Use of structured triacylglycerols containing predominantly stearic and oleic acids to probe early events in metabolic processing of dietary fat

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Abstract Early events in the metabolic processing of dietary triacylglycerol may have an important impact on subsequent development of risk factors for coronary heart disease. We have used structured triacylglycerols containing predominantly stearic or oleic acids at the *sn*-2 position to probe aspects of the processing of dietary fatty acids presented to adipose tissue in chylomicron-triacylglycerol. Studies were conducted on 14 healthy women who were given meals containing 85 g carbohydrate and 60 g of either of the two structured triacylglycerols in random order. Systemic concentrations and arterio-venous differences across adipose tissue for plasma triacylglycerol and non-esterified fatty acids were measured, together with analysis of the fatty acid composition of the relevant fractions. The stereo-specific structure of the ingested triacylglycerol was largely preserved in chylomicron-triacylglycerol. Systemic concentrations of total and individual non-esterified fatty acids were not significantly different after ingestion of the two fats, nor were their rates of release across adipose tissue. The composition of non-esterified fatty acids released from adipose tissue changed after the meal to reflect more closely the composition of the triacylglycerol ingested, but again no significant differences were observed between the two test meals. There was no detectable release of monoacylglycerol from adipose tissue after either test meal. We conclude that the environment for lipoprotein lipase action in adipose tissue in vivo is likely to be highly organized, such that there is no release of monoacylglycerol, nor preferential uptake or release of fatty acids from chylomicron-triacylglycerol according to the nature or the position within triacylglycerol of the fatty acid.—Summers, L. K. M., B. A. Fielding, S. L. Herd, V. Ilic, M. L. Clark, P. T. Quinlan, and K. N. Frayn. **Use of structured triacylglycerols containing predominantly stearic and oleic acids to probe early events in metabolic processing of dietary fat. *J. Lipid Res.* 1999.** 40: **1890–1898.**

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Early events in the metabolic processing of dietary triacylglycerol (TAG) may have a major impact upon the risk of chronic diseases. Persistence of chylomicron remnants in the circulation is a marker for progression of ischemic heart disease (1, 2), and the excessive release of chylomicron-derived fatty acids into the circulation may underlie the common dyslipidemia characterized by elevation of apolipoprotein B concentration (3). Despite the importance of these early metabolic events, there are many aspects of the metabolism of dietary lipids that are not understood.

After the synthesis and secretion of chylomicron-TAG, a key event in its metabolic processing is the action of lipoprotein lipase (LPL) in the capillaries of tissues including adipose tissue, skeletal muscle, and myocardium. LPL releases fatty acids, which may be taken up by the adjacent tissue. The action of LPL is specific for the *sn*-1(3) ester bonds in a TAG molecule, and, in vitro, 2-monoacylglycerol (MAG) will accumulate during LPL action (4). We have shown previously that no MAG is released into venous plasma during high rates of LPL action in subcutaneous adipose tissue in vivo (5, 6), but it is not clear whether that reflects complete hydrolysis of TAG or perhaps tissue uptake of MAG. There is also some evidence of poor hydrolysis by LPL of saturated chains esterified in the *sn*-2 position in TAG (7, 8), raising the question of whether the initial removal of fatty acids from chylomicron-TAG might be fatty acid-specific.

After the hydrolysis of chylomicron-TAG by LPL in adipose tissue, there appears to be regulation of the fate of the fatty acids liberated: uptake and esterification by adipocytes or release into venous plasma as non-esterified fatty

Abbreviations: AUC, area under the curve; GC, gas chromatography; HSL, hormone-sensitive lipase; LPL, lipoprotein lipase; MAG, monoacylglycerol; NEFA, non-esterified fatty acids; OStO, structured triacylglycerol containing predominantly stearic acid at the *sn*-2 position; StOO, structured triacylglycerol containing predominantly oleic acid at the *sn*-2 position; TAG, triacylglycerol; TLC, thin-layer chromatography; VLDL, very low density lipoprotein.

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acids (NEFA) (9, 10). Regulation of this branch-point in fatty acid metabolism may play a fundamental role in determining the lipoprotein profile (10). There is indirect evidence for fatty acid specificity of this branch-point. The plasma NEFA profile tends to become more saturated during the postprandial period, perhaps reflecting preferential tissue retention of unsaturated fatty acids (11, 12). A plausible hypothesis is that the fate of fatty acids released by LPL depends upon their positional distribution within the TAG molecule from which they arise. It might be suggested that if isomerization of the 2-MAG to 1(3)-MAG is required for release of the *sn*-2 fatty acid, then this will occur later in the sequence of events and the *sn*-2 fatty acid might be particularly likely to 'escape' into the venous plasma. Alternatively, it could be argued that if 2-MAG were taken up into the tissue, a point on which there seems to be no evidence, then the *sn*-2 fatty acid would be particularly likely to be retained in the tissue. Fatty acid and positional specificity of these steps could have important implications. For instance, if a saturated fatty acid were to be preferentially released as NEFA into venous plasma by virtue of its nature or its position within the TAG molecule, then this might have adverse consequences in terms of delivery to the liver and effects on hepatic cholesterol metabolism (13).

