Influence of triacylglycerol structure on the postprandial response of factor VII to stearic acid–rich fats[1–3]

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ABSTRACT

Background: The consumption of a synthetic, randomized, stearic acid–rich triacylglycerol results in decreased postprandial lipemia and activated factor VII (FVII:a) compared with cocoa butter (a nonrandomized, symmetrical, stearic acid–rich triacylglycerol). It was hypothesized that this difference is a consequence of the differences in structure between the 2 triacylglycerols.

Objective: The objective was to test whether the consumption of randomized cocoa butter decreases postprandial lipemia and FVII:a.

Design: A randomized crossover trial with 17 male subjects compared the effects of meals containing 50 g fat provided as a symmetrical (cocoa butter) or an asymmetrical (randomized cocoa butter) triacylglycerol on postprandial changes in lipids, chylomicron composition, and FVII:a.

Results: After randomization, the postprandial area under the curve for plasma triacylglycerol decreased by 41% ($P < 0.01$). At 3 h the plasma concentrations of triacylglycerol, palmitic acid, stearic acid, and oleic acid were 26%, 18%, 34%, and 19% lower, respectively. The proportion of oleic acid in the *sn*-2 position of the chylomicron triacylglycerol was reduced from 67.4 mol% to 35.9 mol% and resulted in an increase in the proportion of stearic acid in the *sn*-2 position from 9.2 mol% to 25.4 mol%. FVII:a did not increase 6 h after consumption of the randomized cocoa butter ($\bar{x}$: 1.2; 95% CI: −2.7, 4.6 U/L) but increased significantly ($\bar{x}$: 7.7; 95% CI: 2.5,12.9 U/L) 6 h after consumption of the unrandomized cocoa butter.

Conclusions: Symmetrical stearic acid–rich triacylglycerol with oleic acid in the *sn*-2 position appears to be absorbed more rapidly than is asymmetrical triacylglycerols with long-chain saturated fatty acids in the *sn*-2 position, which leads to activation of FVII. *Am J Clin Nutr* 2003;77:777–82.

KEY WORDS Factor VII, stearic acid, postprandial lipemia, saturated fatty acids, triacylglycerols, digestion

INTRODUCTION

Randomization is a technique being widely adopted by the food industry as an alternative to the partial hydrogenation of fats, because it increases the melting point of fats without leading to the generation of *trans* fatty acids or to significant changes in fat-soluble vitamin concentrations. In this process, totally hydrogenated vegetable fats—which are rich in stearic acid—are interesterified with unhydrogenated oils. This leads to the generation of triacylglycerols with saturated fatty acids in the *sn*-2 position. It was previously reported that a randomized stearic acid–rich triacylglycerol, made from interesterifying totally hydrogenated sunflower oil with unhydrogenated sunflower oil, decreased the postprandial increase in plasma triacylglycerol and activated factor VII (FVII:a) concentrations compared with an oleic acid–rich triacylglycerol (high–oleic acid sunflower oil) (1). In a subsequent study (2), cocoa butter—which is rich in stearic acid—resulted in a similar postprandial increase in plasma triacylglycerol and FVII:a concentrations compared with an oleic acid–rich triacylglycerol (high–oleic acid sunflower oil), but SALATRIM (a synthetic stearic acid–rich triacylglycerol; Danisco Cultor, Ardsley, NY) had a neutral effect. It was proposed that the failure of this synthetic stearic acid–rich triacylglycerol to increase plasma triacylglycerol and FVII:a concentrations postprandially was a consequence of its asymmetrical triacylglycerol structure. However, the synthetic stearic acid–rich triacylglycerol also contains acetic and butyric acids, which could explain the failure. Furthermore, this synthetic stearic acid–rich triacylglycerol does not contain any unsaturated fatty acids in the *sn*-2 position.

