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Regio-distribution of stearic acid is not conserved in chylomicrons after ingestion of randomised, stearic acid-rich fat in a single meal

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Abstract

Stearic acid from conventional food is well absorbed, but the fate of synthetic randomized stearic acid in fat absorption and subsequent metabolism is unclear. In this study, we examined the postprandial triglyceridemia following an ingestion of randomized stearic acid-rich fat. Following a 12-h fast, nine healthy young males ate a hamburger meal with 16.7 g of stearic acid (30% in triacylglycerol (TAG) *sn*-2 position, fully randomized). Postprandial blood samples were collected for 450 min, and the stearic acid content in chylomicron (CM, Svedberg flotation rate >400) TAG and the proportion of stearic acid in the *sn*-2 position were measured by tandem mass spectrometry at peak (180 min) and late (360 min) triglyceridemia. Of all stearic acid in CM TAG, 23% and 22% were in the *sn*-2 position at peak and late triglyceridemia ($P<.004$ and $P<.001$, respectively). This suggests a 68% and 62% conservation of *sn*-2 stearic acid, respectively. Peak postprandial TAG concentration and incremental area under the TAG curve showed a higher correlation with the fasting CM TAG ($r=0.88$, $P<.01$ and $r=0.72$, $P<.05$, respectively) than with total fasting plasma TAG ($r=0.73$, $P<.05$ and $r=0.24$, nonsignificant, respectively). In an earlier study, we showed that the absorption efficiency of the stearic acid of the meal was normal, with only marginal amounts of mainly *sn*-1,3 stearic acid found in the feces. In conclusion, we showed that *sn*-2 stearic acid is underrepresented in the postprandial CM TAG following an ingestion of fully randomized fat.

Keywords: Randomized fat; Stearic acid; Regioisomerism

1. Introduction

Random interesterification is a technique in which the fatty acids (FAs) of a triacylglycerol (TAG) are randomly rearranged on the glycerol backbone in the presence of a chemical catalyst or an enzyme, usually to generate higher melting-point TAGs. In this process, the FAs are equally distributed among the three possible positions (*sn*-1,2,3) [1]. Interesterified fats are increasingly used to replace hydrogenated fats rich in *trans*-FAs, which are associated with risk of coronary heart disease [2,3], while still maintaining physical properties, taste and stability. Cholesterol-neutral stearic acid [4] is the preferred saturated FA (SFA) to be used in interesterification.

Interesterification usually increases the proportion of SFAs in the *sn*-2 position, generating fats similar to animal fats. SFAs in the *sn*-2 position may be absorbed more efficiently [5] and delay intestinal secretion of chylomicron (CM) or the lipolysis of circulating CM TAG [6], resulting in increased and prolonged postprandial triglyceridemia, an important risk factor for atherosclerotic events [7,8]. However, clinical trials with interesterified fats have resulted in decreased triglyceridemia [9–11], a phenomenon which has recently been related to the increased melting point that retards the absorption but does not increase the excretion of fats with high *sn*-2 SFA content [12].

In postprandial plasma CMs, the concentration of *sn*-2 SFA has not been shown to increase after ingestion of interesterified fats [10,12]. However, these measurements were from the peak triglyceridemia (180 min) [10] or from pooled samples from 2 to 6 h after the test meal [12]. Because of the slower absorption and enterocyte secretion

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of *sn*-2-SFA [10,13], as well as their reduced clearance rate from circulation, it is possible that later plasma samples from the late postprandial phase would reveal increases in *sn*-2 SFA in CM TAG. No data are currently available on the relative amount of *sn*-2 SFAs in circulation at peak and late postprandial triglyceridemia after ingestion of interesterified fat.

We have previously shown that stearic acid from an interesterified short- and long-acyl-chain TAG is well absorbed with only marginal fecal stearic acid losses almost exclusively from the *sn*-1 and *sn*-3 positions [14]. The aim of the present study was to estimate the amount of *sn*-2 stearic acid in circulation at peak and late postprandial triglyceridemia by measuring the relative concentration of *sn*-2 stearic acid in circulating CM TAG at 180 and 360 min after ingestion of a hamburger meal containing interesterified fat.

