeletal muscle. *Diabetes* 51: 2684–2690, 2002.
9. Fielding BA, Callow J, Owen RM, Samra JS, Matthews DR, Frayn KN. Postprandial lipemia: the origin of an early peak studied by specific dietary fatty acid intake during sequential meals. *Am J Clin Nutr* 63: 36–41, 1996.
10. Gormsen LC, Jensen MD, Schmitz O, Møller N, Christiansen JS, Jørgensen JO, Nielsen S. Energy expenditure, insulin and VLDL triglyceride production in humans. *J Lipid Res* 47: 2325–2332, 2006.
11. Groot PH, van Stiphout WA, Krauss XH, Jansen H, van Tol A, van Ramshorst E, Chin-On S, Hofman A, Cresswell SR, Havekes L. Postprandial lipoprotein metabolism in normolipidemic men with and without coronary artery disease. *Arterioscler Thromb* 11: 653–662, 1991.
12. Hagenfeldt L. Turnover of individual free fatty acids in man. *Fed Proc* 34: 2246–2249, 1975.
13. Harris WS, Connor WE, Alam N, Illingworth DR. Reduction of postprandial triglyceridemia in humans by dietary n-3 fatty acids. *J Lipid Res* 29: 1451–1460, 1988.
14. Heath RB, Karpe F, Milne RW, Burdge GC, Wootton SA, Frayn KN. Selective partitioning of dietary fatty acids into the VLDL TG pool in the early postprandial period. *J Lipid Res* 44: 2065–2072, 2003.
15. Hixson JE, Vernier DT. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. *J Lipid Res* 31: 545–548, 1990.
16. Hodson L, Fielding BA, Bickerton A, Roberts R, Milne RW, Frayn KN, Karpe F. Incorporation of exogenous and endogenous fatty acids into very low-density lipoprotein (VLDL): a study using stable isotope and immunoaffinity techniques in humans (Abstract). *Diabetologia* 48: A233, 2005.

17. **Jackson KG, Robertson MD, Fielding BA, Frayn KN, Williams CM.** Measurement of apolipoprotein B-48 in the Svedberg flotation rate (S(f))>400, S(f) 60–400 and S(f) 20–60 lipoprotein fractions reveals novel findings with respect to the effects of dietary fatty acids on triacylglycerol-rich lipoproteins in postmenopausal women. *Clin Sci (Lond)* 103: 227–237, 2002.
18. **Jackson KG, Robertson MD, Fielding BA, Frayn KN, Williams CM.** Olive oil increases the number of triacylglycerol-rich chylomicron particles compared with other oils: an effect retained when a second standard meal is fed. *Am J Clin Nutr* 76: 942–949, 2002.
19. **Jensen MD, Sarr MG, Dumesic DA, Southorn PA, Levine JA.** Regional uptake of meal fatty acids in humans. *Am J Physiol Endocrinol Metab* 285: E1282–E1288, 2003.
20. **Jeppesen J, Hollenbeck CB, Zhou MY, Coulston AM, Jones C, Chen YDI, Reaven GM.** Relation between insulin resistance, hyperinsulinemia, postheparin plasma lipoprotein lipase activity, and postprandial lipemia. *Arterioscler Thromb Vasc Biol* 15: 320–324, 1995.
21. **Karpe F, Steiner G, Olivecrona T, Carlson LA, Hamsten A.** Metabolism of triglyceride-rich lipoproteins during alimentary lipemia. *J Clin Invest* 91: 748–758, 1993.
22. **Lang CA, Davis RA.** Fish oil fatty acids impair VLDL assembly and/or secretion by cultured rat hepatocytes. *J Lipid Res* 31: 2079–2086, 1990.
23. **Miles JM, Park YS, Walewicz D, Russell-Lopez C, Windsor S, Isley WL, Coppack SW, Harris WS.** Systemic and forearm triglyceride metabolism: fate of lipoprotein lipase-generated glycerol and free fatty acids. *Diabetes* 53: 521–527, 2004.
24. **Parks EJ, Krauss RM, Christiansen MP, Neese RA, Hellerstein MK.** Effects of a low-fat, high-carbohydrate diet on VLDL-triglyceride assembly, production, and clearance. *J Clin Invest* 104: 1087–1096, 1999.
25. **Peel AS, Zampelas A, Williams CM, Gould BJ.** A novel anti-serum specific to apolipoprotein B-48: application in the investigation of postprandial lipidaemia in humans. *Clin Sci (Lond)* 85: 521–524, 1993.
26. **Robertson MD, Parkes M, Warren BF, Ferguson DJ, Jackson KG, Jewell DP, Frayn KN.** Mobilisation of enterocyte fat stores by oral glucose in humans. *Gut* 52: 834–839, 2003.
27. **Romanski SA, Nelson RM, Jensen MD.** Meal fatty acid uptake in adipose tissue: gender effects in nonobese humans. *Am J Physiol Endocrinol Metab* 279: E455–E462, 2000.
28. **Schmidt DE, Allred JB, Kien CL.** Fractional oxidation of chylomicron-derived oleate is greater than that of palmitate in healthy adults fed frequent small meals. *J Lipid Res* 40: 2322–2332, 1999.
29. **Schneeman BO, Kotite L, Todd KM, Havel RJ.** Relationships between the responses of triglyceride-rich lipoproteins in blood plasma containing apolipoproteins B-48 and B-100 to a fat-containing meal in normolipidemic humans. *Proc Natl Acad Sci USA* 90: 2069–2073, 1993.
30. **Simpson HS, Williamson CM, Olivecrona T, Pringle S, Maclean J, Lorimer AR, Bonnefous F, Bogaievsky Y, Packard CJ, Shepherd J.** Postprandial lipemia, fenofibrate and coronary artery disease. *Atherosclerosis* 85: 193–202, 1990.
31. **Tran K, Sun F, Cui Z, Thorne-Tjomsland G, St Germain C, Lapierre LR, McLeod RS, Jamieson JC, Yao Z.** Attenuated secretion of very low density lipoproteins from McA-RH7777 cells treated with eicosapentaenoic acid is associated with impaired utilization of triacylglycerol synthesized via phospholipid remodeling. *Biochim Biophys Acta* 1761: 463–473, 2006.
32. **Votruba SB, Atkinson RL, Schoeller DA.** Prior exercise increases dietary oleate, but not palmitate oxidation. *Obesity Res* 11: 1509–1518, 2003.
33. **Zilversmit DB.** Atherogenesis: a postprandial phenomenon. *Circulation* 60: 473–485, 1979.