Cocoa butter has an idiosyncratic triacylglycerol structure, with almost all of the stearic acid being present as symmetrical triacylglycerol either as 1,3-distearyl-oleyl glycerol or 1-stearyl, 2-oleyl, 3-palmitoleyl glycerol (3). This unique triacylglycerol structure contributes to the well-known organoleptic properties of cocoa butter, which leads to it melting rapidly at approximately body temperature and being well digested (4). The aim of this present study was to test the hypothesis that triacylglycerol structure determines the extent to which stearic acid–rich triacylglycerol increases plasma triacylglycerol and FVII activation. Consequently, we compared the postprandial response to randomized cocoa butter (asymmetrical triacylglycerol) with that to unrandomized cocoa butter (symmetrical triacylglycerol).

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[2] Supported by the Medical Research Council and King's College London. The experimental fats were provided by Danisco Cultor (Ardsley, NY).

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TABLE 1
Characteristics of the male subjects[1]

	Value
Age (y)	38.2 ± 11.1
Weight (kg)	78.5 ± 11.6
BMI (kg/m^2)	24.5 ± 2.9
Plasma cholesterol (mmol/L)	4.51 ± 0.81
Plasma LDL cholesterol (mmol/L)	2.72 ± 0.77
Plasma HDL cholesterol (mmol/L)	1.29 ± 0.26
Plasma triacylglycerol (mmol/L)	1.04 ± 0.81
Dietary intake	
Energy (MJ)	9.35 ± 2.08
Protein (% of energy)	14.4 ± 2.98
Carbohydrate (% of energy)	50 ± 9.02
Total fat (% of energy)	31.4 ± 7.15
Saturated fat (% of energy)	10.9 ± 4.64
Polyunsaturated fat (% of energy)	5 ± 1.74
Monounsaturated fat (% of energy)	12.2 ± 2.2

[1] $\bar{x}$ ± SD; n = 17.

SUBJECTS AND METHODS

Subjects

Eighteen healthy male subjects were recruited from among staff and students of King's College London, University of London. The exclusion criteria included a history of cardiovascular disease, diabetes, body mass index (in kg/m^2) < 20 or > 35, plasma cholesterol > 7.8 mmol/L, plasma triacylglycerol > 3 mmol/L, current use of antihypertensive or lipid-lowering medication, and a self-reported intake of alcohol of > 28 U/wk (1 U = 10 mL ethanol). Fasting plasma lipoprotein lipid concentrations, body weight, blood pressure, blood cell count, and liver function were confirmed to be within the prescribed limits before entry into the study. Habitual nutrient intake was assessed from a 3-d food intake diary, and nutrient intakes were estimated by using the integrated dietary assessment package (IDA Publications, London). Subject characteristics are shown in **Table 1**. Dietary intakes were close to recommended dietary guidelines. The study protocol was reviewed and approved by King's College Research Ethics Committee, and all participants gave written informed consent.

Study design

A randomized crossover study design was used. Each subject received 2 experimental meals (unrandomized cocoa butter or randomized cocoa butter) with ≥ 1 wk between treatments. Subjects were advised to avoid the consumption of foods high in fat on the day preceding each test meal. To control for physical activity levels, subjects were asked to refrain from strenuous exercise, including cycling and sporting activities, and from the use of alcohol on the day before and on the day of the test meal. Subjects fasted overnight beginning at 2200 and then venous blood samples were obtained between 0800 and 1000 the following morning. The test meal was consumed within 15 min, and additional venous blood samples were obtained 3 and 6 h later. Capillary blood samples were obtained at 1, 2, 4, and 5 h. After the 3-h blood samples were drawn, subjects consumed a standardized lunch (1.7 MJ) consisting of fresh fruit and low-fat yogurt (< 1 g fat). This was previously shown to not interfere with the measurement of postprandial lipemia or the postprandial increase in FVII:a (1, 2).

Formulation of the test meals

The test meals consisted of a muffin and a freshly prepared strawberry milkshake and were formulated to provide 3.13 MJ (749 kcal), 50 g fat, 16 g protein, and 50 g carbohydrate. The milkshake consisted of 150 g fresh strawberries, 150 mL skim milk, and 10 g pasteurized egg white powder. Each muffin contained 50 g test fat, 10 g baking flour, 5 g cornstarch, 5 g cocoa powder, 15 g sugar, 20 mL skim milk, 2 g pasteurized egg white, 2 g vanilla essence, and 2 g baking powder. The test fats consisted of unrandomized or randomized cocoa butter with melting points of 35 and 50 °C, respectively (determined by differential scanning calorimetry analysis provided by Danisco Cultor). Both test fats were manufactured from the same batch of cocoa butter and had the same fatty acid composition following processing and similarly low acid value (0.08) and alkaline number (< 0.02). The muffins were made in a single batch and stored at −20 °C.

