Postprandial response of activated factor VII in elderly women depends on the R353Q polymorphism

Louise I Mennen, Moniek PM de Maat, Gert Meijer, Peter Zock, Diederick E Grobbee, Frans J Kok, Cornelis Kluft, and Evert G Schouten

ABSTRACT
Background: Activated factor VII (FVIIa) is a very potent coagulant and may be a key determinant of the outcome of a cardiovascular event. The main determinants of FVIIa are the R353Q polymorphism and dietary fat intake, which may have an interactive effect.

Objective: The objective was to investigate whether the response of FVIIa to a fat-rich breakfast varies across genotypes of the R353Q polymorphism.

Design: Ninety-one apparently healthy elderly women (> 60 y of age), 56 with the RR genotype and 35 with the RQ or QQ genotype, participated in a randomized, controlled crossover study. Subjects received 5 breakfasts, each on a separate day: 1 low-fat control breakfast and 4 high-fat test breakfasts. Blood samples were taken for measurement of FVIIa at 0800 before each breakfast (fasting) and at 1300 and 1500.

Results: The mean (±SD) fasting FVIIa concentration was 93.3 ± 26.7 U/L in women with the RR genotype, 49.3 ± 19.1 U/L in those with the RQ genotype and 39.5 ± 17.2 U/L in those with the QQ genotype. The mean absolute response to all 4 test breakfasts was 37.0 U/L in those with the RR genotype and 16.1 U/L in those carrying the Q allele (P < 0.001 for difference). Likewise, the FVIIa response relative to fasting FVIIa was significantly higher in women homozygous for the R allele.

Conclusion: This observation may indicate a considerable difference in cardiovascular risk between genotype groups as a result of an increase in FVIIa after a fat-rich diet.


KEY WORDS Coagulation, diet, genetics, elderly women, factor VIIa, factor VII, cardiovascular risk, R353Q genotypes

INTRODUCTION
Coagulation factor VII circulates in plasma mainly as an inactive zymogen; ≈1% of it circulates as activated factor VII (FVIIa). FVIIa is, together with tissue factor, a very potent coagulant, and it is conceivable that a small increase in FVIIa may lead to a pronounced elevation of the risk of a cardiovascular event. This risk may be even more important in elderly people, in whom the prevalence of atherosclerosis is high. Until recently, FVIIa could not be measured. Instead, a functional clotting assay was used (FVII:C), which reflects FVIIa and an unknown part of FVII zymogen. In 2 longitudinal studies a positive association between FVII:C and the risk of fatal ischemic heart disease was observed (1, 2).

In the gene coding for FVII the R353Q polymorphism is the result of a single base change in the codon for amino acid 353 and leads to the replacement of arginine (R) with glutamine (Q) (3). Presence of the Q allele is associated with low concentrations of FVII (3).

Another major determinant of FVIIa is dietary fat intake. Several intervention studies that included mostly small numbers of middle-aged men showed an increase in FVIIa after a fat-rich meal (4–8). It is not yet clear whether this response of FVIIa to fat intake is affected by the R353Q polymorphism. The only study performed on this included only 8 subjects carrying the Q allele. The results showed an absolute difference in the postprandial FVIIa increase, but no difference between the genotypes was observed when the FVIIa increase was calculated relative to the fasting FVIIa concentration (5). We studied the FVIIa response to fat-rich meals in a large number of apparently healthy elderly women who were selected on the basis of their genotype for the R353Q polymorphism.

SUBJECTS AND METHODS
Subjects
Elderly women living independently in retirement communities in Netherlands were asked to participate in the study. The women had to fulfill the following inclusion criteria: were older than 60 y of age, had no diabetes mellitus, had had no myocardial infarction, and had no other major medical disorders.

Postprandial response of activated factor VII in elderly women depends on the R353Q polymorphism

Louise I Mennen, Moniek PM de Maat, Gert Meijer, Peter Zock, Diederick E Grobbee, Frans J Kok, Cornelis Kluft, and Evert G Schouten

ABSTRACT
Background: Activated factor VII (FVIIa) is a very potent coagulant and may be a key determinant of the outcome of a cardiovascular event. The main determinants of FVIIa are the R353Q polymorphism and dietary fat intake, which may have an interactive effect.

Objective: The objective was to investigate whether the response of FVIIa to a fat-rich breakfast varies across genotypes of the R353Q polymorphism.

