Coagulation Factor VII in Elderly People: Genetic Disposition and Diet

Louise Mennen

Promotoren: Dr. ir. F.J. Kok
Hoogleraar in de Humane Epidemiologie

Dr. D.E. Grobbee
Hoogleraar in de Klinische Epidemiologie

Co-promotor: Dr. E.G. Schouten
Universitair hoofddocent bij de Afdeling Humane Voeding en Epidemiologie

Louise Ingeborg Mennen

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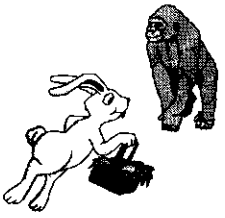
Never let a malignant dominant male destroy your "joie de vivre"!
(J.G. van der Bom, Schiermonnikoog, november 1994)



This is not Bird-science we're talking here!
(O.S. Miettinen, Schiermonnikoog, november 1994)

Aan mijn fantastische Ouders en mijn bijzondere Oma!

ABSTRACT

Coagulation factor VII in elderly people: genetic disposition and diet
PhD thesis by Louise I. Mennen, Division of Human Nutrition and Epidemiology Wageningen Agricultural University, The Netherlands. September 17, 1997

In elderly people an increase in thrombotic tendency may lead to a increase in the risk of a coronary event. Coagulation factor VII affects this thrombotic tendency and has been recognised as a risk indicator for coronary heart disease. It is not known whether the level of factor VII can be influenced at older age. From studies in young subjects it is clear that dietary fat and the R/Q353 polymorphism (alleles R and Q) are the main determinants of factor VII. We studied the relation of factor VII with diet in elderly men and women, taking the R/Q353 polymorphism into account.
In a cross-sectional study among 1158 elderly men and women (>55 y) factor VII coagulant activity (FVII:C) and total factor VII (FVIIt) were investigated in relation to serum-triglycerides, the R/Q353 polymorphism and the habitual diet. FVII:C was inversely associated with dietary fibre and protein and positively with saturated fat intake and serum-triglycerides. These associations were much stronger in subjects with the RR genotype compared to those carrying the Q allele; if the mean intake of dietary fibre would increase with 10 g a day, FVII:C would be expected to decrease with 7.6 % in elderly people homozygous for the R allele versus only 1.9 % decrease in those carrying the Q allele. FVIIt was inversely related to intake of dietary fibre and positively to serum-triglycerides, again the associations being stronger in subjects with the RR genotype.
In a cross-over study among elderly women (> 60 y, 35 RQ/QQ, 56RR) the postprandial response of activated factor VII (FVIIa) to several fat-rich (50 g) breakfasts was evaluated. The response of FVIIa was very similar for meals rich in palmitic acid, rich in stearic acid or rich in linoleic/linolenic acid with a ratio of 3:1 or 15:1. The increase in FVIIa ranged from 14.9 (95% CI:10.6,19.2) IU/mL after the stearic rich breakfast to 21.1 (16.6,25.6) IU/mL after the linoleic/linolenic 15:1 rich breakfasts. After the fat-free control breakfast FVIIa decreased with 8.7 (6.3,11.1) IU/mL. The mean absolute total response to the fat-rich breakfasts combined was 37 IU/mL in subjects with the RR genotype and 16.1 IU/mL in subjects carrying the Q allele. Also the response relative to the fasting FVIIa level differed significantly between the genotype groups (RR: 42%, RQ/QQ: 32%). Serum-triglycerides concentration was not associated with FVIIa.
In elderly people, factor VII is influenced by dietary fibre, total dietary fat and serum-triglycerides and not by fat type. The R/Q353 polymorphism strongly modifies these effects. This indicates that an increase in dietary fibre and a decrease in dietary fat intake may reduce the risk of a coronary event by reducing the level of factor VII, particularly in elderly people with the RR genotype.

CONTENTS

CHAPTER 1

General introduction

Coagulation cascade

Haemostasis is a defense mechanism that serves to protect the integrity of the vascular system after injury of the endothelium of the vessel wall. The haemostatic process consists of platelet activation, blood coagulation and fibrinolysis. This system is normally quiescent but becomes active within seconds after injury. Together with platelet activation, triggering of the coagulation system results in the formation of a blood clot and occlusion of the injured site. The fibrinolytic system dissolves the haemostatic plug in the course of vessel healing and repair.