Collection and handling of blood samples

Venous blood samples were collected by using the evacuated technique with minimal compression necessary to display the vein. The first 10 mL of blood was drawn into a tube containing dipotassium EDTA and the plasma was separated by centrifugation at 4 °C for 15 min at 1500 × *g*. The chylomicron-rich fraction (Svedberg flotation unit > 400) was separated by ultracentrifugation from the 3-h blood sample (5), and plasma lipoprotein concentrations were measured from unfrozen plasma, kept at 4 °C, within 48 h of blood collection. An aliquot of plasma was set aside and frozen at −80 °C for measurement of the plasma total fatty acid content. For FVII:a, blood was collected into a 4.5-mL evacuated tube containing 38 g/L of a trisodium citrate solution, centrifuged at 1500 × *g* for 15 min at 18 °C, divided into aliquots, snap frozen in liquid nitrogen and stored at −80 °C until analyzed for FVII:a. Blood (≈0.5 mL) for analysis of plasma triacylglycerol was collected by fingerprick 1, 2, 4, and 5 h postprandially into a micro-Eppindorf tube containing 1 mg dipotassium EDTA. The blood samples were processed within 1 h of collection.

Analytic methods

Plasma triacylglycerol and total and HDL-cholesterol concentrations were measured by enzymatic assay as previously described (2). Plasma total lipid concentrations of stearic, oleic, and palmitic acids were determined by gas-liquid chromatography (GLC), with pentadecanoic acid as an internal standard (6). Lipids were extracted from the chylomicrons with chloroform:methanol (1:1, by vol), and the triacylglycerol fraction was isolated by thin-layer chromatography on silica gel G plates developed in hexane:diethyl ether:glacial acetic acid (80:20:2, by vol). Bands were detected under ultraviolet light after spraying with 50 mg dichlorofluorescein in 100 mL methanol:water (95:5, by vol). The triacylglycerol fraction was methylated with methanolic HCl, and the fatty acids were analyzed by GLC. The fatty acid composition of the test fats was determined by GLC of the methyl esters, and the triacylglycerol molecular species of the test fats were determined by HPLC with the use of propionitrile as the mobile phase (7) by Michael Jee (Reading Scientific Services, Reading, United Kingdom). The composition of the fatty acids in the *sn*-2 position of the test fats (8) and the chylomicron triacylglycerol fraction were determined by specific enzymatic hydrolysis (5), followed by separation of the 2-monoacylglycerols

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TABLE 2
Triacylglycerol composition of the test fats[1]

	Cocoa butter	
	Randomized	Unrandomized
	mol%	
OOO	2.9	0.3
POO	8.9	2.2
SOO	12.3	3.1
OLP	0.6	0.3
PLP	1.1	1.3
SLP	1.4	1.2
PLS	1.1	2.3
POP	15.1	15.4[2]
SLS	0.5	1.3
POS	21.0	44.0[2]
SOS	12.2	27.5[2]
AOS	0.3	0.7[2]
PPP	2.1	0.0
PPS	7.6	0.3
SSP	8.8	0.1
SSS	3.2	0.1

[1] O, oleic acid (18:1); P, palmitic acid (16:0); S, stearic acid (18:0); L, linoleic acid (18:2n−6); A, arachidic acid (20:0).
[2] Oleate exclusively in the *sn*-2 position.