Design: Ninety-one apparently healthy elderly women (> 60 y of age), 56 with the RR genotype and 35 with the RQ or QQ genotype, participated in a randomized, controlled crossover study. Subjects received 5 breakfasts, each on a separate day: 1 low-fat control breakfast and 4 high-fat test breakfasts. Blood samples were taken for measurement of FVIIa at 0800 before each breakfast (fasting) and at 1300 and 1500.

Results: The mean (±SD) fasting FVIIa concentration was 93.3 ± 26.7 U/L in women with the RR genotype, 49.3 ± 19.1 U/L in those with the RQ genotype and 39.5 ± 17.2 U/L in those with the QQ genotype. The mean absolute response to all 4 test breakfasts was 37.0 U/L in those with the RR genotype and 16.1 U/L in those carrying the Q allele (P < 0.001 for difference). Likewise, the FVIIa response relative to fasting FVIIa was significantly higher in women homozygous for the R allele.

Conclusion: This observation may indicate a considerable difference in cardiovascular risk between genotype groups as a result of an increase in FVIIa after a fat-rich diet.


KEY WORDS Coagulation, diet, genetics, elderly women, factor VIIa, factor VII, cardiovascular risk, R353Q genotypes

INTRODUCTION
Coagulation factor VII circulates in plasma mainly as an inactive zymogen; ≈1% of it circulates as activated factor VII (FVIIa). FVIIa is, together with tissue factor, a very potent coagulant, and it is conceivable that a small increase in FVIIa may lead to a pronounced elevation of the risk of a cardiovascular event. This risk may be even more important in elderly people, in whom the prevalence of atherosclerosis is high. Until recently, FVIIa could not be measured. Instead, a functional clotting assay was used (FVII:C), which reflects FVIIa and an unknown part of FVII zymogen. In 2 longitudinal studies a positive association between FVII:C and the risk of fatal ischemic heart disease was observed (1, 2).

In the gene coding for FVII the R353Q polymorphism is the result of a single base change in the codon for amino acid 353 and leads to the replacement of arginine (R) with glutamine (Q) (3). Presence of the Q allele is associated with low concentrations of FVII (3).

Another major determinant of FVIIa is dietary fat intake. Several intervention studies that included mostly small numbers of middle-aged men showed an increase in FVIIa after a fat-rich meal (4–8). It is not yet clear whether this response of FVIIa to fat intake is affected by the R353Q polymorphism. The only study performed on this included only 8 subjects carrying the Q allele. The results showed an absolute difference in the postprandial FVIIa increase, but no difference between the genotypes was observed when the FVIIa increase was calculated relative to the fasting FVIIa concentration (5). We studied the FVIIa response to fat-rich meals in a large number of apparently healthy elderly women who were selected on the basis of their genotype for the R353Q polymorphism.

SUBJECTS AND METHODS
Subjects
Elderly women living independently in retirement communities in Netherlands were asked to participate in the study. The women had to fulfill the following inclusion criteria: were older than 60 y of age, had no diabetes mellitus, had had no myocardial infarction, and had no other major medical disorders.
**TABLE 1**

General characteristics of the female study population according to genotype

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>RR (n = 56)</th>
<th>RQ (n = 32)</th>
<th>QQ (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>75.7 ± 5.2</td>
<td>75.6 ± 5.2</td>
<td>76.0 ± 5.2</td>
<td>76.0 ± 6.1</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27.7 ± 4.1</td>
<td>28.0 ± 3.9</td>
<td>27.4 ± 4.5</td>
<td>26.1 ± 4.5</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>142 ± 19.4</td>
<td>142 ± 21.9</td>
<td>142 ± 15.2</td>
<td>133 ± 18.6</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>72 ± 9.5</td>
<td>73 ± 9.5</td>
<td>71 ± 9.0</td>
<td>68 ± 17.8</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>6.03 ± 0.86</td>
<td>6.12 ± 0.86</td>
<td>5.90 ± 0.87</td>
<td>5.87 ± 0.34</td>
</tr>
<tr>
<td>Serum triacylglycerol (mmol/L)</td>
<td>1.70 ± 0.67</td>
<td>1.72 ± 0.63</td>
<td>1.69 ± 0.76</td>
<td>1.47 ± 0.51</td>
</tr>
<tr>
<td>Activated factor VII (U/L)</td>
<td>75.2 ± 32.1</td>
<td>93.3 ± 26.7</td>
<td>49.3 ± 19.1</td>
<td>39.5 ± 17.2</td>
</tr>
</tbody>
</table>

1 ± SD. The Q allele had a frequency of 0.09 in the source population of 321 women.
2 Fasting concentrations.
3 Significant among the genotype groups, *P* < 0.05.