2. Methods and materials

The study protocol was approved by the Ethical Committee of the University Hospital of Turku, Finland, and informed consent was obtained from each participant.

2.1. Subjects

Healthy young males were recruited from among University of Turku students. Subjects were 20.4 years old (standard deviation 2.3) and had a body mass index 21.3 (0.7) kg/m^2, plasma TAG 0.8 (0.3) mmol/L, total cholesterol 3.7 (0.3) mmol/L, low-density lipoprotein 2.0 (0.4) mmol/L and high-density lipoprotein 1.4 (0.2) mmol/L. The exclusion criteria included a family history of cardiovascular disease or diabetes, current use of prescription drugs and an immoderate consumption of alcohol. Ten subjects were recruited, but one of them withdrew for personal reasons not related to the study.

2.2. Study design

On the day preceding the postprandial test, the subjects were asked to refrain from strenuous exercise. All food was provided [protein 19 percent energy (E%), carbohydrates 44 E%, fat 37 E%], but drinking was not controlled, except for banning alcohol. The subjects arrived at the trial site at 8 a.m. after a 12-h fast. Adherence to the instructions was checked verbally, an intravenous cannula was placed in a forearm vein, and a basal blood sample was drawn (0 min sample).

The test meal was consumed within 10 min, after which the subjects abstained from all food (water allowed). Additional blood samples were taken at 60, 90, 180, 270, 360 and 450 min. Subjects were requested to remain sedentary, except for visits to the rest room. A standardised meal was served after the last blood sample, and the subjects were then free to leave with the instruction to continue the provided diet until the end of the day. The subjects collected fecal samples from 8 a.m. on the test day for a maximum of 96 h for measurement of fecal fat and FA excretion (results published earlier [14]).

2.3. Test meal

The test meal consisted of a hamburger (Hesburger, Turku, Finland) with 3.8 g stearic acid combined with stearic acid-rich test fat. The test fat consisted of 30 g of a fully randomised mixture of long-chain and short-chain FAs (SALATRIM) (Benefat B-R, Danisco, Copenhagen, Denmark). The dose contained 16.7 g stearic acid, 4.7 g other long-chain FAs, and 5.3 g of short-chain acetic acid. This manufactured fat was chosen because it combines a very high stearic acid content with good emulsification properties necessary for normal enzymatic processing in the intestinal lumen [15].

Representative samples of the meal were pooled, homogenised and freeze-dried for subsequent protein, carbohydrate, and fat analyses. Protein was analysed by the Kjeldahl method using correction factor 6.25. Available carbohydrate was analysed from a defatted sample with an enzymatic kit (Megazymes, Bray, Ireland). Lipids were extracted from a lyophilised meal sample using Soxhlett and chloroform for 5 h. Internal standard (glyceryl triheptadecanoate, Sigma, St. Louis, MO, USA) for quantification was added to the extract and to a sample of SALATRIM. The long-chain FA composition was analysed by methylation by boron trifluoride [16], and the methyl esters were analysed by gas chromatograph–flame ionisation detector (Perkin-Elmer Autosystem, Boston, MA, USA) with a J&W DB23 column (60 m×0.32 mm internal diameter, 0.25 μm film (Agilent, Folsom, CA, USA) [11]. The *sn*-2 FA composition of the test meal was analysed by boron trifluoride methylation of monoacylglycerols (MAGs) from pancreatic lipase treatment [17].

2.4. Biochemical measurements

Plasma and CM TAG and glucose were determined by enzymatic colorimetric methods (Roche 12 Diagnostics, Mannheim, Germany) using an automated instrument (Hitachi 917 Automatic Analyser, Hitachi, Tokyo, Japan). Plasma insulin was measured using time-resolved fluoroimmunoassay (Wallac, Turku, Finland). The incremental area under the curve (iAUC) was calculated using the trapezoidal method, and expressed as pmol∗h/L (insulin) or mmol∗h/L (glucose and TAG).