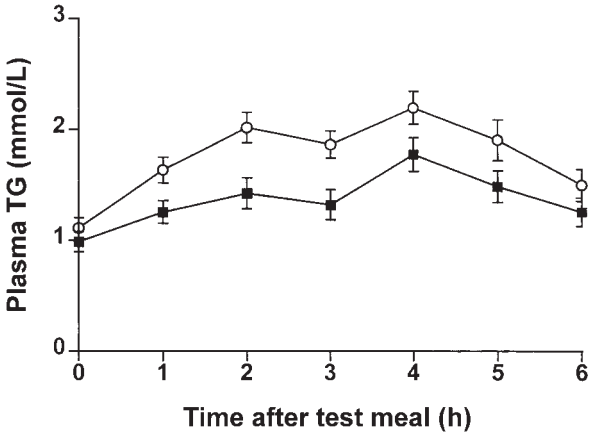

FIGURE 1. Mean (±SEM) plasma triacylglycerol (TG) concentrations in 17 healthy men after consumption of test meals containing either 50 g randomized (■) or 50 g unrandomized (○) cocoa butter. Subjects received a low-fat meal after the 3-h blood sample. The area under the curves are significantly different from each other, $P < 0.01$; randomized: 267 arbitrary units (95% CI: 210, 314); unrandomized: 447 arbitrary units (95% CI: 338, 557).

by thin-layer chromatography and analysis of their fatty acid methyl esters by GLC. The triacylglycerol composition of the test fats is shown in **Table 2**.

FVII:a was measured as previously described in plasma samples stored at −70 °C (9); samples from each subject were analyzed in the same run to avoid between-assay variation.

Statistical analysis

Statistical analyses of the data were carried out by using repeated-measures analysis of variance with SPSS PC version 10 (SPSS Inc, Chicago). Comparisons with fasting values were made by using paired *t* tests with a Bonferroni adjustment for multiple comparisons.

RESULTS

A total of 17 subjects completed the study. The changes in plasma triacylglycerol concentration after the 2 test meals, which appeared to be biphasic for both fats, are shown in **Figure 1**. Plasma triacylglycerol concentrations increased to a lesser extent after consumption of the randomized cocoa butter than after consumption of the unrandomized cocoa butter (meal × times interaction: $P < 0.01$), but the pattern of response was not significantly different between meals. The normalized integrated area under the curve for plasma triacylglycerol was 41% lower ($P < 0.01$) after the randomized cocoa butter than after the unrandomized cocoa butter.

The changes in plasma concentrations of the major fatty acid constituents of the test meal, total cholesterol, and HDL cholesterol are shown in **Table 3**. The postprandial increases in plasma stearic, palmitic, and oleic acid concentrations were all significantly lower ($P < 0.0001$) after the randomized cocoa butter meal than after the unrandomized cocoa butter meal: the increases in plasma triacylglycerol, palmitic acid, stearic acid, and oleic acid at 3 h were 26%, 18%, 34%, and 19% lower, respectively. The analysis of variance for total cholesterol suggested a time effect ($P = 0.064$); the meal × time interaction was not significant. HDL-cholesterol concentrations did not change significantly from baseline after the test meals.

The fatty acid composition of the chylomicron triacylglycerol after both test meals and of the test fat in palmitic, stearic, oleic,

TABLE 3
Plasma total and HDL-cholesterol and plasma fatty acid concentrations at baseline (0 h, fasting) and after the test meals in male subjects[1]

	Randomized	Unrandomized
Total cholesterol (mmol/L)		
0 h	4.22 ± 0.72	4.24 ± 0.63
3 h	4.29 ± 0.75	4.28 ± 0.65
6 h	4.32 ± 0.73	4.26 ± 0.74
HDL cholesterol (mmol/L)		
0 h	1.18 ± 0.25	1.18 ± 0.27
3 h	1.18 ± 0.27	1.15 ± 0.26
6 h	1.18 ± 0.29	1.17 ± 0.28
Palmitic acid (mmol/L)[2]		
0 h	2.16 ± 0.56	2.18 ± 0.44
3 h	2.31 ± 0.61[3]	2.80 ± 0.58[4]
6 h	2.34 ± 0.6[4]	2.56 ± 0.66[4]
Stearic acid (mmol/L)[2]		
0 h	0.75 ± 0.15	0.73 ± 0.10
3 h	0.94 ± 0.2[3,4]	1.43 ± 0.29[4]
6 h	0.92 ± 0.22[3,4]	1.17 ± 0.31[4]
Oleic acid (mmol/L)[2]		
0 h	2.22 ± 0.7	2.25 ± 0.52
3 h	2.50 ± 0.76[3,4]	3.10 ± 0.69[4]
6 h	2.57 ± 0.77[4]	2.79 ± 0.81[4]

[1] $\bar{x}$ ± SD; $n = 17$.
[2] Significant meal × time interaction, $P < 0.01$.
[3] Significantly different from unrandomized cocoa butter, $P < 0.01$ (paired *t* test with Bonferroni correction).
[4] Significantly different from baseline, $P < 0.01$ (paired *t* test with Bonferroni correction).