Many of these poorly understood aspects of the early metabolism of dietary TAG might be probed by using structured TAGs in vivo. In a previous small study we compared the metabolic responses to feeding TAGs consisting predominantly of palmitoyl and oleoyl chains (ratio 1:2), with *sn*-2 palmitic or oleic acids respectively (for shorthand, OPO and POO) (14). No differences in metabolic responses were observed, including no measurable difference in the composition of NEFA released into the adipose tissue venous plasma during LPL action. However, that study had several limitations apart from small size and consequent lack of power to detect differences between the metabolism of the two TAGs. We did not assess the fatty acid positional distribution in chylomicron-TAG and therefore relied upon the assumption that the fatty acid positional distribution of dietary TAG would be largely preserved. More importantly, palmitic and oleic acids normally predominate in plasma NEFA at a molar ratio of approximately 1:2 (14, 15), so that the power to detect small changes above large background levels was limited. We have therefore conducted a larger study using two different structured TAGs, in which palmitic acid has been replaced by stearic acid as the predominant fatty acid at the *sn*-1(3) or *sn*-2 positions (referred to below as StOO and OStO, respectively). As stearic acid is usually a relatively minor component of plasma NEFA, this allowed us greater power to observe differences in fatty acid metabolism in vivo. In addition, it allowed us to test the hypothesis that a stearoyl chain at the *sn*-2 position of chylomicron-TAG might hinder LPL action (8) and reduce chylomicron-TAG clearance (7, 16). We have combined this with assessment of the fatty acid positional distribution in chylomicron-TAG and measurement of production of MAG in adipose tissue venous plasma to give a more complete picture of the early metabolism of dietary TAG in relation to fatty acid and positional specificities.

Some of these results have previously been published in abstract form (17, 18).

METHODS

Subjects

Studies were conducted on 14 healthy female subjects aged 29 to 70 years (median 49 years), body mass index (BMI) 20.6 to 52.8 $kg \cdot m^{-2}$ (median 27.5 $kg \cdot m^{-2}$) on two occasions. Subjects with a range of BMI values were studied to determine any influence of obesity on the metabolic parameters measured, but that aspect will not be presented in this paper. Their median fasting total cholesterol concentration was 4.8 $mmol \cdot l^{-1}$ (3.8 to 9.2 $mmol \cdot l^{-1}$) with a median high density lipoprotein cholesterol concentration of 1.2 $mmol \cdot l^{-1}$ (0.5 to 2.3 $mmol \cdot l^{-1}$) and TAG of 1.1 $mmol \cdot l^{-1}$ (0.6 to 2.4 $mmol \cdot l^{-1}$). Subjects were asked to refrain from smoking, alcohol, and unaccustomed exercise for 24 h prior to the study. They ate a fat-free meal on the evening before the study and then fasted from 8.00 pm, also avoiding caffeinated drinks. Premenopausal female subjects were studied during the same follicular phase of their menstrual cycle on each visit. Whilst estrogen status might be expected to influence lipid metabolism (19), as the study was a within-subjects comparison, this is unlikely to have affected our conclusions. The studies were approved by the Central Oxford Research Ethics Committee and all subjects gave informed consent.

Protocol

A branch of the superficial inferior epigastric vein was cannulated with a 10-cm, 22-gauge Secalon Hydrocath catheter (Ohmeda, Swindon, UK) as described previously (20). A cannula was inserted in a retrograde fashion into a vein draining a hand heated in a box at 60°C, to provide arterialized samples (for simplicity referred to below as arterial). Arterialized blood has been shown to be not detectably different from true arterial blood for many metabolites including NEFA (21, 22). Both cannulae were kept patent by continuous infusion of isotonic saline. A meal consisting of a bowl of cornflakes and a strawberry-flavored milkshake with a cup of decaffeinated tea or coffee, containing 85 g carbohydrate, 60 g fat, and 13 g protein, was given at 0 min. The fat consisted of a structured TAG, either with stearic acid predominantly at the *sn*-1(3) position (StOO) or with stearic acid predominantly at the *sn*-2 position (OStO) (provided by Unilever Research, Bedford, UK) given in random order (**Table 1**). The structured TAGs were not pure, only 61% of 'OStO' being OSO (where S = saturated, almost all of which is 18:0, see Table 1). However, the content of OSx in OStO, where x is another fatty acid, was 88%. The *sn*-2 position fatty acids (analyzed by Unilever Research) were: StOO, 86.9% oleic acid; OStO, 83.3% stearic acid.

At −20, 0, 30, 60, 90, 120, 180, 240, 300, and 360 min, simultaneous blood samples were taken from the artery and the abdominal vein. Adipose tissue blood flow (ATBF) was measured immediately after each blood sample using the ^{133}Xe washout method (23). In brief, 2 MBq ^{133}Xe in saline was injected into the subcutaneous adipose tissue and disappearance of radioactivity was followed over a 10-min period after each blood sample, by external radioactivity monitoring (24). At all time points whole-blood lactate, glycerol, and 3-hydroxybutyrate (3-OHB), plasma NEFA, TAG, and glucose concentrations were measured in arterial and venous samples. Insulin concentrations were measured at the same time-points in arterial samples only. Gas chromatographic

TABLE 1. Composition of the triacylglycerols used in the test meals

Fatty Acid Composition			Molecular Species Composition		
Triacylglycerol Fatty Acid	StOO	OStO	Molecular Species[a]	StOO	OStO
	wt%	*wt%*		*wt%*	*wt%*
12:0	0.0	1.8	SSS	0.0	1.2
14:0	0.0	1.7	SOS	5.8	0.0
16:0	2.8	1.6	OSS	1.9	11.1
16:1	0.1	0.3	SLnS	1.2	0.0
18:0	30.7	30.8	SSLn	0.7	0.0
18:1	56.9	54.0	SOO	82.0	4.3
18:2	8.4	8.6	OSO	0.0	61.3
18:3	0.0	0.0	OSLn	0.0	15.3
20:0	0.3	0.1	SOLn	5.0	1.1
20:1	0.2	0.2	OOO	2.8	3.5
			>3DB	0.8	2.3

Data were provided by the manufacturers (Unilever Research, Bedford, UK); molecular species information was obtained by silver-phase chromatography.

[a] StOO and OStO are defined in the text; S, all saturated fatty acids; O, oleic acid; Ln, linoleic acid; >3DB, triacylglycerols with more than 3 double bonds.

analysis of the specific fatty acid composition of arterial and venous plasma NEFA was performed at all time-points and of chylomicron-TAG at hourly intervals from 60 min onwards.

