Effects of palm oil and transesterified palm oil on chylomicron and VLDL triacylglycerol structures and postprandial lipid response

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Abstract The effects of positional distribution of triacylglycerol (TAG) fatty acids to TAG structures in chylomicrons and VLDL, and to postprandial lipemia, were studied in 10 healthy premenopausal women using a 6-h oral fat load test and a randomized, double-blind cross-over design. Molecular level information of TAG regioisomerism was obtained with a tandem mass spectrometric method. The positional distribution of fatty acids in chylomicron TAGs was similar to the respective dietary fat; 79% of the analyzed regioisomers in palm oil and 84% of the analyzed regioisomers in transesterified oil were found in chylomicron TAGs 3 h after the oral fat loads. VLDL TAGs were equal after the two fat loads in all but one regioisomer. Similarities in the fatty acid compositions of chylomicron TAGs suggest that palmitic acid was absorbed equally from both test fats. The proportion of palmitoleic acid in the chylomicrons was increased. Fat with palmitic acid predominantly in the *sn*-1 and *sn*-3 positions caused a larger incremental area of total TAGs in plasma and reduced plasma insulin values at the beginning of the postprandial response (0–90 min) compared with fat with palmitic acid randomly distributed.▪ The relationship between TAG molecular structures in dietary fats and in lipoproteins provides new means for understanding the effects of fatty acid positonal distribution on human lipid metabolism.—Yli-Jokipii, K., H. Kallio, U. Schwab, H. Mykkänen, J-P. Kurvinen, M. J. Savolainen, and R. Tahvonen. **Effects of palm oil and transesterified palm oil on chylomicron and VLDL triacylglycerol structures and postprandial lipid response.** *J. Lipid Res.* 2001. **42:** 1618–1625.

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The positional distribution of fatty acids in triacylglycerols (TAGs) varies greatly among fats and oils of different origin. The lipases in the digestive tract hydrolyze the fatty acids in the *sn*-1 and *sn*-3 positions, whereas the long-chain fatty acids in the *sn*-2 position predominantly remain in this location and are absorbed as 2-monoacylglycerols. Because the long-chain saturated fatty acids can form insoluble soaps with Ca^{2+} and Mg^{2+} in the gut, stearic acid and palmitic acid are better absorbed if situated in the *sn*-2 position than if situated in the *sn*-1 and *sn*-3 positions (1–3). Furthermore, saturated fatty acids in the *sn*-2 position of dietary TAGs have been shown to slow down the clearance of chylomicrons in animals. The 2-monoacylglycerols may remain on the chylomicron surface, causing changes in the physical properties of the surface layer (4–6). Therefore, the positional distribution of saturated fatty acids may influence lipid metabolism postprandially.

Because TAGs with saturated fatty acids in the *sn*-2 position may be absorbed more efficiently and cleared from circulation more slowly than TAGs with saturated fatty acids in the *sn*-1 and *sn*-3 positions, feeding these dietary TAGs may result in a more pronounced postprandial lipemia, which is an independent risk factor for coronary artery disease (7–9). However, Zampelas et al. (10) and Summers et al. (11, 12) found no significant differences in plasma lipids after meals with TAGs of different positional distributions. The authors suggested that either the fats studied were digested and cleared from blood at the same rates or the differences in digestion and clearance cancelled each other out.

VLDL TAGs are highly asymmetrical. Saturated fatty acids are located predominantly in the *sn*-1 position and unsaturated fatty acids in the *sn*-2 and *sn*-3 positions (13). The fatty acid composition of a diet has an effect on the fatty acid composition of VLDL TAGs and consequently possibly also on the positional distribution of fatty acids in VLDL TAGs (14).

The relationship between TAG molecular structures in dietary fats, chylomicrons, and VLDL provides new means

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Abbreviations: ACN, acyl carbon number; DB, double bond; GIP, glucose-dependent insulinotropic polypeptide; TAG, triacylglycerol.
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for understanding the effects of fatty acid positional distribution on human lipid metabolism. In this study, a newly developed tandem mass spectrometric method (15–17) was used to determine the regioisomers of chylomicron and VLDL TAGs isolated from postprandially collected blood samples. Two fat blends with identical fatty acid compositions but different positional distributions of fatty acids were incorporated into an oral fat load and served to healthy female subjects in order to study the effects of TAG structure on chylomicron and VLDL TAG structures, the absorption of the fats, and their clearance from circulation. The study produced detailed molecular level information that is impossible or highly laborious to determine with traditional enzymatic methods. To our knowledge, information of individual molecular species of human lipoprotein TAGs during postprandial lipemia has not been published before.

MATERIALS AND METHODS

Study design

The study had a randomized double-blind cross-over design. Postprandial responses to two meals containing either palm oil or transesterified palm oil were measured over 6 h on two occasions 4 weeks apart.