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TABLE 4
Fatty acid composition of venous chylomicron-triacylglycerol at 180 min and of the test fat in male subjects

	Chylomicron triacylglycerol[1]		
	Randomized cocoa butter	Unrandomized cocoa butter	Test fat
	mol%		*mol%*
Palmitic acid	35.7 ± 4.1[2]	33.2 ± 1.3	29.0
Stearic acid	18.9 ± 4.3[3]	28.8 ± 4.2	35.8
Oleic acid	36.5 ± 4.3[3]	32.7 ± 2.6	32.8
Linoleic acid	8.9 ± 2.3[3]	5.2 ± 2.1	2.4

[1] $\bar{x}$ ± SD; n = 17.
[2,3] Significantly different from unrandomized cocoa butter (paired t test): [2] $P < 0.05$, [3] $P < 0.01$.

TABLE 5
Plasma activated factor VII concentrations in male subjects after the test meals[1]

	Cocoa butter	
	Randomized	Unrandomized
0 h	33.7 ± 11.2	34.9 ± 11.5
3 h	32.7 ± 11.6	38.4 ± 12.4[2]
6 h	34.9 ± 12.7	42.7 ± 16.3[2]
Change from 0 h at 3 h	−1.0 (−4.6, 2.6)	3.4 (1.0, 5.9)[2]
Change from 0 h at 6 h	1.2 (−2.7, 4.6)	7.7 (2.5, 12.9)[2]

[1] $\bar{x}$ ± SD; n = 17. 95% CI in parentheses. Two-factor repeated-measures ANOVA showed significant effects for meal × time interactions (P = 0.038), time (P = 0.004), and meal (P = 0.002). Analysis of the deviations from fasting showed a significant effect of time (P = 0.018) and meal (P = 0.02).
[2] Significantly different from randomized cocoa butter, $P < 0.05$ (paired t test with Bonferroni correction for multiple comparisons).

and linoleic acids is shown in **Table 4**. The proportion of stearic acid was 34% lower and the proportions of palmitic, oleic, and linoleic acids were proportionately higher (7.5%, 11.7%, and 71.1%, respectively) after the randomized than after the unrandomized cocoa butter meal. The distribution of fatty acids in the *sn*-2 position of the chylomicron triacylglycerol reflected the distribution in the test fat (**Figure 2**). Compared with fasting values, the proportion of oleic acid in the *sn*-2 position of the chylomicron triacylglycerol was reduced from 67.4 mol% to 35.9 mol% and that of stearic acid increased from 9.2 mol% to 25.4 mol% after the randomized and unrandomized cocoa butter meals, respectively. Fasting FVII:a concentrations were not significantly different before the 2 test meals (**Table 5**). FVII:a concentrations did not increase from baseline 3 and 6 h after the randomized cocoa butter meal, but did increase significantly 3 and 6 h after the unrandomized meal ($P < 0.006$). At 3 and 6 h, FVII:a was significantly higher after the unrandomized cocoa butter meal than after the randomized cocoa butter meal (both $P < 0.05$). No other significant differences were noted.