One subject had a cannula inserted arterially rather than into an arterialized vein on both study days. In one subject venous samples were not obtained for all time-points on one study and therefore this subject was only included for arterial analyses. In four subjects it proved impossible to obtain samples from the adipose tissue vein sufficient for gas chromatography analysis at all time-points on both visits; these subjects, therefore, were not included in the statistical analysis of the gas chromatography results. Numbers of subjects contributing to each measurement are shown with figure legends.

In separate studies to confirm the preservation of TAG structure in chylomicron-TAG, three normal subjects ingested the same test meals and blood samples were taken at 4 h for preparation of a chylomicron-rich fraction ($S_f > 400$) from plasma.

Analytical methods

A small portion of each arterial blood sample was heparinized and was used for blood gas analysis and hematocrit estimation. A portion of both arterial and venous samples was rapidly deproteinized with 7% (w/v) perchloric acid and the remainder was used to prepare plasma. Arterial and venous whole-blood lactate, glycerol, and 3-OHB, plasma NEFA and glucose concentrations were measured using enzymatic methods on an IL Monarch centrifugal analyzer (Instrumentation Laboratory (UK) Ltd, Warrington, Cheshire, UK). Plasma total acylglycerol concentrations were also measured enzymatically with correction for free glycerol (25) and will be referred to as TAG because, as shown below, mono- and diacylglcyerol concentrations are very low. Plasma insulin was measured in the arterial samples using a double-antibody radioimmunoassay method (Pharmacia & Upjohn Ltd., Milton Keynes, UK).

A chylomicron-rich fraction was prepared from plasma by layering 0.75-ml portions of plasma underneath a solution with a density of 1006 g/L in 11 × 34 mm centrifuge tubes and centrifuging at 4°C in a rotor type TLS 55 at 30,000 rpm for 20 min in an Optima TLX ultracentrifuge (Beckman Instruments (UK) Ltd, High Wycombe). The chylomicron-rich fraction was separated by slicing. For analysis of specific fatty acids, lipids were extracted from plasma or from the chylomicron-rich fraction using chloroform–methanol 2:1 (v/v). After separation of the lipid classes by thin-layer chromatography (TLC) and methylation of fatty acids with methanoic sulfuric acid, gas chromatography (GC) was used to analyze the fatty acid composition of the plasma NEFA, chylomicron-, and plasma TAG fractions. Details have been given previously (26). The absolute concentrations of the individual fatty acids were calculated by reference to internal standards added to the plasma at the stage of lipid extraction. These were heptadecanoic acid for NEFA and triheptadecanoyl glycerol for chylomicron- and total TAG. The sums of the individual fatty acids were calculated to give the total lipid concentration in each fraction. Samples of the meal were also analyzed using gas chromatography to establish their specific fatty acid composition.

In four subjects, paired arterial and adipose tissue venous samples taken during the postprandial period (240 or 300 min) were analyzed for specific TAG, diacylglycerol (DAG) and monoacylglycerol (MAG) concentrations. Lipid extraction and separation on TLC were as described by Fielding et al. (27), except that in addition, internal standards were added to the plasma at the stage of lipid extraction. These were triheptadecanoyl glycerol, diheptadecanoyl glycerol, and monoheptadecanoyl glycerol for TAG, DAG, and MAG, respectively. After separation the concentrations were measured by GC of fatty acid methyl esters as above.

In the separate studies in three subjects, the fatty acids making up the *sn*-2 position in the chylomicron-TAG fraction were analyzed by specific enzymatic hydrolysis (28). The chylomicron-rich fraction (200 μl) was incubated at 37°C for 7.5 min with 240 μl Tris buffer (0.2 mol/l, pH 8.0), 5 μl calcium chloride (18% w/v) and 5 μl porcine pancreatic lipase (Sigma-Aldrich Chemical Co., Poole, UK; 100,000 units in 1 ml sodium phosphate, 0.1 mol/l, pH 7.0). These conditions were shown in preliminary studies to produce almost complete hydrolysis of TAG with generation of MAG, assumed to represent the *sn*-2 fatty acid of the TAG. After lipid extraction, MAG was isolated by TLC as above and the fatty acid composition was determined by GC. A portion of each test fat was also extracted and analyzed in an identical manner.

Calculations and statistics

Adipose tissue blood flow (ATBF) was calculated as described by Larsen, Lassen, and Quaade (23). The partition coefficient for ^{133}Xe was taken as 10 ml·g^{-1}. Repeated measures analysis of variance was used to compare metabolite concentrations in each subject, analyzing the effects of time, with the site from where the sample was taken (arterial or venous) and the type of TAG as within-subjects factors.

In order to place confidence limits on the degree of (dis)agreement between responses to the two types of fat, the following approach was used. Responses were summarized as total and incremental areas-under-curves (AUCs), where the total AUC is from 0 to 360 min and the incremental AUC is total AUC minus mean baseline value extrapolated over this period. AUCs were divided by 360 to give time-averaged postprandial values. For each major variable to be analyzed, the difference between incremental AUCs for StOO and OStO was calculated within each subject. In order to express this as a percentage variation, and to allow comparison between different variables, it was then expressed as a percentage of the total AUC within that person (average of StOO and OStO responses). Mean and 95% confidence intervals were then calculated over all subjects.

To increase the power to find differences in the key variable of the relative release of the *sn*-1(3) and *sn*-2 fatty acids from adipose tissue, we added data from our previous study with POO and OPO (14). In this case the end-point was the ratio of oleic to

palmitic acid release from adipose tissue (n = 5). The data were analyzed in an identical manner.

RESULTS

Triacylglycerol

Plasma TAG concentrations rose after the meals to reach peak values at 240 to 300 min (**Fig. 1**). Chylomicron-TAG concentrations (Fig. 1) also rose to a maximum, at 240 min after the meal containing StOO and 300 min after the meal containing OStO (Fig. 1). Comparing the two test meals, there were no significant differences in plasma- or chylomicron-TAG concentrations; the magnitude of the actual (nonsignificant) differences is considered later.