Subjects

Eleven female volunteers were recruited from the staff and student population of the University of Kuopio, Finland. The subjects recruited met the following criteria: age 18–45 years, body mass index 18.5–25 kg/m^2; fasting values of serum total cholesterol <5.5 mmol/l, LDL cholesterol <3.5 mmol/l, HDL cholesterol 0.8–2 mmol/l (at least one-fifth of total cholesterol), serum TAGs <2 mmol/l, plasma glucose 4.2–6 mmol/l, blood pressure <140/90 mmHg, hemoglobin >120 g/l; and normal liver, kidney, and thyroid functions. The age of volunteers was 26.9 ± 2.56 years (mean ± SD) and the body mass index was 20.5 ± 1.81 before the test with palm oil and 20.6 ± 1.88 before the test with transesterified palm oil. Similarly, fasting serum lipids (millimoles) before palm oil treatment and transesterified palm oil treatment were 3.96 ± 0.59 and 3.93 ± 0.46 for total cholesterol, 2.23 ± 0.39 and 2.24 ± 0.32 for LDL cholesterol, 1.48 ± 0.30 and 1.49 ± 0.24 for HDL cholesterol, and 0.67 ± 0.32 and 0.76 ± 0.35 for TAGs, respectively. There were no statistical differences in the fasting values between the treatments. Four volunteers used oral contraceptives, and the subjects were asked to keep their medication unchanged during the study. The volunteers were at the same stage of their menstruation cycle during both tests. Each subject provided a written consent for the study, and they were free to discontinue their participation in the study at any point without explanation. The study plan was approved by the Ethics Committee of the University of Kuopio and Kuopio University Hospital.

One volunteer did not participate in the second visit of the experiment for personal reasons, and all of her results were excluded. Due to a technical reason, the 6-h sample from one volunteer during the test with transesterified fat was missed, and both of her 6-h samples were omitted when incremental areas were calculated.

The subjects were asked to fast overnight (14 h) and advised not to consume alcohol nor engage in strenuous exercise for 5 days before the test. The subjects kept food diaries from Wednes-

day to Saturday the week before the test week, and they were advised to eat as habitual. The energy percentages from fat, protein, and carbohydrate calculated from the 4-day food records with Micronutrica software (Version 2.5) (18) did not differ between the treatments, but the percentage of total energy from saturated fat was greater before the test with palm oil than before the test with transesterified palm oil ($P = 0.031$).

Oral fat load

A palm oil fraction was obtained from an oil processing plant of Raisio Group (Raisio, Finland). The fraction was used as the experimental fat both as such and after transesterification. The two fats had identical fatty acid compositions (**Table 1**), but their positional distribution was different (**Table 2**). The different positional distributions influenced the melting qualities of the fats, because at 20°C transesterified palm oil contained 17.8% solid fat but palm oil was totally melted, as measured by pulse nuclear magnetic resonance (Bruker Minispec NMS-100, Germany). However, both fats were liquid at body temperature.

The amount of fat in the test meal was 55 g per body square meter area according to the Dubois body surface chart. The fat was melted in a microwave oven and blended with UHT-treated skim milk low in lactose so that the fat percentage of the resulting mixture was 30. Vanillin (0.05 g) (Merck, Darmstad, Ger-

TABLE 1. Fatty acid compositions of palm oil and transesterified palm oil

Fatty Acid	Palm Oil	Transesterified Oil
12:0	0.7	0.7
14:0	1.1	1.1
16:0	32.8	32.8
16:1(n-7)	0.3	0.3
18:0	4.4	4.4
18:1(n-9)	45.7	46.0
18:1(n-7)	0.9	1.0
18:2(n-6)	12.9	12.6
18:3(n-3)	0.3	0.2
18:4(n-3)	0.0	0.1
20:0	0.4	0.4
20:1(n-9)	0.2	0.2
22:0	0.1	0.1
Others	0.1	0.3

Values are given as molar proportions.

TABLE 2. Major regioisomers of palm oil and transesterified palm oil analyzed by tandem mass spectrometry

Triacylglycerol	Palm Oil	Transesterified Oil
sn-16:0-18:1-18:1 + sn-18:1-18:1-16:0	33.7	18.1
sn-16:0-18:1-16:0	11.8	5.4
sn-16:0-18:2-16:0	7.5	1.4
sn-16:0-18:2-18:1 + sn-18:1-18:2-16:0	7.3	3.4
sn-16:0-18:1-18:2 + sn-18:2-18:1-16:0	5.4	4.4
sn-18:1-16:0-16:0 + sn-16:0-16:0-18:1	4.4	13.2
sn-18:2-16:0-16:0 + sn-16:0-16:0-18:2	2.1	3.2
sn-16:0-18:2-18:2 + sn-18:2-18:2-16:0	2.0	1.7
sn-18:1-16:0-18:1	1.3	10.2
sn-18:1-16:0-18:2 + sn-18:2-16:0-18:1	1.1	3.9
sn-14:0-18:1-18:1 + sn-18:1-18:1-14:0	0.7	0.4
sn-18:2-16:0-18:2	0.4	0.4
sn-18:1-14:0-18:1	0.2	0.7
Others	22.0	33.5

Values given as molar percentages of total fat.

many) and 0.5 g sweetener with 3% aspartame and 97% malto-dextrin (G.D. Searle & Company Ltd, France) were added to the blend. The blend was rapidly cooled to $10-15°C$. Five minutes before ingestion of the test meal, a rice cake (7.3 g) topped with low fat cheese [10 g cheese (12% fat, 7% saturated fat)/70 kg body weight; Valio Ltd., Finland] was served. Fat from the cheese accounted for 1–2% of total fat.