If the balance between coagulation and fibrinolysis is disturbed, extensive clot formation can take place, which may influence the development of cardiovascular disease[1-4]. The coagulation system is a sequential activation of certain plasma proteases (proenzymes) to their enzyme forms. This cascade of activation reactions will finally lead to the formation of fibrin. The coagulation system as such will be clarified in detail below, but for reason of simplicity not all inhibitors or external factors influencing the cascade are described.

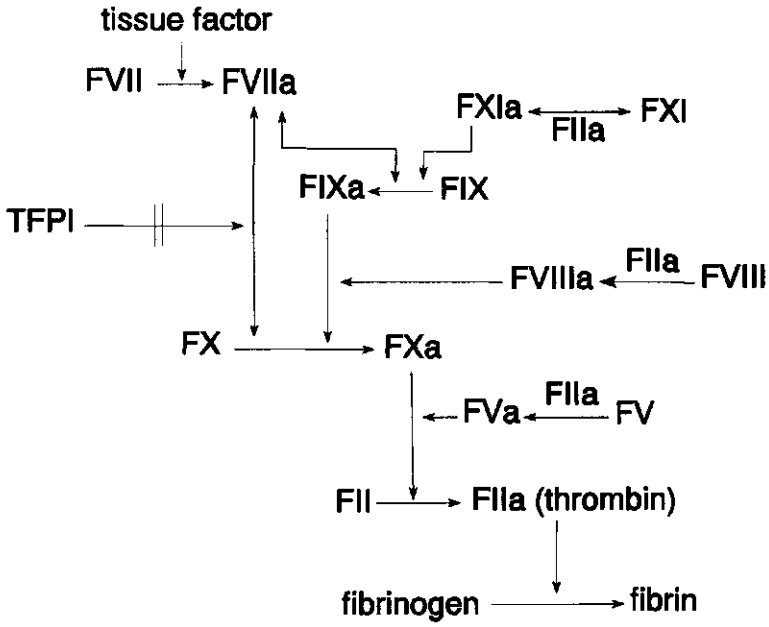
After injury, blood coagulation is activated by the release of tissue factor from the vessel wall (Figure 1.1). Tissue factor binds to factor VII and forms a complex with it. If factor VII is activated it will activate factor X to Xa and factor IX to IXa. Factor IXa will form a complex with factor VIIIa and this complex is a second activator of factor X[5].

The protease responsible for the initial activation of factor VII is unknown, but once clotting is activated, several proteases further along the pathway, like factor Xa and IXa, can in turn activate factor VII. This will lead to an acceleration of the factor VII activation. Although factor VIIa is able to activate factor X, the activation of factor X by IXa is the main route, since the factor VIIa/tissue factor complex will be rapidly inhibited by tissue factor pathway inhibitor (TFPI). Once thrombin is formed the activation of factor XI is catalyzed. Once factor XIa is generated, there is an additional mechanism for augmenting the activation of factor IX, which makes the factor VII/tissue factor complex at that moment redundant for the activation of factor X.

Factor Xa in complex with factor Va activates prothrombin into thrombin (factor IIa). Thrombin cleaves fibrinogen into monomeric fibrin, which then polymerizes to form the fibrin clot. This whole process takes place on different cell-surface membranes, which play an important role in the formation of several complexes[5].

In short the tissue factor/factor VII complex is needed to activate factor X and start the initial generation of thrombin, after which the activation of factor IX and factor XI will result in an explosive formation of fibrin.

Figure 1.1. The coagulation cascade



Factor VII

Factor VII is one of the coagulation factors found to be associated with cardiovascular disease[2,6]. Two prospective studies have shown that factor VII is higher in persons who died from coronary heart disease than in persons without the disease[2,6]. It is conceivable that a small increase of FVIIa may lead to an extensive formation of fibrin and thereby increase the chance of a vessel occlusion after rupture of an atherosclerotic plaque.
To increase understanding of the behaviour of factor VII in relation to its determinants some biochemical aspects of factor VII will be described.
Factor VII is a single-chain glycoprotein, with a molecular weight of approximately 50,000[7]. This mature protein is composed of 406 amino acids[8]. It is a vitamin K dependent coagulation factor and has a high degree of homology with factor II, IX, X, protein C and protein S. It is synthesized by the liver and secreted into the blood as an inactive zymogen. It has the most rapid turnover rate of any coagulation factor, with a half-life of 2 to 7 hours after activation[7]. The single chain form of factor VII as found in plasma