Blood samples taken 180 and 360 min after the meal (peak and late postprandial triglyceridemia, respectively) were subjected to more detailed analysis. Plasma was immediately separated from blood samples by centrifugation. CM lipoproteins [Svedberg flotation (S_f) >400] were isolated from plasma by ultracentrifugation [18]. Lipids in plasma and CM were extracted with chloroform/methanol (2:1) [19], and TAG separated from the extracted lipid mixture with silica columns [20].

The molecular weight distribution of the TAG was determined by ammonia-negative ion chemical ionisation with a triple quadrupole tandem mass spectrometer (TSQ-700, Finnigan MAT, San Jose, CA), and the relative molar proportions of different molecular weight species were calculated using the corrected $[M–H]^-$ ion abundance as described elsewhere [11,21]. Shortly, TAG regioisomerism was determined with a tandem mass spectrometric analysis based on negative ion chemical ionisation and collision-induced dissociation with argon gas. The results were calculated with the MSPECTRA program (Nutrifen, Turku, Finland). The regioisomers, selected on the basis of their abundance and propensity to carry stearic acid, were analysed in quadruplicate.

2.5. Statistical analysis

Random positional distribution of FA in TAG was tested with *t* test, and correlations were analysed by Pearson correlation. Analyses were carried out with SPSS (version 11.0, Chicago, IL, USA). Values are expressed as the mean of nine determinations. TAG data were log-transformed before analysis. A statistical difference at $P<.01$ was deemed significant.

3. Results

3.1. Meal composition and lipoprotein FAs

The test meal (359±12 g) contained 4594 kJ energy (1098 kcal), 44 g protein, 62 g available carbohydrate, and 86 g fat, of which SALATRIM contributed 30 g. The test meal, plasma and CM lipoprotein FA composition are shown in Table 1. Stearic acid content in the meal was 26.8±0.3% of all long-chain FAs, but only 19.0±9.9% (not different from the meal, $P<.05$) and 12.1±4.5% (different from the meal, $P<.01$) in CM at 180 and 360 min after ingestion of the meal, respectively. A lower-than-expected level of stearic acid in the CM (71% and 45% of the percentage content in the meal at 180 and 360 min, respectively) was compensated by an increase in all the other major FAs: oleic acid (108% and 115%), linoleic acid (117% and 119%) and palmitic acid (114% and 147%). In CM, the between-subject variation in stearic acid concentration (coefficient of variation 52% and 37% at 180 and 360 min, respectively) was notably higher than that of the other FAs (12–22% and 6–9%, respectively) (see Fig. 1).

Table 1
FA composition of the test meal, plasma and CM at 180 and 360 min

FA	Test meal, %	Plasma, %		CM, %	
		180 min	360 min	180 min	360 min
14:0	1.1	1.4±0.4	1.3±0.3	0.9±0.7	1.4±0.4
16:0	10.9	16.0±1.3	17.3±1.0	12.4±2.8	16.0±1.3*
16:1(n-7)	1.1	1.8±0.2	2.1±0.4	1.1±0.6	1.8±0.2*
18:0	26.8	12.1±4.5	4.8±0.4	19.0±9.9†	12.1±4.5*
18:1(n-9)	37.9	43.7±2.6	46.4±1.1	41.0±4.8	43.7±2.6*
18:1(n-7)	1.8	2.3±0.1	2.6±0.2	2.2±0.1*	2.3±0.1*
18:2(n-6)	12.9	15.3±1.3	17.7±1.4	15.1±2.8†	15.3±1.3*
18:3(n-3)	5.1	4.5±0.5	4.8±0.6	4.9±0.9	4.5±0.5
20:0	0.8	0.4±0.1	0.2±0.1	0.7±0.2	0.4±0.1*
22:0	0.2	0.0±0.0	0.0±0.0	0.0±0.1	0.0±0.1*
other	1.5	2.3±1.0	2.7±1.0	2.6±1.9	2.2±1.0

Values are presented as a mean of 3 analyses (meals) or as a mean of 9 subjects (±standard deviation). Different from the meal (for CM only). FA fatty acid, CM chylomicron.