Triacylglycerol fatty acid composition

After both meals, the chylomicron-TAG was enriched in stearic and oleic acids, similar to the test fats (**Table 2**). Comparing the two meals, there were no significant differences in the proportions of stearic, oleic, or palmitic acid making up chylomicron-TAG.

TABLE 2. Composition of arterial chylomicron-TAG (average values from 180 to 360 min after test meals) and of the test fats

	Chylomicron-TAG			
	Meal Containing StOO	Meal Containing OStO	Test Fats StOO	Test Fats OStO
	mol%	*mol%*	*mol%*	
16:0	11.4 ± 2.0	10.9 ± 1.7	3.1	1.8
18:0	23.3 ± 2.1	24.2 ± 1.2	31.2	30.3
18:1	51.5 ± 1.6	49.6 ± 1.6	58.2	54.7

The fatty acid composition of StOO and OStO is from our own analyses and may be compared with the data supplied by Unilever Research (Table 1); n = 13 for Chylomicron-TAG data. Values are mean ± SE.

Positional distribution of fatty acids making up chylomicron-triacylglycerol

Our analysis of *sn*-2 fatty acids in the triacylglycerols used for the test meals agreed closely with data supplied by the manufacturer (**Fig. 2**), confirming the accuracy of the methodology. The chylomicron-TAG composition was analyzed in a separate study in three normal subjects (see Methods). The *sn*-2 fatty acids were largely preserved in the chylomicron fraction (Fig. 2).

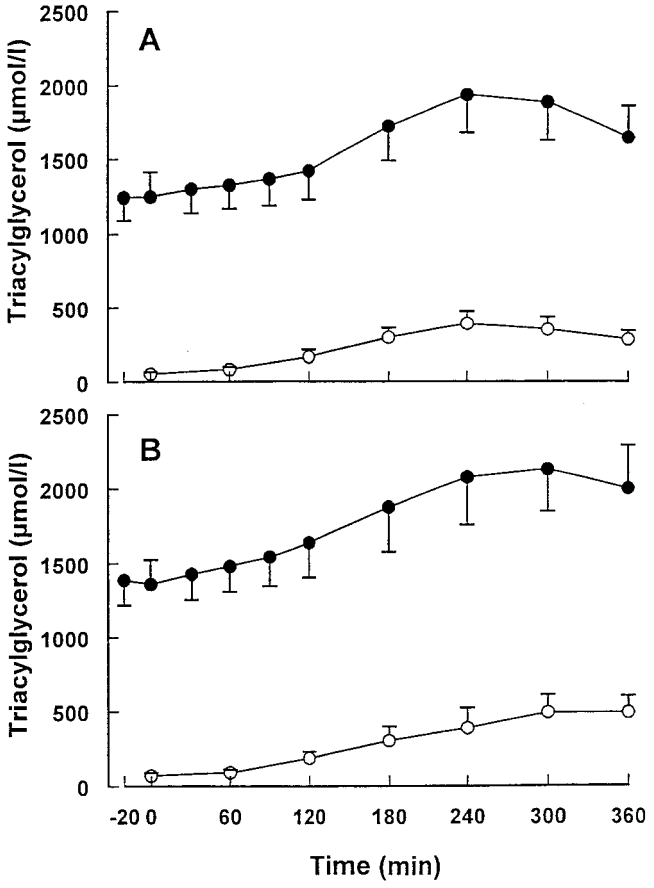

Fig. 1. Arterial TAG concentrations in plasma (●, n = 14), and the chylomicron fraction (○, n = 13), before and after meals containing structured triacylglycerols: panel A, StOO; panel B, OStO (defined in the text). Repeated measures ANOVA shows significant changes with time for plasma-TAG concentrations ($P < 0.001$) and chylomicron-TAG concentrations ($P < 0.005$), with no significant differences between plasma- or chylomicron-TAG concentrations after the two meals.

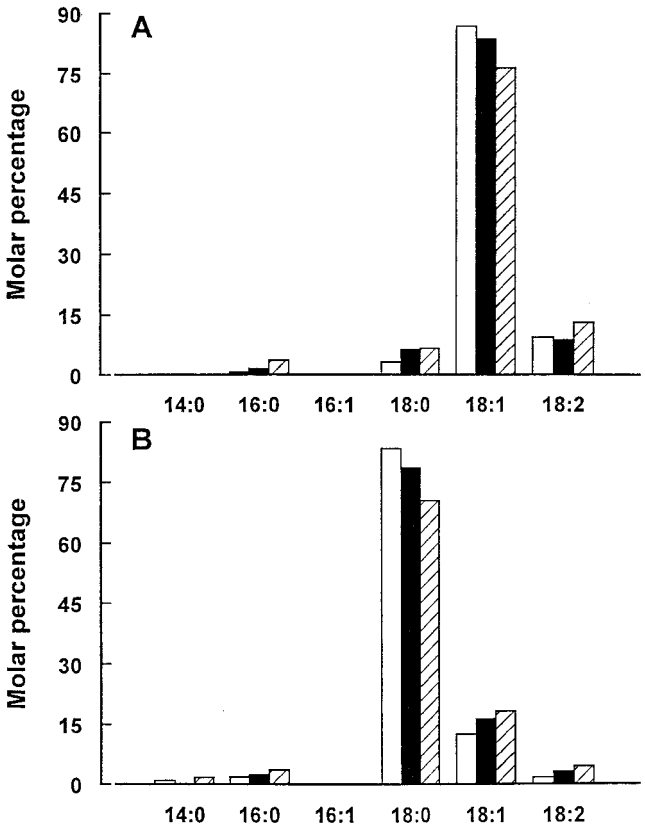

Fig. 2. *sn*-2 Fatty acid composition of the triacylglycerols used in the test meals and of the chylomicron fraction. Panel A, StOO; panel B, OStO (defined in the text). Data are molar percentage of each fatty acid at the *sn*-2 position. Open bars, data provided by Unilever Research (Bedford, UK); solid bars, our analysis of the triacylglycerols used in the test meals; hatched bars, chylomicron-TAG composition at 4 h, median values from three subjects.