Laboratory methods

On both mornings of the study, a cannula was inserted into an antecubital vein for blood collection. After the fasting blood samples were taken, the subjects ate the rice cake with cheese and 0.5 dl of water. Five min after ingesting the rice cake with cheese, the subjects were asked to take the liquid meal (0 min) in 5 min. Another 0.5 dl of water was also provided. Blood samples for TAG, cholesterol, glucose, insulin, and free fatty acid measurements were collected at 20-min intervals during the first hour, at 30-min intervals during the second hour, and hourly thereafter up to 6 h. After the first 2 h, the subjects were provided water ad libitum.

The isolation of chylomicrons and VLDL from plasma and precipitation of LDL was performed as previously described (19). Cholesterol and TAG concentrations from chylomicron and VLDL-rich fractions and plasma were determined by enzymatic colorimetric methods (Monotest Cholesterol and Triglyceride GPO-PAP; Boehringer Mannheim, Germany) using an automated instrument (Kone Specific Clinical Analyzer, Kone Ltd, Espoo, Finland). Free fatty acids in serum were determined by a turbidometric method and analyzed with the Kone Specific Clinical Analyzer. Plasma insulin was measured by a radioimmunoassay method (Phadeseph Insulin RIA 100; Pharmacia Diagnostics, Uppsala, Sweden). Plasma glucose was analyzed using a glucose oxidase method (Glucose Auto&Stat model GA-110; Daiichi, Kyoto, Japan). Incremental areas under the response curves for TAGs and cholesterol in chylomicron, VLDL, and serum samples and for plasma free fatty acids were calculated using Canvas™ software (Version 6; Deneba Software Inc, Miami, FL).

Lipids from the chylomicron samples collected at 90 min to 6 h postprandially were extracted, and TAGs were separated from the extracted lipid mixture as described elsewhere (20, 21). Fatty acid methyl esters were prepared from the TAG fraction as described elsewhere (22). The fatty acid methyl esters were dissolved in hexane and analyzed twice by gas chromatography (Perkin Elmer AutoSystem, Norwalk, CT) on a WCOT column (NB-351, 25 m × 0.32 mm i.d., 0.20 μm film thickness, Nordion, Finland).

The molecular weight distributions of the TAGs of the test fats and the TAGs extracted from the 3-h chylomicron samples and 4-h VLDL samples were determined by ammonia negative ion chemical ionization with a triple quadrupole tandem mass spectrometer (TSQ-700; Finnigan MAT, San Jose, CA) (16). The sample was introduced into the ion source with a direct exposure probe. Chemical ionization with ammonia resulted in the formation of deprotonated TAG ions [M-H]⁻, which were analyzed by scanning the mass range from m/z 500 to 1,000. The combined number of acyl carbons and double bonds in the acyl chains of TAGs were calculated according to the m/z values of the [M-H]⁻ ions. Relative molar proportions of different molecular weight species were calculated using the [M-H]⁻ ion abundancies. The amount of naturally occuring ^{13}C was taken into account when calculating the proportions of TAGs. The analysis parameters were set according to the optimization carried out earlier in our laboratory (23). Each sample was analyzed in quadruplicate.

TAG regioisomerism was determined with a tandem mass spectrometric (TSQ-700, Finnigan MAT) analysis based on negative ion chemical ionization and collision-induced dissociation with argon gas (15, 24). The results were calculated by the TAGs-100 program (Nutrifen, Turku, Finland) (17). The regioisomers of the test fats and the most abundant molecular weight species of the TAGs extracted from the chylomicron samples at 3 h postprandially and from the VLDL samples at 4 h postprandially were determined in quadruplicate. The following molecular weight species were analyzed: ACN:DB (acyl carbon number: number of double bonds) 50:1, 52:2 and 52:3 from the chylomicron TAGs and ACN:DB 52:2 and 52:3 from the VLDL TAGs.

