Factor VIIa Response to a Fat-Rich Meal Does Not Depend on Fatty Acid Composition
A Randomized Controlled Trial

Louise Mennen, Moniek de Maat, Gert Meijer, Peter Zock, Diederick Grobbee, Frans Kok, Cornelis Kluit, Evert Schouten

Abstract—A fat-rich meal increases activated factor VII (FVIIa), but it is not clear whether this increase depends on the fatty acid composition of the meal. Therefore, we studied the FVIIa response to fat-rich meals with different fatty acid composition in a randomized controlled crossover trial and investigated whether this response is mediated by an increase in serum triglycerides. Elderly women (≥ 60 years, n=91) received on separate days four different fat-rich breakfasts (50 energy percent [en%] of fat) and a control breakfast (1.5 en% fat; crossover). The fat-rich breakfasts differed in fatty acid composition: one rich in palmitic acid (21.7 g), one in stearic acid (18.6 g), and the other two in linoleic and linolenic acid—one with a ratio 3:1 (12.5/3.9 g) and the other with a ratio of 15:1 (18.8/1.2 g). At 8 AM before the breakfast (fasting) and at 1 and 3 PM, blood samples were taken, in which FVIIa and serum triglycerides were measured. FVIIa response to the fat-rich meals ranged from 11.6 mU/mL (95% confidence interval: 8.3,14.9) on the stearic meal to 15.9 mU/mL (12.0,19.8) on the linoleic/linolenic 15:1 meal at 1 PM and from 14.9 mU/mL (10.6,19.2) to 21.1 mU/mL (16.6,25.6) for the same meals at 3 PM. The responses did not differ between the fat-rich meals. After the control breakfast, FVIIa decreased, with 6.3 mU/mL (3.9,8.7) at 1 PM and 8.7 mU/mL (6.3,11.1) at 3 PM. The triglyceride response was lower after both linoleic/linolenic rich breakfasts compared with the palmitic and stearic breakfast (P<.05) and was not associated with the FVIIa response at any of the blood sampling occasions. The results of this study show that the response of FVIIa to a fat-rich meal is independent of its fatty acid composition and is not mediated by serum triglycerides. (Arterioscler Thromb Vasc Biol. 1998;18:599-603.)

Key Words: postprandial factor VIIa ■ dietary fat ■ elderly women

Factor VII is a vitamin K–dependent coagulation factor, which circulates in plasma mainly as an inactive zymogen. About one percent of this factor VII circulates in the activated form, factor VIIa (FVIIa). A small increase in FVIIa may, at release of tissue factor, lead to an explosive formation of thrombin and thereby to an increase in the risk of arterial occlusion. This risk may be even more important in elderly people, in whom the prevalence of atherosclerosis is high.

FVIIa can be measured directly, but also the coagulant activity of factor VII (FVII:C) can be measured. The direct measurement of FVIIa has only recently become available; therefore FVII:C was often used in previous studies. This measurement, however, not only reflects FVIIa but also an unknown part of factor VII zymogen. Furthermore, different reagents hamper comparability of these studies. In two longitudinal studies, a positive association between FVII:C and fatal ischemic heart disease was observed.1,2 It is by now accepted that FVII:C increases postprandially after intake of dietary fat.3 It is, however, not yet clear whether this increase is dependent on fatty acid composition of the fat consumed. Five studies have evaluated this subject for FVII:C, but the results were contradictory, and sample sizes were small (n=4 to 10).4-8 In a few experimental studies, a postprandial increase in FVIIa has also been observed,9-12 but none of these studies investigated different fatty acids. Furthermore, FVIIa is higher in postmenopausal women compared with men of the same age,13 but (elderly) women have rarely been included in studies on FVIIa and dietary fat.

One of the mechanisms to explain an effect of dietary fat on factor VII is based on activation of factor VII during lipolysis of triglyceride-rich lipoproteins.3,14 In two studies, a positive association between postprandial concentrations of serum triglycerides (which partly reflect the amount of triglyceride-rich lipoproteins) and factor VII was observed.15,16

We studied the FVIIa and serum triglyceride response to fat-rich meals with different fatty acid composition in a...
randomized controlled crossover trial in a large number of apparently healthy elderly women.