The genotype for the R353Q polymorphism was assessed in 321 women. Thirty-five of the 55 women found to carry the Q allele were able to participate and 56 women with the RR genotype were randomly selected. Thus, a total of 91 women were included in the intervention study. The study was approved by the Medical Ethics Committee of the Wageningen Agricultural University and written informed consent was obtained from all participants.

### Intervention

Participants received 5 breakfasts, each on a different day. At 0800 (after an overnight fast) the first blood sample was taken and within 0.5 h the subjects ate their breakfast. Additional blood samples were taken at 1300 and 1500. The participants stayed in the same room during the entire procedure and received fat-free foods after the breakfast if they requested them.

All breakfasts had the same total energy content, but 4 breakfasts contained 50% of energy as fat (test breakfast), whereas the control breakfast contained only 1.5% of energy as fat (fat was exchanged for carbohydrates). The test breakfasts consisted of a high-fat bun with 20 g margarine 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 and 30 g jam, 200 mL low-fat yogurt mixed with 65 g energy-rich powder (Caloreen; Clintec, Utrecht, Netherlands), 20 g sugar, 1 cup of tea, and 100 mL energy-rich drink (Nutrical; Nutricia, Zoetermeer, Netherlands). For the purposes of a separate research question, the test breakfasts differed in fatty acid composition (1 was rich in palmitic acid, 1 in oleic acid, and 2 in linoleic and linolenic acids, either with a ratio of 3 to 1 or with a ratio 15 to 1), but we did not observe a difference in FVIIa response between these test breakfasts (9).

### Measurements

Blood was collected in siliconized evacuated tubes containing 0.129 mol sodium citrate/L (Becton Dickinson, Meylan, France). Samples were centrifuged for 30 min at 1500 × g and 20°C. Citrated plasma was snap frozen and stored at −80°C until analyzed. FVIIa was measured on an STA instrument (Boehringer, Mannheim, Germany) with a clotting assay using soluble recombinant tissue factor (Staclot; Diagnostica Stago, Asnières, France) (10). The recorded clotting time is inversely related to the FVIIa activity expressed as U/L. The CV of this measurement was 8% and the intraindividual variation (calculated with measurements of 5 fasting blood samples for each person) was 2.09 U/L.

DNA was extracted from mouth swabs according to Meulenberg et al (11). DNA polymorphisms were determined as described previously (12). Briefly, genomic DNA was amplified by using the polymerase chain reaction with oligonucleotide primers as described by Lane et al (13). The reaction components were incubated at 94°C for 4 min, followed by 32 cycles of 94°C for 1 min, 59°C for 1.5 min, and 72°C for 2 min. Ten milliliters polymerase chain reaction product were digested with 5 U MspI (Gibco BRL, Rockville, MD) at 37°C. MspI digestion yielded a constant 40–base pair (bp) band. The common R allele gave bands of 205 bp and 67 bp and the Q allele gave a band of 282 bp as described previously (3). Because the number of persons with the QQ genotype was too small for meaningful analysis, they were combined with subjects having the RQ genotype.

### Data analyses

The response of FVIIa to each meal was calculated by subtraction of the fasting FVIIa concentration from the concentrations at 1300 and 1500. The mean of the responses of FVIIa to the 4 test breakfasts was used in this study because there was no significant difference in response of FVIIa between the test breakfasts (9). To take into account what would have happened without the fat in the meal, the response to the control breakfast at 1500 was subtracted from the mean response to the test breakfasts at 1500. We called this the mean absolute response. The mean relative total response is the mean absolute response as a percentage of the fasting FVIIa concentration. With paired Student’s *t* tests, we evaluated whether the mean absolute and relative total responses differed between the genotype groups. To allow for further adjustment for the fasting concentration of FVIIa, a linear regression analysis was performed with the mean absolute response as the dependent variable and the genotype group and fasting FVIIa concentration as the independent variables. The statistical analysis program SAS (version 6.11; SAS Institute, Cary, NC) was used for the analyses.

### RESULTS

General characteristics of the study population according to genotype are presented in Table 1. The frequency of the Q allele in the source population of 321 women was 0.09. Compared with women homozygous for the R allele, fasting FVIIa was...
47% lower (49.3 compared with 93.3 U/L) in women with the RQ genotype and 58% lower (39.5 compared with 93.3 U/L) in women homozygous for the Q allele.