Statistical methods

Normal distribution of the data was tested. A paired t-test was used on the dietary data and on fasting plasma values before treatments. The Wilcoxon matched pairs signed ranks test was used when the areas of total TAGs, chylomicron TAGs, chylomicron cholesterol, VLDL TAGs, and VLDL cholesterol were compared, and paired samples t-test was used when areas of free fatty acids and slopes of TAG curves were compared. The proportions of palmitic, palmitoleic, stearic, oleic, and linoleic acids in chylomicron TAGs at different time points were compared by ANOVA for repeated measures. Each time point was compared with the one preceding it. Proportions of palmitic, palmitoleic, stearic, oleic, and linoleic acids in chylomicron TAGs at corresponding time points between the treatments were compared using the paired t-test. Proportions of different molecular weight classes and regioisomers in test fat, chylomicron, and VLDL were compared using ANOVA and, as post hoc tests, Tukey's test when

TABLE 3. Fatty acids of chylomicron triacylglycerols

|	Meal	90 min	2 h	3 h	4 h	5 h	6 h
After palm oil meal							
16:0	32.8	27.7 ± 1.5	29.5 ± 0.8**	28.4 ± 2.3	30.0 ± 1.4*	29.7 ± 1.1	30.3 ± 1.6
16:1(n-7)	0.3	1.2 ± 0.6	1.0 ± 0.5	0.6 ± 0.5	0.4 ± 0.3	0.3 ± 0.3	0.2 ± 0.2
18:0	4.4	4.0 ± 0.5	4.0 ± 0.4	4.1 ± 0.4	4.2 ± 0.2	4.2 ± 0.3	4.2 ± 0.3
18:1(n-9)	45.7	41.7 ± 2.8	44.8 ± 1.4**	44.7 ± 1.9	45.8 ± 1.2	45.8 ± 1.1	45.1 ± 1.5
18:2(n-6)	12.9	13.5 ± 1.2	13.6 ± 0.8	13.4 ± 1.7	12.3 ± 0.6	12.1 ± 0.5	12.0 ± 0.7
After transesterified oil meal							
16:0	32.8	28.6 ± 2.1	29.6 ± 1.2	28.8 ± 1.8	29.6 ± 1.1	29.9 ± 1.3	30.6 ± 1.2
16:1(n-7)	0.3	1.3 ± 0.8	1.1 ± 0.7	0.7 ± 0.6	0.6 ± 0.4	0.6 ± 0.4	0.3 ± 0.4
18:0	4.4	4.0 ± 0.3	4.1 ± 0.2*	4.2 ± 0.3	4.1 ± 0.2	4.1 ± 0.2	4.2 ± 0.2
18:1(n-9)	46.0	41.6 ± 2.1	44.1 ± 1.5*	43.5 ± 2.1	45.1 ± 1.2	45.2 ± 1.5	44.7 ± 1.0
18:2(n-6)	12.6	12.9 ± 1.0	13.0 ± 0.6	12.5 ± 1.6	12.5 ± 0.6	12.2 ± 0.5	12.0 ± 0.5

Values are given as mean percentages ± SD. Data marked with asterisks differ from the preceding time point (* $P < 0.05$, ** $P < 0.005$).

variances were homogeneous and Tamhane's test when variances were not homogeneous. ACN:DB classes and regioisomers of VLDL after the palm oil meal and transesterified oil meal were compared using Student's *t*-test. All procedures were carried out using the SPSS-PC$^+$ statistical package (Version 10; SPSS Inc., Chicago, IL).

RESULTS

The proportions of major fatty acids in chylomicron TAGs were similar after the two meals and during the course