**Methods**

**Subjects**

Elderly women living independently in retirement communities in the surroundings of Wageningen, the Netherlands, were invited to participate in the study. They had to fulfill the following inclusion criteria: older than 60 years of age, no diabetes mellitus, no myocardial infarction in the year before the investigation, no use of vitamin K antagonists, no use of hormone replacement therapy, and no medical problems after fat consumption. For purposes of a separate research question, subjects were selected on the genotype of the R/Q353 polymorphism of the gene coding for factor VII, to have no medical problems after fat consumption. For purposes of a multiple regression analysis, comparisons were made with the Tukey test to investigate whether the FVIIa response differed between the fat-rich breakfasts. Multiple comparisons were made with the Tukey test to investigate whether the FVIIa response differed between the fat-rich breakfasts.

**Intervention Procedure**

Every participant received each of the five different breakfasts with at least one week in between, in different order (crossover). For each participant, every test was performed on the same day of the week. For practical reasons, there were no tests on Mondays or on weekends.

Participants gathered in the communal room in their apartment building at 8 AM after an overnight fast. Within half an hour after taking blood samples with minimal stasis, they ate their breakfast. At 1 PM and 3 PM subsequent blood samples were taken. The participants remained in the communal room during the whole procedure and went home after the last blood sampling. At 10 AM and 2 PM, coffee or tea with a fat-free snack were served and at noon a fat-free meal. Except for the breakfast, they were free to choose from the fat-free foods (fruits, fat-free cake, and fat-free bread) we supplied, but were not allowed to eat anything else.

The response of FVIIa at each meal was calculated by subtraction of the mean fasting FVIIa, triglycerides, and total cholesterol concentration in the study population. The response of FVIIa at each meal was calculated by subtraction of the mean fasting FVIIa, triglycerides, and total cholesterol concentration in the study population.

**Laboratory Measurements**

Blood was collected in siliconized evacuated tubes (Vacutainer) containing 0.129 mol/L sodium citrate (Becton Dickinson). The first 5 mL was used for measurement of blood lipids. Samples were centrifuged for 30 minutes at 1500g and 20°C. Citrated plasma was snap-frozen and stored at −80°C until laboratory analysis. FVIIa was measured in all samples on the STA instrument (Boehringer), with a clotting assay using soluble recombinant tissue factor (Staclot, Diagnostica Stago). The mean clotting time is inversely related to the FVIIa level (expressed in milliliters per milliliter). The coefficient of variation of this measurement was 8%, and the intrapersonal variation (calculated with the measurements of the five fasting blood samples for each person) was 2.09 mU/mL. Serum triglycerides were measured with a colorimetric assay using a Kodak Ektachem 250 Analyzer. Serum total cholesterol was determined using an automated enzymatic procedure.

**Data Analysis**

For every participant, the five fasting measurements were combined with similar total energy content. Four breakfasts contained 50 energy percent (en%) of fat (fat-rich breakfast); the control breakfast contained only 1.5 en% of fat (Table 2). In the control breakfast, fat was exchanged for carbohydrates (46 en% versus 94 en% in fat-rich and control breakfast, respectively). The fat-rich breakfasts differed in fatty acid composition: one was rich in palmitic acid (C16:0, 21.7 g), one in stearic acid (C18:0, 18.6 g), and the other two in linoleic (C18:2) and linolenic acid (C18:3); one with a ratio of 3:1 (12.5/3.9 g) and another with a ratio of 15:1 (18.6/1.2 g). The fat-rich breakfasts consisted of a high-fat bun with 20 g margarine (90% fat) and 30 g jam, 200 mL orange juice, and 67 g cake. The control breakfast consisted of a low-fat bun with 10 g low-fat spread (3% fat) and 30 g jam, 200 mL low-fat yogurt mixed with 65 g carbohydrate-rich powder containing 62.4 g carbohydrates (Caloreen, Clintec Utrecht), 20 g sugar, one cup of tea, and 100 mL carbohydrate-rich drink containing 31 g carbohydrates (Nutralin, Nutricia Zoetermeer). Each participant received the breakfasts in a different order.

**Breakfast**

Each day, the participants received a different breakfast with similar total energy content. Four breakfasts contained 50 energy percent (en%) of fat (fat-rich breakfast); the control breakfast contained only 1.5 en% of fat. The fat-rich breakfasts differed in fatty acid composition: one was rich in palmitic acid (C16:0, 21.7 g), one in stearic acid (C18:0, 18.6 g), and the other two in linoleic (C18:2) and linolenic acid (C18:3); one with a ratio of 3:1 (12.5/3.9 g) and another with a ratio of 15:1 (18.6/1.2 g). The fat-rich breakfasts consisted of a high-fat bun with 20 g margarine (90% fat) and 30 g jam, 200 mL orange juice, and 67 g cake. The control breakfast consisted of a low-fat bun with 10 g low-fat spread (3% fat) and 30 g jam, 200 mL low-fat yogurt mixed with 65 g carbohydrate-rich powder containing 62.4 g carbohydrates (Caloreen, Clintec Utrecht), 20 g sugar, one cup of tea, and 100 mL carbohydrate-rich drink containing 31 g carbohydrates (Nutralin, Nutricia Zoetermeer). Each participant received the breakfasts in a different order.
linear regression analysis was used to evaluate the association of FVIIa with serum triglycerides.