The absolute total response of FVIIa at 1300 and 1500 is shown in Figure 1 for the genotype groups separately. The mean absolute response of FVIIa was 37.0 U/L in the group with the RR genotype and 16.1 U/L (P < 0.001) in those carrying the Q allele. Note that the R353Q polymorphism was an independent predictor of the FVIIa response even after adjustment for fasting FVIIa concentration in the linear regression model (β = −11.8 U/L; 95% CI: −20.0, −3.6). The mean relative total response in FVIIa was 42% and 32% (P = 0.01 for difference) in women homozygous for the R allele and in those carrying the Q allele, respectively.

**DISCUSSION**

The results of this study in elderly women clearly show that the absolute as well as the relative response to a fat-rich meal depends on the genotype of the R353Q polymorphism. The subjects in this study were selected on the basis of genotype to include similar numbers of women with the RR and RQ or QQ genotypes. The frequency of the Q allele in the source population of 321 women, however, was similar to the frequency reported previously in European whites (12, 14).

The rise in FVIIa found in our study may reflect a true increase in FVIIa or may reflect an increase in the amount of lipids in the blood samples. It may be that the amount of triacylglycerol in the sample influences the measurement of FVIIa. Although there have been a few studies investigating the influence of lipids on the measurement of factor VII:C, none included measurement of FVIIa, and results were inconsistent (5, 15). Furthermore, if the measurement of FVIIa were dependent on the amount of triacylglycerol, we would expect a clear association between serum triacylglycerol and FVIIa in our study. This was not the case, however, in any of the blood sampling occasions (9).

**FIGURE 1.** Mean activated factor VII (FVIIa) in elderly women before and after test and control breakfasts according to genotype.

![Graph showing the absolute total response of FVIIa](image-url)
In 5 earlier studies an increase in FVIIa was found after a fat-rich meal (4–8). Miller et al (4) observed a 19% increase in FVIIa 6.5 h after a high-fat (64% of energy as fat) meal and no response to a low-fat (9% of energy as fat) meal. In a study by Sanders et al (7), it was found that FVIIa increased in response to 90 and 120 g of fat, with a peak at 7 h, but not in response to 15 or 60 g of fat. Kapur et al (8) found an increase in FVIIa 6 h after a fat-rich meal (30 g/m²). Two studies by Silveira et al (5, 6) in 31 male postinfarction patients and 24 healthy men showed an increase in FVIIa after 6 h during a fat-tolerance test. The absolute increase in FVIIa was higher in postmyocardial infarction patients with the RR genotype than in patients carrying the Q allele. However, in contrast with our study, the percentage increase in FVIIa did not differ between the genotypes (5). This discrepancy may be explained by different types of subjects: men with myocardial infarction in the study of Silveira et al (5) and healthy elderly women in our study. However, we think it is more likely that Silveira et al (5) did not find a difference in percentage response between the genotypes because they studied a much smaller number of subjects (31, of whom 8 had the RQ genotype).

One of the mechanisms that may explain an effect of dietary fat on factor VII is based on activation of factor VII by negatively charged surfaces during lipolysis of triacylglycerol-rich lipoproteins (16). The fact that our data show differences in activation for the R and Q alleles, independent of the fasting activity of FVIIa, suggest a molecular difference between the 2 types of FVIIa in this respect. The results of our study do not clarify how the R353Q polymorphism could influence the activation of FVII, but it may be that the change in charge ensuing from the substitution of the positively charged arginine with a neutral glutamine affects the interaction of FVII with lipid surfaces (17). Furthermore, the R353Q polymorphism and the −323Ins10 polymorphism in the promoter region of the FVII gene show strong linkage disequilibrium and allelic association (18). Although a polymorphism in the promoter region is more likely to affect basal FVIIa, we cannot exclude the possibility that in fact this polymorphism may cause the difference in the postprandial FVIIa increase.

In conclusion, the results of this study suggest that FVIIa increases profoundly after a fat-rich meal in elderly women with the RR genotype of the R353Q polymorphism. Because this may contribute to the risk of a cardiovascular event or modify its prognosis, a diet with a low to moderate fat intake may be recommended for elderly subjects with the RR genotype.

We thank all participants of this study for their enthusiastic cooperation. Furthermore, we thank Linda Huisman for the analysis of blood lipids, factor VII, and DNA, and Suzanne van Gaans, Celine van der Hoofd, and Marlon Louisa for their assistance during the data collection.

REFERENCES