Triacylglycerol fractional extraction, and di- and monoacylglycerol concentrations

Extraction of plasma TAG across adipose tissue increased after the meals, to maximal values at 240–300 min ($P < 0.05$). Mean fractional extraction of plasma TAG ± SE (over the whole experiment, based on AUCs) was: for StOO meal, 15.1 ± 2.5%; for OStO meal, 14.3 ± 2.3%. Extraction of chylomicron-TAG across adipose tissue was very variable from person to person and is analyzed further below.

Plasma DAG and MAG concentrations were measured in the postprandial period only. They were low in relation to plasma TAG concentrations, accounting for less than 1% of total acylglycerols after both test meals. Whilst there was considerable extraction of TAG across the adipose tissue bed, there were no changes in concentration of either MAG or DAG across adipose tissue (**Fig. 3**). Although total concentrations appear different between the two test meals on Fig. 3, the data do not come from the same timepoints in each subject.

Non-esterified fatty acids

Plasma NEFA concentrations, both arterial and adipose tissue-venous, decreased after the meals with maximal suppression occurring at 90 to 120 min (**Fig. 4**). As expected, there was net release of fatty acid from adipose tissue both before and after the meal, indicated by a large veno-arterial (V-A) difference for NEFA. This V-A difference decreased after the meals and was lowest at the times of maximal NEFA suppression (Fig. 4). There were no significant differences between the two meals in the NEFA concentrations or V-A differences; the magnitude of the actual (non-significant) differences is considered later.

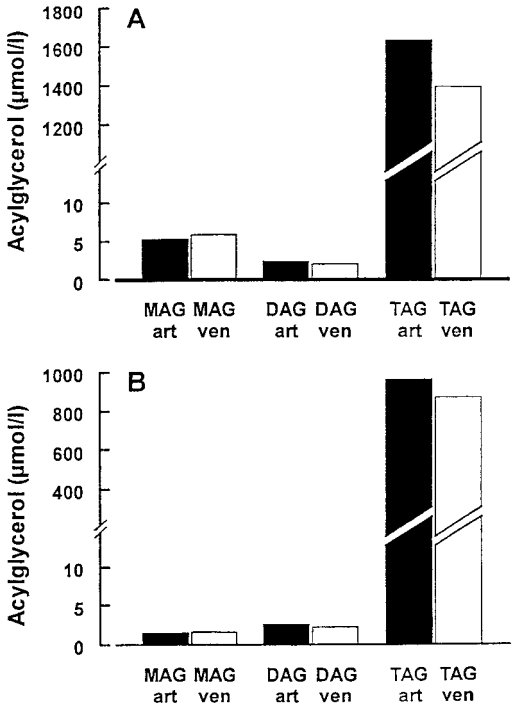

Fig. 3. Acylglycerol concentrations in plasma at 4–5 h after meals containing structured triacylglycerols. Panel A, OStO; panel B, StOO (defined in the text). Solid bars, arterial concentrations; open bars, adipose tissue venous concentrations. Mean values from four subjects.

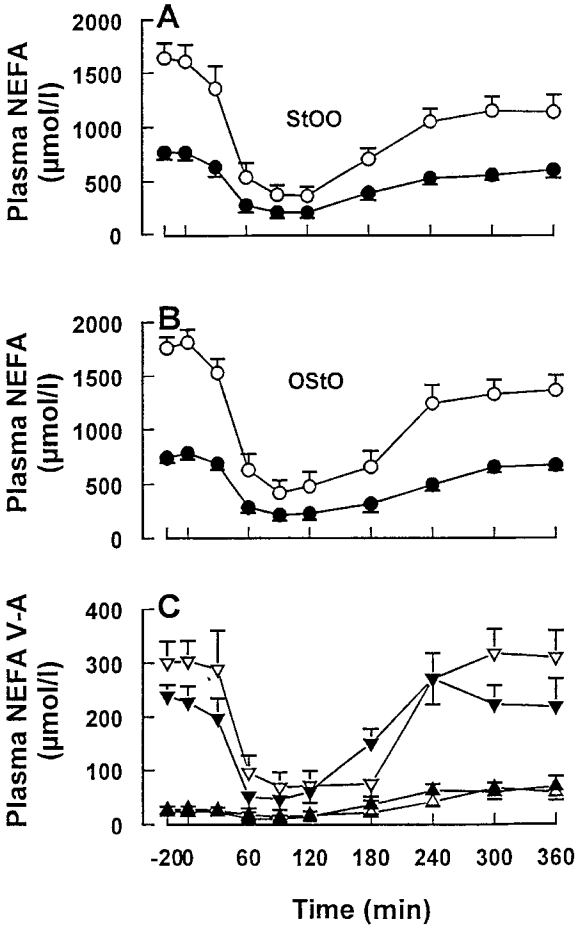

Fig. 4. NEFA concentrations in arterial (●, n = 13) and in adipose tissue venous plasma (○, n = 13), before and after meals containing structured triacylglycerols: panel A, StOO; panel B, OStO (defined in the text). Panel C shows veno-arterial (V-A) differences for oleic acid (downward triangles) and stearic acid (upward triangles) after the two meals: ▼, oleic acid, StOO; ▽, oleic acid, OStO; ▲, stearic acid, StOO; △, stearic acid, OStO; n = 13. Repeated measures ANOVA shows significant changes with time for arterial NEFA concentrations ($P < 0.001$) and for the NEFA (V-A) differences ($P < 0.001$). There were no significant differences between the meals for plasma NEFA concentrations or for the NEFA V-A differences.