**Results**

The FVIIa responses did not differ between the fat-rich breakfasts (Fig 1). FVIIa response to the fat-rich meals ranged from 11.6 mU/mL (95% CI: 8.3,14.9) on the stearic meal to 15.9 mU/mL (12.0,19.8) on the linoleic/linolenic 15:1 meal at 1 PM and from 14.9 mU/mL (10.6,19.2) to 21.1 mU/mL (16.6,25.6) for the same meals at 3 PM. When the results on the fat-rich breakfast were combined, FVIIa increased from 74.9 mU/mL (SD 32.3) to 88.4 mU/mL (SD 38.6) at 1 PM and to 93.0 mU/mL (SD 41.0) at 3 PM (mean response at 3 PM: 19.5 mU/mL [15.8,23.2]). After the control breakfast, FVIIa decreased, with 6.3 mU/mL (3.9,8.7) at 1 PM and 8.7 mU/mL (6.3,11.1) at 3 PM. The mean difference between the FVIIa response to the four fat-rich breakfasts combined and the response to the control breakfast in the 3 PM sample was 28.0 mU/mL (CI: 24.1,31.9).

After the control breakfast, triglyceride concentration increased slightly, up to 1.77 mmol/L (SD 0.89) in the 3 PM sample. The triglyceride responses on the fat-rich breakfasts were highest in the 1 PM sample, but were still increased in the 3 PM sample (Fig 2). The triglyceride level at 1 PM after the palmitic breakfast was 3.44 mmol/L (SD 1.64) and after the stearic breakfast 3.38 mmol/L (SD 1.60), while the level was lower after the linol 3:1 (3.07 mmol/L, SD 1.50) and linol 15:1 breakfast (3.03 mmol/L, SD 1.63). The difference at 1 PM between the palmitic and stearic breakfast compared with linol 3:1 and 15:1 breakfast ranged from 0.28 mmol/L for stearic versus linol 15:1 to 0.39 mmol/L for palmitic versus linol 3:1 (P<.05 for differences between breakfasts). There was no association between the response of FVIIa and the response to the control breakfast in the 3 PM sample was 28.0 mU/mL (CI: 24.1,31.9).

Figure 1. Response of FVIIa for each breakfast separately. Linol 3:1 indicates linoleic and linolenic acid with ratio of 3:1 and linol 15:1, linoleic and linolenic acid with ratio of 15:1.

After the control breakfast, triglyceride concentration increased slightly, up to 1.77 mmol/L (SD 0.89) in the 3 PM sample. The triglyceride responses on the fat-rich breakfasts were highest in the 1 PM sample, but were still increased in the 3 PM sample (Fig 2). The triglyceride level at 1 PM after the palmitic breakfast was 3.44 mmol/L (SD 1.64) and after the stearic breakfast 3.38 mmol/L (SD 1.60), while the level was lower after the linol 3:1 (3.07 mmol/L, SD 1.50) and linol 15:1 breakfast (3.03 mmol/L, SD 1.63). The difference at 1 PM between the palmitic and stearic breakfast compared with linol 3:1 and 15:1 breakfast ranged from 0.28 mmol/L for stearic versus linol 15:1 to 0.39 mmol/L for palmitic versus linol 3:1 (P<.05 for differences between breakfasts). There was no association between the response of FVIIa and the response to the control breakfast in the 3 PM sample was 28.0 mU/mL (CI: 24.1,31.9).

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**Discussion**

The results of this randomized controlled trial clearly demonstrate that the FVIIa increase in elderly women after a fat-rich breakfast is independent of the fatty acid composition of the meal.

The breakfasts in our study contained a high amount of fat compared with what elderly are used to eating at breakfast (approximately 15 g fat) to maximize the chance of an FVIIa response. In fact, on a normal day, FVII:C would probably hardly start to rise until after lunch. For feasibility reasons, we decided to put the amount of fat usually eaten at breakfast and lunch together in one fat-rich breakfast. Therefore, these results do not necessarily reflect the FVIIa change at these hours in a normal situation.