† $.05>P>.01$.

* $.01\geq P$.

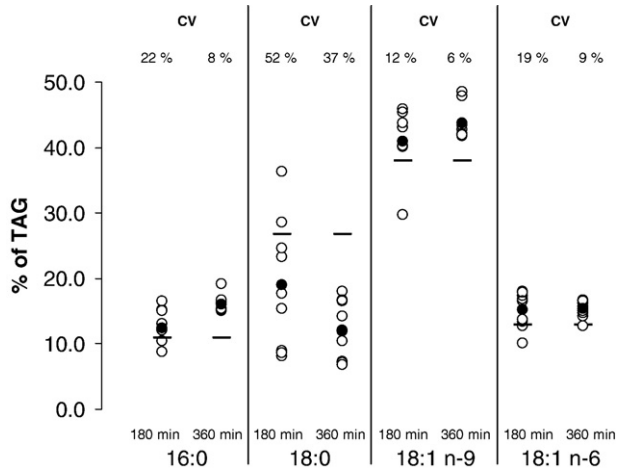

Fig. 1. Between-subject variation of the major FAs in plasma CM at 180 and 360 minutes after the meal. Open symbols indicate subject's CM FA content after the test meal; filled symbols, mean of all subjects' CM FA content; dash, meal FA content. CV coefficient of variation.

3.2. Stearic acid distribution in lipoprotein TAG

Stearic acid was randomly distributed in the test fat (30.0% in *sn*-2 position). The acyl carbon number:double bond (ACN:DB) profile of the CM TAG at 180 and 360 min is shown in Fig. 2. ACN:DB species 52:1, 52:2, 52:3, 54:1, 54:2, 54:3, 54:4 and 54:5 were chosen for the triple-quadruple tandem mass spectrometric analysis (MS-MS). This was based on their concentration in the CMs (≥3% of the CM TAG) (see Fig. 2) and their potential to carry stearic acid-containing TAG. The stearic acid distribution is shown in Table 2. Only 23% and 22% of CM stearic acid was in the *sn*-2 position at 180 and 360 min after the meal, a distribution that was nonrandom ($P<.001$), and differing significantly from that of the test fat ($P<.004$ and $P<.001$, respectively). The proportion *sn*-2 stearic acid seemed to increase with increasing unsaturation of the TAG, but the trend was not statistically significant. There was no statistically significant difference in stearic acid in *sn*-2 CM TAG at 180 and 360 min. No correlation was found between total and *sn*-2 stearic acid content in CM TAG.

3.3. Correlation of CM FAs and postprandial events

The postprandial TAG in plasma and CM peaked at 180 min after the meal (see Fig. 3A). Insulin peaked at the first