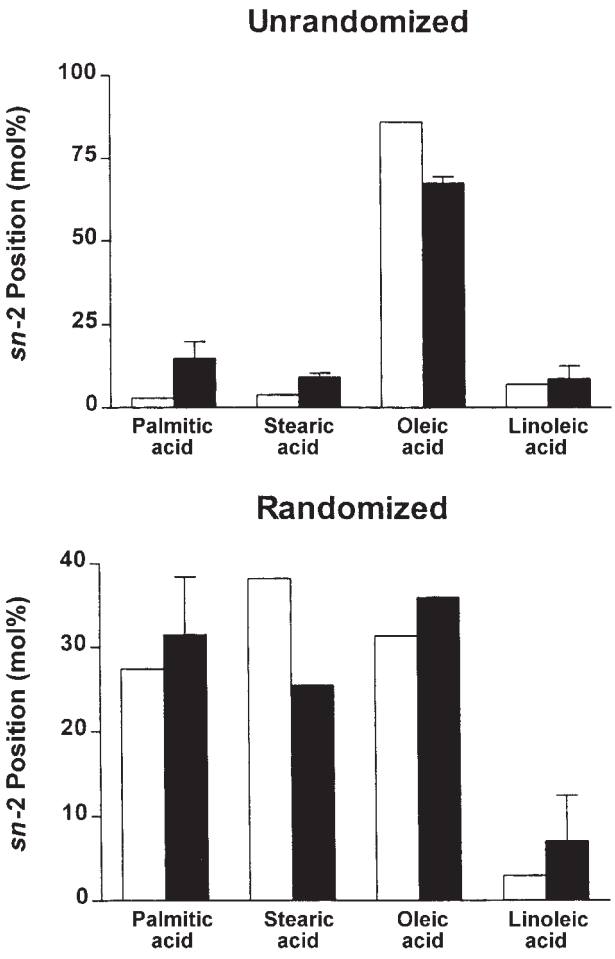

FIGURE 2. Composition of palmitic, stearic, oleic, and linolenic acids in the *sn*-2 position of the chylomicron triacylglycerol (■) and in the test meals (□). Bars indicate the 95% CIs. Note that the *y* axes of the 2 panels differ.

DISCUSSION

The aim of this study was to further investigate why different dietary stearic acid–rich triacylglycerols result in different postprandial effects on lipids and FVII:a. Unlike in previous studies, the fats used in the current study differed only with regard to the triacylglycerol structure. Randomization led to a marked decrease in the proportion of the symmetrical triacylglycerol molecules 1,3-distearyl-oleyl glycerol and 1-stearyl, 2-oleyl, 3-palmitoleyl glycerol and to increases in the proportions of asymmetrical triacylglycerols such as 1,2-distearyl, 3-oleyl glycerol and 1-palmityl, 2-stearyl, 3-oleyl glycerol and of triacylglycerols with higher melting points and containing 3 saturated fatty acids. The consequence of randomization was that the postprandial increase in plasma triacylglycerol was diminished. Plasma stearic acid concentrations were more markedly lowered than were those of palmitic acid or oleic acid. Analysis of the chylomicron triacylglycerol showed that the proportion of stearic acid was 34% lower after the randomized cocoa butter meal. However, the proportions of palmitic acid, stearic acid, and oleic acid in the *sn*-2 position were broadly similar to those in the dietary triacylglycerol, even though the proportion of stearic acid in the *sn*-2 position of the chylomicron triacylglycerol tended to be lower than in the test fat. These observations suggest that triacylglycerols containing stearic acid in the *sn*-2 position are less well absorbed or that their release into the circulation is delayed. These findings taken together are consistent with the view that asymmetrical stearic acid–containing

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triacylglycerols are less well absorbed than are symmetrical stearic acid–containing triacylglycerols (10).

Summers et al (5) compared the effects on postprandial lipid metabolism of meals containing 60 g fat providing stearic acid either predominantly in the *sn*-1 position (SOO meal) or the *sn*-2 position (OSO meal). They found no difference in the extent of the postprandial lipemia measured as the area under the curve but chylomicron triacylglycerol concentrations reached a maximum 4 h after the SOO meal and 5 h after the OSO meal. In common with the current study, the proportion of fatty acids in the *sn*-2 position of the chylomicron triacylglycerol was very similar to that of the dietary fat, showing that the fatty acids in the *sn*-2 position of dietary fats are conserved in the same position on absorption. As in our previous studies (1, 2), subjects in the current study consumed a low-fat meal after the 3-h blood sample. This light meal was given to make the procedure acceptable to the subjects but also to mimic the normal physiologic state, in which persons rarely go without food for long periods of time. In the current study, we observed a biphasic response in serum triacylglycerol concentration similar to that observed by Fielding et al (11), which may reflect a second-meal effect.