of postprandial lipemia (**Table 3**). Palmitic acid was not fully absorbed from either of the test fats: the fats given to the subjects contained 32.8% palmitic acid, but of the fatty acids in chylomicrons (based on the mean of six time points), only 29.3% and 29.5% were palmitic acid after the palm oil and transesterified oil meals, respectively. The fatty acid composition of chylomicron TAGs at 90 min after the ingestion of the fat was, of the time points studied, least comparable to that of ingested fat. This was especially apparent for oleic acid. There was a decreasing trend in the proportion of palmitoleic acid dur-
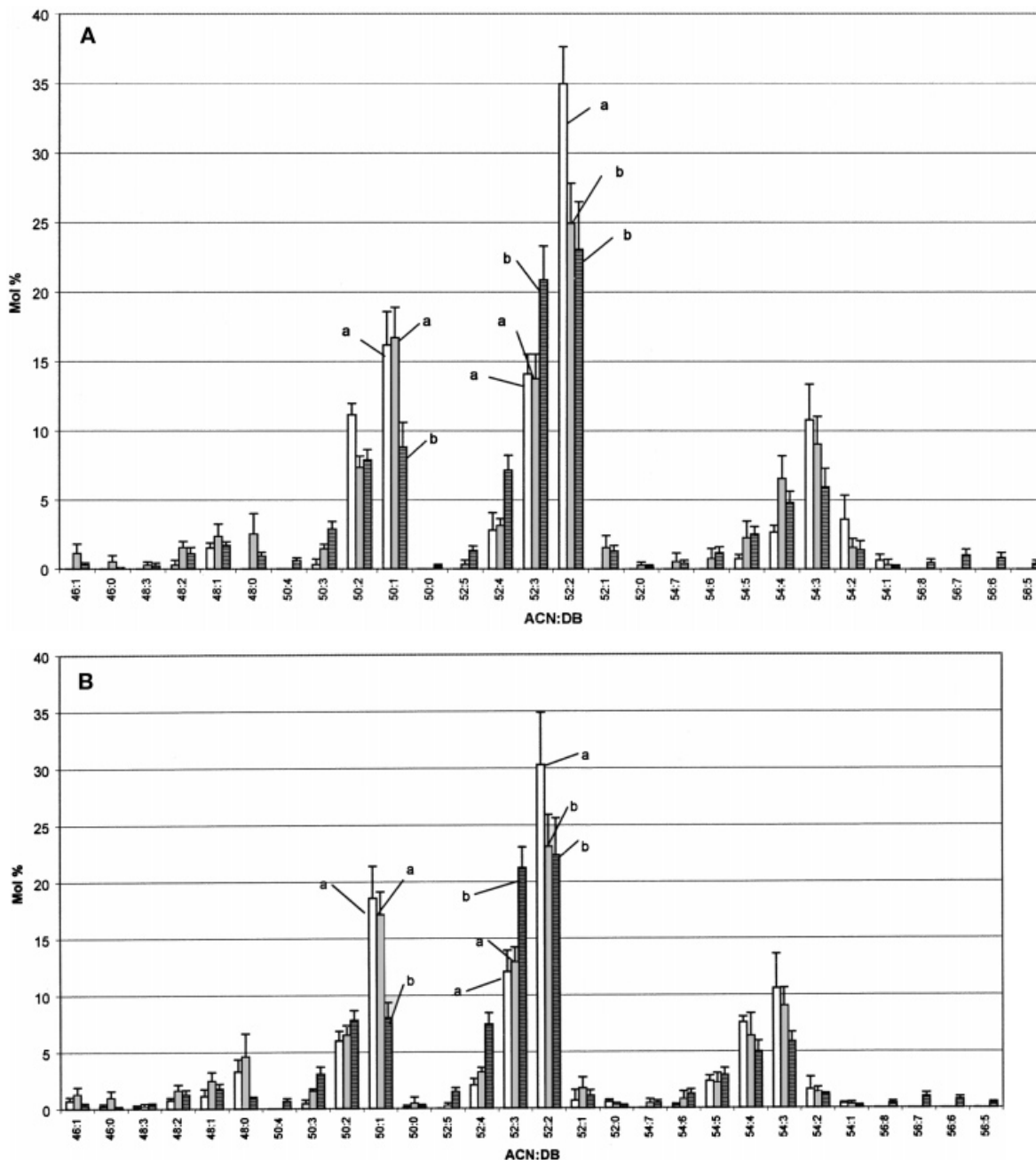
Fig. 1. Molecular weight distributions (mean and SD) of test oil TAGs (open bars), 3–h chylomicron TAGs (light gray bars), and 4-h VLDL TAGs (dark gray bars) in palm oil treatment (A) and transesterified palm oil treatment (B). Data marked with different letters are statistically different ($P < 0.05$) in ACN:DB fractions 50:1, 52:3, and 52:2.

ing the course of postprandial lipemia, but the within-subject variation was large, and no time points were statistically significantly different from the preceding ones. The only statistically significant difference in the proportions of palmitic, stearic, oleic, and linoleic acids after the two fat loads was the significantly larger (13.6% vs. 13.0%, $P = 0.016$) amount of linoleic acid after the palm oil meal than after the transesterified oil meal at the 2-h time point.

The molecular weight distributions of the test fats and the TAGs extracted from the chylomicron samples (3 h post-prandially) and from the VLDL samples (4 h postpran-dially) are displayed in **Fig. 1**. The proportion of TAGs with two oleic acid residues and one palmitic acid residue (ACN:DB 52:2) decreased from 35% in palm oil to 25% in chylomicron TAGs ($P < 0.001$) and similarly from 30% to 23% ($P = 0.004$) in the treatment with transesterified oil. The proportions of the two other major molecular weight species, ACN:DB 50:1 (one oleic acid and two palmitic acid residues) and 52:3 (one palmitic, one oleic, and one linoleic acid residue), were similar in test fats and in chylomicrons in both treatments. TAG molecular weight species absent from the test fats or chylomicrons were detected in VLDL.

The regioisomers of chylomicron TAGs after the two fats were different and resembled closely the isomers of the appropriate fats served (**Table 4**). In spite of the large variability of the data, the content of both dioleoylpal-mitoleoylglycerol regioisomers was increased in chylomi-crons compared to the fats ingested, indicating the presence of endogenous palmitoleic acid in chylomicron TAGs. Of the analyzed regioisomers, 79% of the regioisomers in

palm oil and 84% of the regioisomers in transesterified oil were found in chylomicron TAGs 3 h after the meal. There were no significant differences in the structures of the VLDL TAGs between the treatments, except that the proportion of the regioisomer sn-16:0-18:1-18:2 + sn-18:2-18:1-16:0 was significantly higher ($P = 0.041$) after the palm oil than after the transesterified oil.