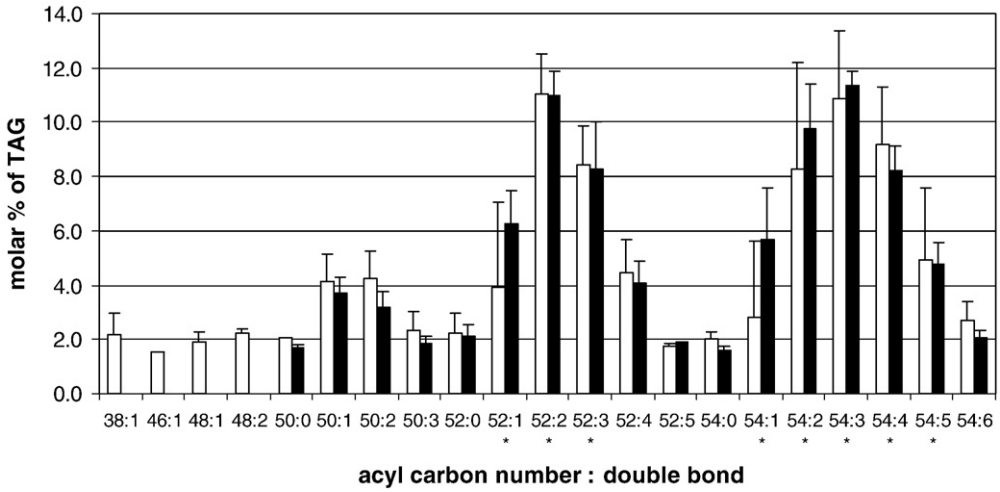

Fig. 2. ACN:DB profile of CM TAG at 180 and 360 min after the test meal. Only regioisomers found in three or more samples are presented. Asterisks indicate selected for MS-MS analysis; open and filled bars, ACN:DB at 180 and 360 min, respectively.

postprandial sampling point at 60 min, whereas glucose remained relatively stable throughout the follow-up (Fig. 3B). All variables had returned to fasting level at 450 min.

The correlations of fasting parameters and postprandial TAG measures after the test fat meal are shown in Table 3. Peak plasma TAG concentration at 180 min correlated with fasting plasma TAG and, especially, with fasting CM TAG. The association between peak CM TAG and fasting plasma and CM TAG was somewhat weaker [r=0.62 and r=0.75, respectively (nonsignificant)]. A nearly significant association was seen between plasma TAG iAUC and fasting CM TAG (r=0.72). Peak and iAUC glucose significantly correlated with the fasting glucose (r=0.80 and r=0.85, respectively). Inspection of correlations of individual FAs at TAG peak at 180 min showed a distinct difference between

Table 2
Stearic acid proportion in selected CM TAG ACN:DB species at 180 and 360 min after the test meal

ACN:DB	% of total TAG	Stearic acid concentration, %			P
		sn-1,2,3	*sn*-2	*sn*-2/*sn*-1,2,3	
CM 180 min					
52:1	3.9±3.1	33.3±0.0	9.7±3.8	29.1±11.5	
52:2	11.1±1.5	7.9±3.2	1.7±0.9	21.5±8.9	
52:3	8.5±1.4	4.4±0.9	1.4±0.5	33.2±10.1	
54:1	2.8±2.8	66.2±1.1	18.0±5.1	27.1±7.6	
54:2	8.3±3.9	37.0±2.3	7.5±2.5	19.9±5.7	
54:3	10.9±2.5	17.1±5.4	3.1±2.2	17.0±8.1	
54:4	9.2±2.1	9.2±3.6	2.0±1.3	26.2±19.9	
54:5	5.0±2.6	3.6±3.0	1.8±2.0	38.3±28.2	
Total	59.6±5.6	22.7±9.0	5.0±2.4	22.9±3.7	P<004[a]
CM 360 min					
52:1	6.3±1.2	33.6±0.4	8.5±1.6	25.4±4.9	
52:2	11.0±0.9	8.6±2.6	1.7±1.1	19.8±10.9	
52:3	8.3±1.8	4.4±1.8	1.8±1.1	38.3±13.1	
54:1	5.7±1.9	66.4±0.6	17.9±3.1	27.0±4.7	
54:2	9.8±1.7	38.4±2.3	7.2±3.5	18.4±8.5	
54:3	11.4±0.5	17.7±3.6	2.1±1.0	12.4±5.2	
54:4	8.2±0.9	11.8±2.7	3.0±2.1	23.8±14.5	
54:5	4.8±0.8	5.6±2.1	2.5±1.2	40.8±18.6	
Total	65.1±1.8	21.6±4.8	4.9±1.4	22.02.8	P<001[a]

Values are presented as a mean of 9 subjects (±standard deviation).

[a] Statistical significance of the difference of CM *sn*-2 stearic acid from the test meal *sn*-2 stearic acid.