Compared with the unrandomized cocoa butter meal, the randomized cocoa butter meal failed to elicit an increase in FVII:a. This observation is similar to that observed in our previous studies, in which we used SALATRIM (2), and is consistent with the observations in 2 other studies, in which a synthetic stearic acid fat produced from tristearin, which was interesterified randomly with high–oleic acid sunflower oil (1, 12). Although, it is tempting to attribute this effect to the lower postprandial increase in plasma triacylglycerol, it was previously shown that the extent of the increase in plasma triacylglycerol was not proportional to the increase in FVII:a (2). The intake of stearic acid was 36 g in the study by Sanders et al (1) and 30–38 g in the study by Tholstrup et al (13). In the study by Sanders et al (1), tristearin, distearoyl, and monostearyl triacylglycerol accounted for 12%, 49%, and 39% of the stearic acid in the dietary fat, respectively, with ≈36% being present in the *sn*-2 position (TAB Sanders, unpublished observations, 2000). Mennen et al (14) did not observe a statistically significant difference in the postprandial increase in FVII:a after a stearic acid–rich test meal compared with meals rich in palmitic acid or linoleic acid. However, there was a tendency for the increase to be lower after the high–stearic acid meal (11.6 compared with 15.9 U/L after a high–linoleic acid meal) even though the postprandial area under the curve was greater after the stearic acid–rich meal than after the linoleic acid–rich meal. In that study, no information was provided about the source of the stearic acid in the test fat. The intake of stearic acid in the test meal served by Mennen et al was 18.6 g, which was similar (17 g) to that used in the current study. After the unrandomized cocoa butter meal, most of the stearic acid was present in the triacylglycerol containing oleic acid in the *sn*-2 position, and this was accompanied by a significant increase in FVII:a. Consequently, it appears that symmetrical stearic acid–rich triacylglycerol with oleic acid in the *sn*-2 position is absorbed and metabolized more rapidly, leading to activation of FVII, than is asymmetrical triacylglycerol in the *sn*-2 position with long-chain saturated fatty acids.

The mechanism for the postprandial activation of FVII after fatty meals is not fully understood. Although there is a relation between the fasting plasma triacylglycerol concentration and FVII coagulant activity, there appears to be no clear relation between the extent of postprandial lipemia and FVII:a (2). It has been proposed that factor XII (FXII) activated during the lipolysis of triacylglycerol-rich lipoprotein would result in FVII activation. However, this hypothesis appears to be refuted by the finding that postprandial activation of FVII occurs in patients with a complete deficiency of factor XII (15) and a lack of change in activated FXII (FXII:a) after a high-fat test meal (16). FVII:a is associated with plasma phospholipid concentrations (17), and it is known that FVII can be activated by synthetic phospholipid particles containing negatively charged phospholipids (18). Membrane microparticles can be shed from activated platelets and leukocytes (19), and this may well occur postprandially, when lipid transfer reactions are active. It is plausible that these reactions proceed at a slower pace after consumption of triacylglycerol with stearic acid in the *sn*-2 position. Indeed, lipoprotein lipase (EC 3.1.1.34) and cholesterol ester transfer protein activities were found to be lower after meals rich in stearic acid (14). The pathophysiologic significance of changes in FVII:a concentrations in relation to the risk of coronary heart disease remains uncertain because the FVII:a concentration was paradoxically associated with a decreased risk of coronary heart disease in a prospective cohort study (20). It was also postulated that the postprandial increase in FVII:a may be related to activation of the ATP-binding cassette transporter A-I (21), which may be stimulated by the production of nascent HDL of intestinal origin. Further studies are required to examine the mechanisms leading to FVII activation.

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TABS conceived and devised the study and contributed to the analysis and the writing of the manuscript, SEEB organized and conducted the study and contributed to the writing of the manuscript, and GJM supervised the FVIIa analysis and contributed to the writing of the manuscript. The authors had no financial our commercial interest in any company or organization sponsoring the research.

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