The participants in our study may have been a somewhat healthy selection of the general elderly population because of the inclusion criteria we used to prevent interference by medication and disease. Additionally, the subjects were selected on genotype of the R/Q353 polymorphism. In a general population, about 20% of the people carry the Q allele, which is associated with lower levels of factor VII. In our study population, 38% carried this allele, and the mean FVIIa level is therefore lower. Furthermore, since the FVIIa response to a fat-rich meal is lower in subjects carrying the Q allele than in those with the RR genotype, the increase in FVIIa after a fat-rich meal as observed in our study probably underestimates the true increase. However, when the data were analyzed for each genotype separately, again no difference in the FVIIa response between the fat-rich breakfasts was found in either genotype group. Finally, only elderly women were included in our study. Postmenopausal women have higher values of FVIIa compared with men of the same age. It is, however, not yet known whether the response of FVIIa to a fat-rich meal in postmenopausal women is also...
higher than in men. Since the response of FVIIa seen in healthy elderly women in our study was similar to that seen in young and middle-aged subjects, \textsuperscript{9–12} it may be expected that in these subjects also, no difference in FVIIa response between different fat types occurs.

For practical reasons, it was not possible to obtain more than three blood samples per day in these elderly women. Although Fig 1 suggests that after the fat-rich breakfast, FVIIa is still rising at 3 PM, it is expected from the literature that the peak in the FVIIa response occurred at some point between 1 PM and 3 PM.\textsuperscript{7,9–12}

Five studies have been published in which the effect of fatty acid composition on FVII:C was investigated\textsuperscript{4–8} (Table 3). Only one of three studies, comparing the effect on FVII:C of a saturated fat-rich meal with one rich in unsaturated fat, showed a difference between the meals.\textsuperscript{4,6} Sanders et al\textsuperscript{7} observed an increase in FVII:C after an olive oil meal compared with a meal rich in medium-chain triglycerides. Tholstrup et al\textsuperscript{8} showed no increase after myristic acid and a minor increase after stearic acid, although this finding was not significant. Summarizing, we think that the results of these small experimental studies combined with the results of our large trial support the view that the factor VII response to a high dietary fat intake is independent of the type of fat.

One of the mechanisms that could explain the effect of dietary fat on factor VII involves triglyceride-rich lipoprotein (TRLP).\textsuperscript{14} During lipolysis of TRLP, factor VII becomes activated. In our study we measured serum triglycerides as an estimate of circulating postprandial TRLP. Although the serum triglyceride response differed significantly between fat-rich breakfasts with mainly unsaturated fat and those with mainly saturated fat, this variation was not reflected in a similar difference in the FVIIa response. Furthermore, the concentration of serum triglycerides was not associated with FVIIa. This result was also observed in one previous study.\textsuperscript{12} Silveira et al\textsuperscript{10} however, did observe a positive association between FVIIa and serum triglycerides, measured 6 hours after a fat load. This discrepancy in results may indicate either that serum triglycerides do not reflect TRLP or the metabolism of TRLP is more important for the activation of factor VII than the absolute circulating amount of TRLP.

FVIIa is a very potent coagulant, which in complex with tissue factor may induce thrombomembolic occlusion of diseased blood vessels. The lower the circulating level of FVIIa, the lower the risk of atherothrombotic complications, especially in elderly persons. The postprandial rise of FVIIa should therefore be kept as low as possible. The results of our study show that this is best achieved by reducing the total amount of fat in the diet rather than by changing fat composition.

In conclusion, our study shows that in elderly women, the FVIIa response to a fat-rich meal is not dependent on the fatty acid composition and that this response is not mediated by a postprandial increase in triglycerides.

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### Table 3. Previous Studies on the Response of FVII:C to a Fat-rich Meal

<table>
<thead>
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<td>5 men, 4 women, healthy</td>
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<tr>
<td>5</td>
<td>SAFA vs PUFA</td>
<td>10 men, middle aged</td>
<td>–</td>
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<tr>
<td>6</td>
<td>SAFA vs PUFA</td>
<td>4 men, 1 woman, healthy</td>
<td>SAFA ↑</td>
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<td>Olive oil vs MCT fat</td>
<td>4 men, 2 women, age 22-43</td>
<td>Olive oil ↑</td>
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<tr>
<td>8</td>
<td>C18:0 vs C14:0</td>
<td>10 men, age 21–28</td>
<td>C18:0 ↑ (NS)</td>
</tr>
</tbody>
</table>

SAFA indicates saturated fat; PUFA, polyunsaturated fat; MCT, medium chain triglycerides; C18:0, stearic acid; C14:0, myristic acid; –, no difference; ↑, increased; and NS, not significant.

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