Non-esterified fatty acid fraction composition

The plasma concentration of palmitic acid in the NEFA fraction suppressed after the meals, and the proportion of total arterial plasma NEFA made up of palmitic acid decreased markedly (not shown). The concentration of stearic acid in the NEFA fraction decreased at 60–120 min after the meals before increasing above baseline values (Fig. 4C), and the proportion of stearic acid in plasma NEFA increased steadily. The concentration of oleic acid fell sharply after eating before returning to fasting values (Fig. 4C), and the proportion of oleic acid in plasma

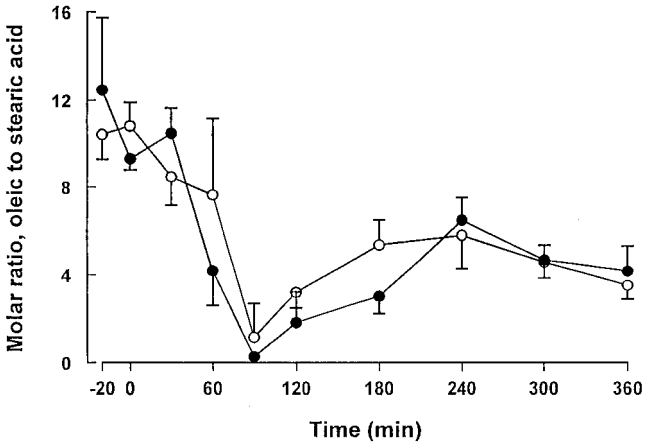

Fig. 5. Ratio of stearic to oleic acid in the NEFA veno-arterial (V-A) difference across adipose tissue (i.e., ratio of stearic to oleic acid release from adipose tissue), before and after meals containing structured triacylglycerols: ●, StOO; ○, OStO (defined in the text); n = 9. Repeated measures ANOVA shows significant changes with time for stearic to oleic acid ratio ($P < 0.001$) but with no differences between the two meals.

NEFA behaved similarly. Figure 4C shows V-A concentration differences for stearic and oleic acids. There were no significant differences between the meals in absolute concentrations or proportions of stearic, palmitic, or oleic acid in the arterial or adipose-tissue venous plasma NEFA fraction.

The ratio of oleic to stearic acid in the V-A difference of the plasma NEFA fraction was analyzed as the key indicator of preferential uptake or release of the *sn*-2 position fatty acid in chylomicron-TAG (**Fig. 5**). This ratio fell sharply after the meals to a value below 2, reflecting suppression of intracellular lipolysis and the beginning of the hydrolysis of the exogenous TAGs in chylomicron-TAG. (The ratio of oleic to stearic acid in the two structured TAGs was about 1.8:1; Table 1). The ratio of oleic to stearic acid in the NEFA V-A difference remained below fasting levels for the remainder of the study. There were no differences between the two meals in the ratios of palmitic and oleic acids to stearic acid in the V-A difference of the plasma NEFA fraction. **Table 3** shows the composition of the NEFA V-A difference across adipose tissue for the period 4–6 h after the meals, when the ratio of oleic to stearic acid release was about half its fasting value (indicating a substantial contribution of LPL-derived fatty acids to adipose tissue NEFA release). There were no significant differences between the meals in the composition of the NEFA released from adipose tissue at this time.

TABLE 3. Composition of adipose tissue non-esterified fatty acid release from 4 to 6 h after meals containing structured triacylglycerols, StOO or OStO

Fatty Acid	Meal Containing StOO	Meal Containing OStO
10:0	0.4 ± 1.0	0.1 ± 0.1
12:0	0.3 ± 0.3	1.0 ± 0.7
14:0	2.3 ± 1.9	3.9 ± 0.8
16:0	16.5 ± 6.4	17.6 ± 5.6
16:1	6.1 ± 4.8	5.3 ± 3.1
18:0	12.5 ± 6.8	11.4 ± 5.0
18:1	47.9 ± 7.2	45.7 ± 4.1
18:2	13.3 ± 4.2	14.3 ± 2.4
18:3	0.8 ± 0.7	0.9 ± 0.8
18:1/18:0 ratio	5.1 ± 2.8	4.9 ± 2.3

Values are molar percentages except for 18:1/18:0 ratio; n = 11. Values given as mean ± SD.

Differences between responses to the test fats

Although no significant differences in any response were noted when comparing one fat with another, it was important to put limits on the potential magnitude of any real differences. **Figure 6** shows a summary of differences in responses calculated as described under Statistical Methods. All end-points are normalized for comparison with each other by expressing the absolute differences