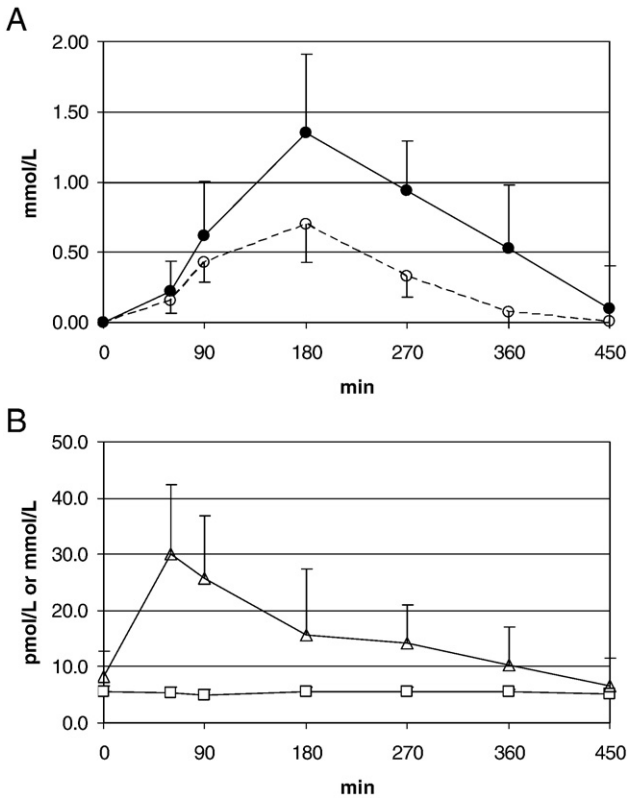

Fig. 3. Postprandial plasma and CM TAG (mmol/L) (A) and plasma glucose (mmol/L) and insulin (pmol/L) (B). Filled circles indicate plasma TAG; open circles, CM TAG; squares, glucose; triangles, insulin.

Table 3
Pearson correlation of fasting, peak and iAUC glucose, insulin, plasma TAG and CM TAG

		Fasting				Peak				iAUC			
		Glucose	Insulin	Plasma TAG	CM TAG	Glucose	Insulin	Plasma TAG	CM TAG	Glucose	Insulin	Plasma TAG	CM TAG
Fasting	Glucose	1	0.18	0.30	−0.23	0.80*	0.20	−0.05	−0.01	−0.85*	0.32	−0.38	−0.06
	Insulin	–	1	0.74†	0.26	0.14	0.68†	0.30	0.11	−0.03	−0.65	−0.07	0.21
	Plasma TAG	–	–	1	0.56	0.09	0.50	0.73†	0.62	−0.20	−0.14	0.24	0.31
	CM TAG	–	–	–	1	−0.20	0.25	0.88*	0.75†	0.31	0.09	0.72†	−0.12
Peak	Glucose	–	–	–	–	1	0.54	−0.09	−0.05	−0.42	0.24	−0.20	−0.04
	Insulin	–	–	–	–	–	1	0.26	0.26	0.27	−0.33	0.06	0.29
	Plasma TAG	–	–	–	–	–	–	1	0.89*	0.09	0.11	0.82*	0.22
	CM TAG	–	–	–	–	–	–	–	1	0.11	0.29	0.72†	0.43
iAUC	Glucose	–	–	–	–	–	–	–	–	1	–0.34	0.37	0.10
	Insulin	–	–	–	–	–	–	–	–	–	1	0.13	0.21
	Plasma TAG	–	–	–	–	–	–	–	–	–	–	1	0.22
	CM TAG	–	–	–	–	–	–	–	–	–	–	–	1

For glucose and insulin, peak time point was defined individually, whereas for plasma and CM TAG, 180 min was used.
† .05>*P*>.01.
* .01≥*P*.

stearic acid and the three other major FAs (Table 4). Palmitic, oleic and linoleic acids in circulation postprandially correlated positively with fasting CM TAG concentration, whereas stearic acid showed a negative correlation (all correlations not significant). Stearic acid correlated with postprandial CM TAG concentration only at 360 min, whereas the other major FAs showed significant correlation with TAG at both the peak (180 min) and the late (360 min) postprandial phase. Palmitic, oleic and linoleic acids also correlated with plasma TAG at 180 min (.05>*P*>.01), whereas stearic acid showed no correlation.