The mean postprandial lipemic responses after both treatments are shown in **Table 5** and **Fig. 2**. Contrary to expectations, the incremental area of TAGs in serum was significantly greater after the palm oil than after the trans-esterified palm oil ($P = 0.047$). The chylomicron TAGs tended to be greater after the palm oil than after the transesterified oil ($P = 0.074$). The slope of the TAG curve 40 to 120 min postprandially was steeper after palm oil than after transesterified oil ($P = 0.006$). Insulin, glu-cose, and cholesterol responses expressed as incremental areas or incremental areas under the free fatty acid re-sponse 0 to 120 min postprandially did not differ between the treatments. The insulin value peaked at the 90-min time point after palm oil and at the 60-min time point after transesterified oil. The responses for glucose and cholesterol fluctuated within a narrow range during the course of postprandial lipemia.

DISCUSSION

In this study, the effects of fatty acid positions in TAGs of palm oil and transesterified palm oil on TAG structures in chylomicrons and VLDL and on postprandial lipemia

TABLE 4. Regioisomers of the test fat, chylomicron (3 h after the meal), and VLDL (4 h after the meal) triacylglycerols

	Palm Oil	Palm Oil Chylomicron	Palm Oil VLDL	Transesterified Oil	Transesterified Oil Chylomicron	Transesterified Oil VLDL
50:1	16.2 ± 2.4^a	16.7 ± 2.2^a	8.8 ± 1.8^b	18.5 ± 2.7^a	17.1 ± 2.0^a	8.0 ± 1.3^b
16:0/16:0/18:1						
sn-16:0-16:0-18:1 + sn-18:1-16:0-16:0	27.4 ± 4.8	34.3 ± 5.5		70.9 ± 4.1	67.9 ± 5.6	
sn-16:0-18:1-16:0	72.6 ± 4.8	65.7 ± 5.5		29.2 ± 4.1	32.3 ± 5.5	
52:3	14.1 ± 1.4^a	13.7 ± 1.8^a	20.9 ± 2.4^b	12.0 ± 1.9^a	12.9 ± 1.3^a	21.2 ± 1.8^b
16:0/18:2/18:1						
sn-16:0-18:2-18:1 + sn-18:1-18:2-16:0	51.7 ± 4.9	44.7 ± 7.6	49.3 ± 12.9	28.3 ± 3.1^a	35.7 ± 4.4^b	62.5 ± 16.2^c
sn-16:0-18:1-18:2 + sn-18:2-18:1-16:0	38.6 ± 3.2	35.6 ± 6.0	$38.5 \pm 12.2*$	36.5 ± 2.8	34.4 ± 6.4	$25.4 \pm 14.4*$
sn-18:2-16:0-18:1 + sn-18:1-16:0-18:2	$7.7 \pm 4.6^{a,b}$	12.8 ± 6.1^a	4.8 ± 4.2^b	32.5 ± 3.2^a	23.0 ± 8.5^a	5.0 ± 4.3^b
16:1/18:1/18:1						
sn-18:1-18:1-16:1 + sn-16:1-18:1-18:1	1.6 ± 1.2^a	$3.9 \pm 1.2^{a,b}$	5.5 ± 3.2^b	1.6 ± 1.8^a	$4.2 \pm 20^{a,b}$	6.0 ± 3.4^b
sn-18:1-16:1-18:1	0.4 ± 0.8^a	3.2 ± 1.4^b	$1.8 \pm 1.7^{a,b}$	1.3 ± 1.1	2.8 ± 2.4	1.1 ± 1.3
52:2	35.0 ± 2.7^a	24.9 ± 2.9^b	23.1 ± 3.4^b	30.2 ± 4.5^a	23.1 ± 2.8^b	22.4 ± 3.3^b
16:0/18:1/18:1						
sn-16:0-18:1-18:1 + sn-18:1-18:1-16:0	96.3 ± 2.5	90.8 ± 3.9	96.9 ± 4.2	59.6 ± 8.2^a	77.1 ± 4.2^a	94.6 ± 3.8^b
sn-18:1-16:0-18:1	$3.7 \pm 2.5^{a,b}$	8.9 ± 3.9^a	2.9 ± 3.5^b	33.8 ± 8.4^a	22.9 ± 4.2^a	5.2 ± 3.6^b
16:0/18:2/18:0						
sn-16:0-18:2-18:0 + sn-18:0-18:2-16:0				0.8 ± 1.6		0.1 ± 0.3
sn-18:2-16:0-18:0 + sn-18:0-16:0-18:2			0.3 ± 0.8	3.4 ± 2.4^a		0.1 ± 0.4^b
sn-16:0-18:0-18:2 + sn-18:2-18:0-16:0				2.6 ± 1.9^a		0.1 ± 0.2^b

Data marked with different letters are statistically different in each treatment. The VLDL and ACN:DB species marked with asterisks differ from the same regioisomer or triacylglycerol sizes in the other treatment ($P < 0.05$). Standard deviations are analytical in test fats and between subjects in chylomicron and VLDL.