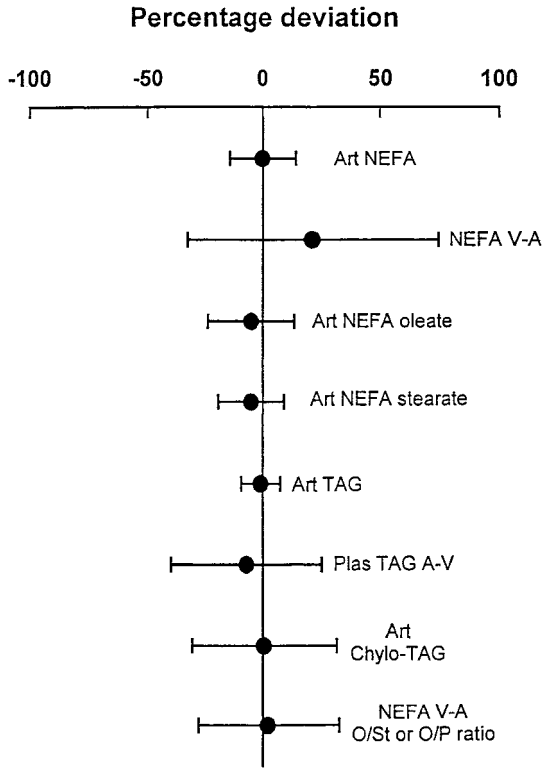

Fig. 6. Analysis of magnitude of differences between postprandial responses to StOO and OStO. Each major variable was analyzed as described in the text; within-subject differences between incremental AUCs following StOO and OStO were normalized by expression as a percentage of mean AUC_{0-360} for that variable. Data shown are mean values where 0 represents identical responses to StOO and OStO, and 95% confidence limits expressed as percentage of mean response. Data shown are (top to bottom): arterial NEFA concentration (n = 14); V-A difference across adipose tissue for NEFA (n = 13); arterial concentrations of non-esterified oleic and stearic acids (n = 13); arterial TAG concentration (n = 14) and A-V difference across adipose tissue for plasma TAG (n = 13); arterial chylomicron-TAG concentration (n = 13), and the ratio of release from adipose tissue of oleic acid to saturated fatty acid, either stearic acid (following StOO, OStO; n = 9) or palmitic acid (following POO or OPO in previous studies (14); n = 5).

(StOO − OStO) as a percentage of the total AUC_{0-360} (average of values for StOO and OStO meals) for that variable. For the key variable of the ratio of release of nonesterified oleic and stearic acids, the power was increased by addition of data from a previous study (14) using the structured TAGs POO and OPO (see Introduction).

The central tendency was close to zero for most measurements although the confidence intervals varied. In general the data for fatty acid metabolism were more closely defined than those for TAG metabolism, and the data for arterial concentrations were more closely defined than those for arterio-venous differences.

The differences for chylomicron-TAG arterio-venous difference, when expressed in this way, showed wide confidence limits. However, that reflected in part the method chosen to express the differences. Some subjects had low chylomicron-TAG extraction across adipose tissue on both occasions (close to zero), giving large numerical values when differences were normalized. If data from one study were plotted against those from the other (**Fig. 7A**) it can be seen that agreement was reasonably close ($r = 0.87$, $P < 0.01$). The slope of the regression line for chylomicron-TAG A-V difference for OStO versus StOO was 0.61 (95% confidence limits 0.33 to 0.89), suggesting poorer extraction of chylomicron-TAG enriched in OStO.

This method of expressing the data was also applied to the results of the key variable of the relative release of the *sn*-1(3) and *sn*-2 fatty acids from adipose tissue. Both total AUCs (0–360 min) and incremental AUCs were plotted, and again the data from the previous study with POO and OPO were included. The results lay clustered around the line of identity (Fig. 7B), strongly suggesting lack of differential metabolism of the *sn*-2 position fatty acid.

Adipose tissue blood flow, insulin, and metabolites

As expected (24, 29), there was a significant postprandial rise in ATBF ($P < 0.005$), with a peak at 30 min. ATBF did not differ differ significantly between the two test meals.

Arterial plasma concentrations of glucose and insulin and blood concentrations of glycerol, lactate, and 3-OHB are given in time-averaged form in **Table 4**. Plasma glucose and insulin concentrations rose significantly after both meals ($P < 0.001$, $P < 0.005$, respectively), reaching a peak at 30 min. Glycerol concentrations decreased postprandially ($P < 0.001$) to a minimum at about 60 to 120 min after both meals. 3-Hydroxybutyrate and lactate concentrations also changed with time ($P < 0.001$), 3-hydroxybutyrate reaching a trough at 90 to 120 min and lactate concentrations reaching a peak at 60 min. There were no significant differences between the meals in arterial concentrations of these metabolites nor in the uptake (glucose) or release (glycerol) across adipose tissue.

DISCUSSION

It has been demonstrated in previous studies that there is substantial conservation of the fatty acid at the *sn*-2 position of the ingested TAG in the chylomicron-TAG after digestion (30–32). This was essential for our study and our results clearly confirmed it.

There were no differences in the arterial metabolite or insulin concentrations after the two meals, confirming the results of two previous studies (14, 33).

Our measurements of the release of MAG from adipose tissue bear out previous findings in vivo (5, 6): even during relatively high rates of LPL action on chylomicron-TAG in vivo, there is no net liberation of MAG into the plasma. This contrasts strongly with findings showing accumulation of MAG during the action of LPL in vitro (4) or in vivo after displacement of LPL by heparin (27). The findings suggest that LPL may operate in vivo in a highly structured environment in which lipoprotein particles are

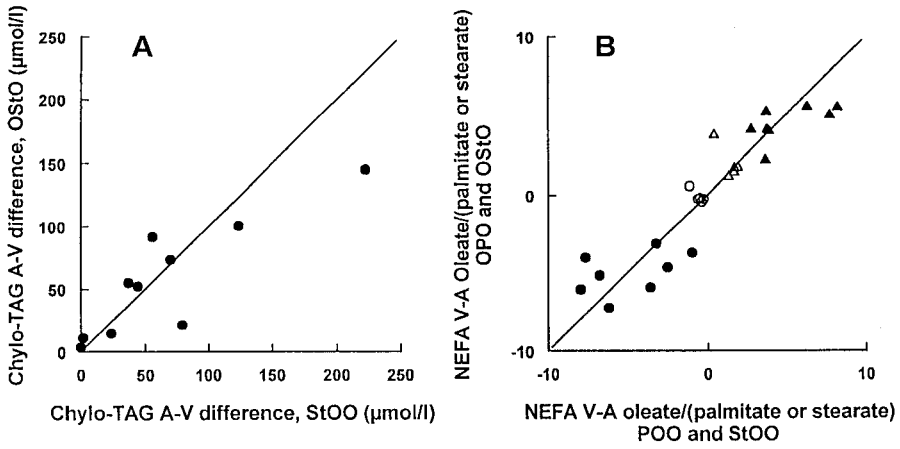

Fig. 7. Responses to StOO and OStO compared directly. On both panels the line of identity is shown. Panel A, arterio-venous differences for chylomicron-TAG expressed as time-averaged values derived from total AUC_{0-360} (n = 10). Panel B: total and incremental $AUCs_{0-360}$ for the ratio of release from adipose tissue of oleic to saturated fatty acid, either stearic acid (following StOO, OStO; n = 9) or palmitic acid (following POO or OPO in previous studies (14); n = 5); ●, incremental AUC after StOO / OStO; ○, incremental AUC after POO / OPO; ▲, total AUC after StOO / OStO; △, total AUC after POO / OPO. For ● one outlier at −9.87 (StOO), −17.3 (OStO) is not shown.