4. Discussion

The aim of the present study was to measure the amount of *sn*-2 SFA in circulation at peak and late postprandial triglyceridemia. We found that the amount of *sn*-2 stearic acid remained stable, with less than 23% of stearic acid in the *sn*-2 position of CM TAG. Ingestion of randomized stearic acid-rich fat does not result in a quantitative accumulation of *sn*-2 stearic acid in CM TAG during postprandial triglyceridemia. Our findings do not support the view of an increased postprandial triglyceridemia as a result of reduced clearance of *sn*-2-SFA.

It has been suggested that TAG with *sn*-2 stearic acid would result in a slower hydrolysis and absorption of the TAG [22] and delayed release of CM TAG [10], resulting ultimately in a greater enrichment of *sn*-2 stearic acid in the late postprandial phase. Our findings do not support these hypotheses. On the other hand, a reduced rate of lipolysis of TAG with *sn*-2 SFA has been reported in animal and in vitro studies [6,23]. One hypothesis has been that the lipoprotein lipase-mediated hydrolysis product, *sn*-2-stearoylglycerol, would be more difficult to remove from circulation due to an altered affinity of the particle surface for the apolipoproteins as a result of the high melting-point of the MAG [6]. This would result in an accumulation of CM particles with S_f<400, and an increased atherogenic potential. Our study on the *sn*-2 stearic acid content in intact CM TAG molecules supports the hypothesis that the reduced lipolysis is due to the differences in MAG, not TAG properties. In fact, the higher correlation of postprandial stearic and palmitic acids with changes in total plasma TAG than with changes in CM TAG, as opposed to what was observed for unsaturated FAs, supports the increased formation of small remnant particles residing in the S_f<400 fraction from CM TAG carrying SFAs. Due to the atherogenic nature of the small TAG-rich particles, the effect of the meal FA composition on TAG-rich lipoprotein size in the postprandial period needs to be studied further.

The *sn*-2 position of the ingested TAG is generally believed to be highly conserved in the CM (78–87%) [24–26]; based on 75% conservation, we would expect to find at least 28% of the stearic acid in the *sn*-2 position.

Table 4
Pearson correlations of individual FA concentrations in CM (mmol/L) (180 and 360 min) and total plasma and CM TAG concentration (mmol/L) (fasting, 180 and 360 min)

CM FA	Plasma TAG			CM TAG		
	Fasting	180 min	360 min	Fasting	180 min	360 min
180 min						
Palmitic acid	0.59	0.84†	n.r.	0.66	0.96†	n.r.
Stearic acid	0.37	0.05	n.r.	−0.31	0.18	n.r.
Oleic acid	0.38	0.79†	n.r.	0.63	0.93†	n.r.
Linoleic acid	0.37	0.81†	n.r.	0.70	0.91†	n.r.
360 min						
Palmitic acid	0.19	n.r.	0.57	−0.31	n.r.	0.86*
Stearic acid	0.10	n.r.	0.51	−0.40	n.r.	0.76†
Oleic acid	0.17	n.r.	0.53	−0.35	n.r.	0.84*
Linoleic acid	0.16	n.r.	0.52	−0.35	n.r.	0.84*

n.r., not reported.
† .05>*P*>.01.
* .01≥*P*.

In the present study, we observed 68% (180 min) and 66% (360 min) conservation of *sn*-2 stearic acid. This is in conflict with some [12,13,23,27] but not all [10,28] earlier investigations using randomised SFA-rich fats. Our results are in line with those of Emken et al. [29], who, using isotope-labeled TAGs, found that the other common SFA, palmitic acid, also undergoes substantial isomerisation after absorption, whereas the position is conserved with polyunsaturated linoleic acid. The anticipated increased fecal stearic acid losses from the *sn*-2 position [10,30] can be excluded as only marginal amounts were found in the feces [14].