TABLE 5. Triacylglycerol and cholesterol responses to palm oil and transesterified palm oil expressed as incremental area under the response curves

	Palm Oil	Transesterified Palm Oil
	mmol/l	
Triacylglycerols		
Total	2.33 ± 1.20^{a}	1.77 ± 0.53^{b}
Chylomicron	1.51 ± 0.72	1.09 ± 0.32
VLDL	0.63 ± 0.41	0.61 ± 0.33
Cholesterol		
Chylomicron	0.29 ± 0.27	0.17 ± 0.10
VLDL	0.19 ± 0.13	0.23 ± 0.13

Values given as mean ± SD. Data marked with different letters in each row differ from each other in statistical terms ($P < 0.05$).

were investigated. Such detailed information would have been impossible to achieve from the physiological samples by traditional enzymatic methods. Fatty acid composition only, on the other hand, fails to provide information of the positional distribution of fatty acids in TAGs, when fatty acids in different positions are metabolized differently by lipases in the digestive tract and human plasma.

Food records revealed that the percentage of total energy from saturated fat was greater before the test with palm oil than before the test with transesterified palm oil. This result was obviously undesired. In a recent study, the postprandial plasma TAG concentrations were higher after a diet rich in monounsaturated fatty acids than after a diet rich in saturated fatty acids, but no differences were found in the TAG-rich lipoprotein fraction (25), whereas in our study, the TAG concentration was higher after the meal associated with larger dietary saturated fat values. Furthermore, there were 1 to 3 days in between the last

day of food record keeping and the oral fat loads. Taken together, our belief is that the results of this study cannot be explained by the difference in the amounts of saturated fat recorded in the food diaries, although its influence cannot be excluded.

Dissimilar behavior was observed in TAGs with different fatty acid combinations. Unlike the TAGs with ACN:DB 50:1 and 52:3, the proportion of TAGs with two oleic acid residues and one palmitic acid residue (ACN:DB 52:2) dropped significantly. The lower production of ACN:DB 52:2 postprandially may indicate the synthesis of endogenous TAGs other than ACN:DB 52:2 via the glycerol-3-phosphate pathway.

The similarities of the positional distributions of fatty acids in the original oils and in chylomicron TAGs at 3 h postprandially are explained by the absorption of 2-monoacylglycerols from the diet. In palm oil and in transesterified oil, 79% and 84%, respectively, of the analyzed TAGs had identical regioisomerism with the respective chylomicron TAGs 3 h after the meal. The dilution of original positional distribution by endogenous fatty acids has been reported previously (26, 27). Our finding is in agreement with the estimated magnitude of the glycerol-3-phosphate pathway (28, 29) but suggests that the fatty acid positional distribution of dietary fat might have an effect on the magnitude of the glycerol-3-phosphate pathway. It is also possible that TAGs similar to transesterified palm oil are formed by the glycerol-3-phosphate pathway in greater amounts than TAGs similar to palm oil. Only the seven most abundant regioisomers were studied. Whether the results apply to the molecular weight species found in small quantities in chylomicrons is not known.

The positional distribution of fatty acids in the meal was not seen in the VLDL TAGs 4 h postprandially, thus re-
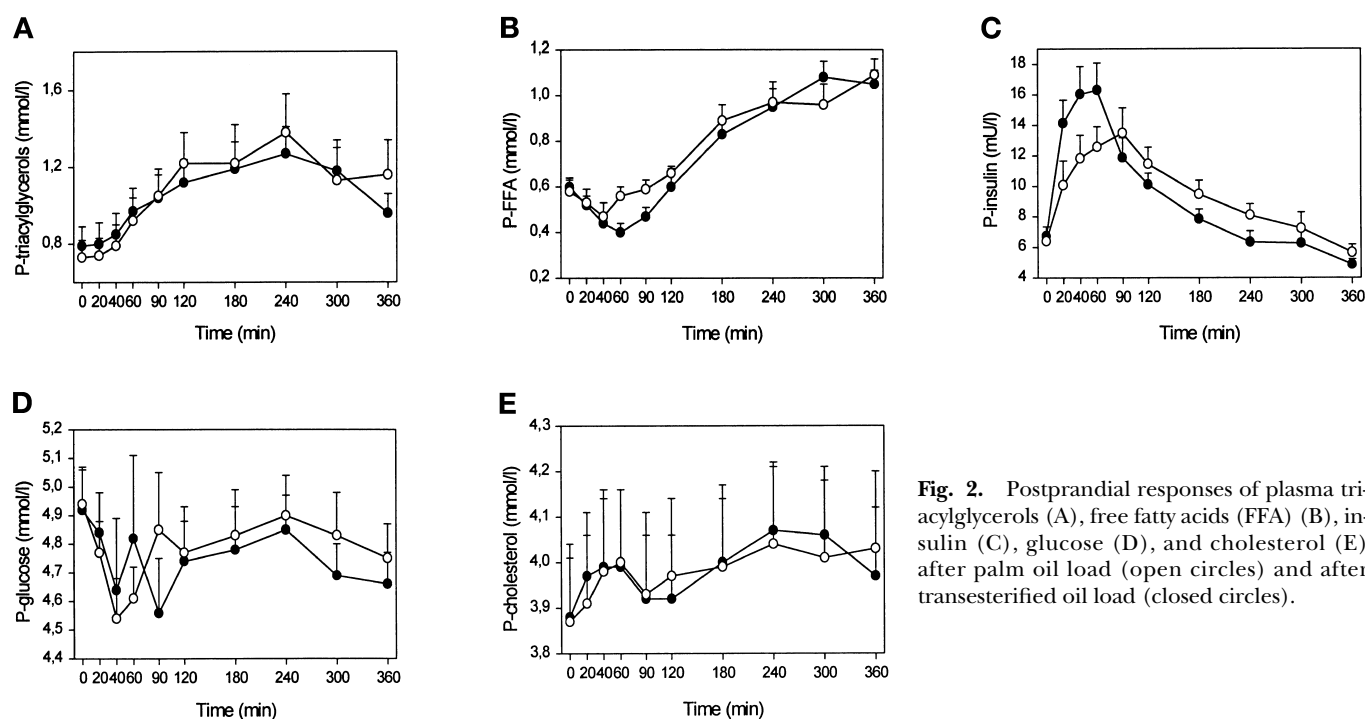
Fig. 2. Postprandial responses of plasma triacylglycerols (A), free fatty acids (FFA) (B), insulin (C), glucose (D), and cholesterol (E) after palm oil load (open circles) and after transesterified oil load (closed circles).