TABLE 4. Arterial insulin and metabolite concentrations determined after subjects had consumed meals containing structured triacylglycerols, either StOO or OStO

Metabolite	Meal Containing StOO Basal	Meal Containing OStO Basal	Meal Containing StOO Postprandial	Meal Containing OStO Postprandial
Glucose (mmol/l)	5.3 ± 0.32	5.4 ± 0.29	6.0 ± 0.33	6.0 ± 0.26
Insulin (mU/l)	15.8 ± 7.0	16.0 ± 6.7	37.1 ± 13.0	36.3 ± 11.3
Lactate (μmol/l)	633 ± 44	642 ± 52	675 ± 54	676 ± 48
3-OHB (μmol/l)	134 ± 16	117 ± 22	95 ± 10	89 ± 13
Glycerol (μmol/l)	101 ± 8.2	100 ± 6.7	72.8 ± 6.1	71.8 ± 5.8

Values given as mean ± SE were time-averaged values based on mean basal and postprandial area under the curve; n = 14. Glucose and insulin were measured in plasma, the remainder in whole blood.

anchored for sufficient time for isomerization of 2-MAG to 1(3)-MAG and thus complete hydrolysis. Clearly this occurs even when stearic acid is esterified at the *sn*-2 position. The only alternative explanation would be that 2-MAG is taken up by the tissue. However, the findings with regard to release of individual fatty acids (discussed below) do not bear this out.

The major emphasis of our study was to investigate the possible selective uptake or release of fatty acids according to stereo-specific position in chylomicron-TAG. Calculations of the source of NEFA released from adipose tissue in the postprandial period (34) suggest that plasma TAG hydrolysis by LPL was a major source of these fatty acids for most of the postprandial period. This can be seen clearly from the ratio of oleic to stearic acid released (Fig. 5), which fell after ingestion of the meals to a value below 2, similar to that in the TAGs fed. This implies that chylomicron-TAG hydrolysis by LPL predominated as a source of NEFA leaving adipose tissue at that time, reflecting insulin suppression of adipose tissue hormone-sensitive lipase (HSL), and yet there was no difference between the two test meals in the relative proportions of stearic and oleic acid released. During the period 4–6 h after the meal, the ratio of oleic to stearic acid released from adipose tissue was around 5. If a ratio of around 11 (as in the fasting state) is considered to represent 'baseline lipolysis' and a ratio around 1.8 to reflect LPL hydrolysis of chylomicron-TAG, then a value around 5 suggests that about two-thirds of the stearic acid leaving adipose tissue is derived from chylomicron-TAG hydrolysis, but still no differences between the meals were seen in the composition of NEFA released from adipose tissue.

These results therefore strongly refute the hypothesis that the fatty acid at the *sn*-2 position of chylomicron-TAG, especially if saturated, would be preferentially released into the adipose tissue venous plasma. However, they also show clearly that the alternative hypothesis of tissue uptake of 2-MAG with preferential release of the *sn*-1 and *sn*-3 fatty acids into the venous plasma is incorrect. It should be noted that these conclusions apply to fatty acids of 16 and 18 carbon chain length: there is evidence for effects on metabolic availability of the very long-chain n–3 polysunsaturated fatty acids according to positional distribution in dietary TAG (35).

Although these results bear out our previous findings (14), they do so with much greater power. However, measurements of fasting and postprandial lipid metabolism in vivo are inherently somewhat variable within subjects (36), and more so when catheterization of subcutaneous adipose tissue drainage is added. This may be seen clearly from the data in Figs. 6 and 7, which allow us to put limits on the degree to which we can claim no effect of dietary TAG structure. The fact that the central tendency for all variables examined in this way was close to zero suggests a lack of effect of TAG structure. However, we could not rule out (at a confidence level of 5%) a difference of 15–20% for average postprandial arterial concentrations of total NEFA or of non-esterified oleic or stearic acids. For the arterial plasma TAG concentration, the equivalent figure was 9%. For the key variable of ratio of release of oleic and stearic acids (or of oleic to palmitic acids in the earlier experiments with POO, OPO) the mean results agreed to within 2% although the confidence limits were ±30%; however, graphical analysis of the data (Fig. 7B) strongly suggests a lack of effect on average. Other variables such as the V-A difference for plasma NEFA concentration were imprecisely defined, emphasizing the variability of such data.

Despite the fact that the power of these studies to look at TAG metabolism was generally less than that for NEFA metabolism, it was interesting that an examination of the extraction of chylomicron-TAG across adipose tissue suggested that a saturated fatty acid at the *sn*-2 position somewhat reduced removal compared with the presence of an unsaturated fatty acid. This finding is in accord with findings in rat studies using chylomicron-like emulsions (7, 16, 37). More data would be needed to confirm this interesting preliminary observation.

The use of structured TAGs has therefore allowed us to probe early events in the metabolic processing of dietary fat. Contrary to expectations based largely upon data obtained in vitro and from animal studies, no differences were detectable in the handling of individual fatty acids in the systemic circulation or in subcutaneous adipose tissue. These results highlight the efficiency of TAG hydrolysis by LPL in adipose tissue in vivo. Even with a saturated fatty acid present at the *sn*-2 position in chylomicron-TAG, we found no evidence for release of 2-MAG and no evidence for selective fatty acid uptake or release. We conclude that these early metabolic events after LPL-hydrolysis of chylo-

micron-TAG are largely unaffected by the nature or the position of fatty acids within dietary TAG.

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