Already in 1972, O'Doherty et al reported that a portion of *sn*-2-stearoylglycerol is slowly isomerised to *sn*-1-stearoylglycerol during the monoacylglycerol pathway [31]. As isomerisation of *sn*-MAGs commonly occurs as a function of time, the high-melting-point *sn*-2-stearoylglycerol could well be subject to increased migration of stearic acid. We cannot exclude the possibility of very long absorption times resulting in *sn*-2-stearoyl-TAGs appearing in circulation after completion of the follow-up (7.5 h). By that time, plasma and CM TAG had reached fasting levels, rendering significant levels of intestinal TAG secretion unlikely. The low conservation may also be an artefact of the molecular structure of SALATRIM, in which ca. 50% of FAs are short-chain FAs. After normal hydrolysis in the intestinal lumen, the short-chain acetic acid is either oxidised for energy in the mucosal cells or transported via portal blood to the liver. *sn*-2-Stearoylglycerol may therefore be exposed to prolonged residence in the enterocytes, waiting for FAs to be incorporated into *sn*-1 and *sn*-3 positions. The observed low conservation could also relate to changes in the regioisomerism taking place prior to absorption. In the stomach, short-chain FAs are preferentially hydrolysed by gastric lipase from *sn*-1 and *sn*-3 positions, leaving room for acyl migration from *sn*-2 into *sn*-1 or *sn*-3, and thereby enabling a dilution of the proportion of stearic acid at *sn*-2. The rate of this conversion is, however, unclear.

The TAG responses to the test fat meal were highly individual. This was mainly related to the circulating stearic acid concentration, which was more varied than that of the other FAs (see Fig. 1), an observation also made by earlier investigators [13]. Interestingly, the variation in stearic acid concentration at peak triglyceridemia in the CMs [between 0.05 and 0.31 mmol/L (6.2% and 33.7% of all FAs of the fraction, respectively)] did not correlate with the overall TAG concentration in CMs or plasma. A closer inspection revealed that though the fasting TAG concentrations highly significantly predicted the concentration of all the major FAs in the CMs, fasting TAG bore no relation to stearic acid at 180 or 360 min. This drastic difference between stearic acid and the other dietary FAs may be caused by the rapid conversion of stearic acid to oleic acid [15,32]. Further studies on the role of stearic acid as an independent variable in postprandial triglyceridemia are warranted.

Limitations of the study must be addressed. Firstly, half of the postprandial TAG were in the S_f<400 fraction. Therefore, we made additional analyses of the stearic acid distribution in plasma TAG at 180 min in the three most prominent ACN:DB species (52:2, 54:2 and 54:3). As we found only 16.5±7.5% of the stearic acid in *sn*-2 (P<001), we do not believe that the exclusion of the small intestinal lipoproteins in the S_f<400 fraction biases the results. Secondly, the analysis of too few ACN:DB species could lead to the exclusion of some unlikely *sn*-2-stearoyl-TAGs. The risk was minimised by analysing ca. 60% of the total TAG, specifically chosen for their propensity to carry stearic acid.

The correlation between TAG concentration in fasting and postprandial plasma is well established [33,34]. We showed that the best predictor of postprandial peak and iAUC TAG, risk factors for atherosclerosis, is the fasting TAG in CMs. To our knowledge, this was the first time such a connection has been reported. The concentration of TAG in large lipoprotein particles persisting in circulation even after a 12-h fast could well be a function of the TAG clearance system efficiency. We also found that, unlike the other major test meal FAs, the concentration of stearic acid in circulating CM did not correlate with the total CM TAG concentration, indicating significant differences in the metabolism of stearic acid compared with that of the other saturated or unsaturated FAs.

In conclusion, we were able to show that the low postprandial triglyceridemia following the ingestion of a randomised stearic acid-rich fat is accompanied by a concomitant loss of stearic acid in the *sn*-2 position of TAG. Since fecal losses were not increased, we suggest that the slow metabolism of the stearic acid allows a substantial isomerisation of *sn*-2-stearoyl-MAGs to *sn*-1-or *sn*-3-stearoyl-MAGs during the TAG resynthesis in the enterocytes. Additionally, fasting TAG in CMs is a potential marker for estimating the degree of postprandial triglyceridemia and, as such, is worth further investigation.

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