flecting the synthesis of TAGs in the liver via the glycerol-3-phosphate pathway. However, it is possible that the composition of fatty acids in the diet influences VLDL TAG structures over the long term, because the VLDL TAGs of people on a diet rich in olive oil have been shown to contain more triolein than the VLDL TAGs of people consuming traditional Western diets (14). Zock, Gerritsen, and Katan (30) found a greater quantity of palmitic acid in fasting plasma TAGs after a diet rich in palmitic acid in the *sn*-2 position than after a diet rich in palmitic acid in the *sn*-1 and *sn*-3 positions. In the present study, we could not confirm the possible transfer of lipids between chylomicrons and other lipoproteins, as suggested by Zock, Gerritsen, and Katan. However, the latter observations were made after the subjects had been on the studied diet for several weeks.

The proportion of palmitic acid was lower in chylomicrons than in the fats ingested, but the proportions were similar after both oral fat loads, which indicates that there were no differences in the absolute amounts of palmitic acid absorbed despite the differences in the positional distribution of fatty acids. In addition to incomplete absorption, it is possible that the endogenous contribution of fatty acids other than palmitic acid was larger than that of palmitic acid. The statistically significant difference of linoleic acid at one time point after the two fat loads is not likely to have physiological importance.

Contrary to what was expected, fat with palmitic acid predominantly in the *sn*-1/3 (palm oil) positions caused a larger incremental area of total TAGs in plasma than did fat with palmitic acid randomly distributed to all of the *sn* positions. The amount of TAGs in chylomicrons tended to be greater as well. The difference was, however, quite small and possibly influenced by the 360-min time point. Unfortunately, most of the subjects did not reach fasting values during the observed time period; it would have been interesting to follow the plasma TAGs longer. Although the incremental areas for insulin, glucose, or cholesterol were not significantly different between the treatments, more insulin was secreted at the beginning of the postprandial response after the meal with transesterified palm oil than after the meal with palm oil. The lower insulin excretion after palm oil could explain the behavior of free fatty acid and TAG concentrations in plasma. Because lingual and gastric lipases have affinity to the short chain and polyunsaturated fatty acids of the *sn*-3 position, it is possible that the transesterified fat may be hydrolyzed faster, causing more enhanced glucose-dependent insulinotropic polypeptide (GIP) and insulin secretion than palm oil. The GIP secretion is very sensitive to acute and chronic changes in dietary lipid content (31). The differences observed might also be caused by the different melting characteristics of the fats, because transesterified oil contained TAGs with a higher melting point than the native palm oil. However, both fats were liquid at body temperature. These questions are important because postprandial TAG concentrations have been suggested to be important factors in the development of coronary artery disease (8, 9).

Fatty acid positional distribution may cause differences in lipid metabolism beyond the postprandial state. In rats, the amounts of arachidonic and palmitoleic acids in plasma have been noted to be related to the amounts of linoleic and palmitic acids, respectively, in the *sn*-2 position of dietary TAGs (32). Diets containing palmitic acid in the *sn*-2 position have caused higher plasma TAG values than diets containing palmitic acid in the *sn*-1/3 positions both in rats (32) and in human infants (33). However, no significant differences were found in one adult trial (27), whereas another study (34) reported larger LDL cholesterol concentrations caused by diets including palmitic acid in the *sn*-2 position in men but not in women.

The tandem mass spectrometric analysis applied to the study of positional distribution of dietary fat, chylomicron TAGs, and VLDL TAGs provided detailed information about human lipoprotein TAG structures. Despite clear differences in fatty acid positional distribution in both test fats and chylomicron TAGs, similar structures of VLDL TAGs were seen after the two meals. The relationship of these findings to postprandial lipemia and long-term lipid metabolism requires further research. ■

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