becomes activated (factor VIIa) after enzymatic cleavage and has a 60- to 80-fold increased coagulant activity[7]. Cleavage can be performed by factor Xa, factor XIIa, factor IXa and thrombin[8-10]. This happens normally when the vessel wall is damaged and tissue factor is released, but also cold storage and contact activation with glass can activate factor VII zymogen[7]. In each case, a single Arg 152 - Ile 153 internal bond is cleaved, which results in the formation of a two-chain active enzyme composed of a light chain and a heavy chain held together by a disulphide bond.
About 1% of the total amount of factor VII in the circulation exists as activated factor VII, the other part exists as the inactive factor VII zymogen. Several methods are available to measure factor VII. The activated part of factor VII can be measured directly (factor VIIa), but also the coagulant activity of factor VII (factor VII:C) can be analyzed. Furthermore two methods exist to measure the total amount of factor VII (factor VIIt) in plasma. These methods will be described in more detail in Chapter 2.

Determinants of factor VII

Factor VII increases with age and with use of oral anticonceptives or hormone replacement therapy. It is positively associated with body mass index and is higher in women than in men especially after menopausal age[11-14]. Furthermore, factor VII was found to be associated with blood pressure, alcohol intake, smoking, physical exercise and intake of caffeine[11,12,15-18], but these findings are not consistent. The major known determinants of factor VII, however, are diet, blood lipids and genetic factors[19-23].
One cross-sectional study among rural Finnish men showed a positive correlation of total fat intake and fasting factor VII[24]. Results from intervention studies show that factor VII increases when the intake of dietary fat increases. This effect is seen in long-term studies on fasting factor VII as well as in short-term studies on postprandial factor VII level[19,20,25-32]. The effect of the fatty acid composition of the diet used in these intervention studies is not quite clear. In some studies a difference in factor VII between diets which differ in fatty acid composition was found, while this is not the case in other studies[20,25,28,29,32]. These studies will be extensively described in Chapter 2.
Other dietary factors which may influence factor VII are dietary fibre and protein. Factor VII was found to be inversely related to dietary fibre and positively to protein[33-36], but clear evidence is missing.
In many cross-sectional studies a positive association between serum-triglycerides and factor VII has been observed[16,37-43]. This relation is much less apparent in intervention studies on the effect of dietary fat on factor VII in which triglyceride concentration is

measured as an intermediating factor[19,20,25-27].
The R/Q353 polymorphism in the gene coding for factor VII is the result of a single base change in the codon for amino acid 353 and leads to the replacement of arginine (R) by glutamine (Q)[23]. The Q allele is associated with lower levels of factor VII.
The combined effect of diet and genetic factors was investigated in a very small intervention study among coronary heart disease patients[106]. It was found that the response of factor VII on a high-fat meal was much higher in patients with the RR genotype compared to those with the RQ genotype. This suggests that the R/Q353 polymorphism may modify the relation of factor VII with diet and blood lipids, but there is no extensive evidence for this hypothesis.

Rationale and outline of this thesis

Arterial thrombosis becomes more important in elderly people, since they have a high prevalence of atherosclerosis. Elevated levels of factor VII, in combination with the existing atherosclerosis, may increase their risk of a coronary event. However, no information on factor VII in elderly is available and it is not known whether in elderly people factor VII can be altered by diet. Therefore, we studied factor VII in relation to its major determinants in elderly people.
A cross-sectional study and an intervention study were performed to answer the following questions:

1a. What is the magnitude of the association of factor VII:C and factor VIIt with diet and serum-triglycerides in elderly men and women?
b. Do these associations vary across genotype groups of the R/Q353 polymorphism?
2a. Is, in elderly women, the response of factor VIIa on a fat-rich meal dependent on the fatty acid composition of the meal?
b. Do these responses vary across the genotype groups of the R/Q353 polymorphism?

This thesis describes the results of a cross-sectional and an intervention study. To give background information on the relation of factor VII with dietary fat, the next chapter describes the state of the art on this topic at the start of the project. In Chapter 3 a cross-sectional study on the relation of factor VII with diet in a large population of elderly people is evaluated. The effects of the R/Q353 polymorphism on the association of factor VII with diet and blood lipids in subjects from the extreme quintiles of the factor VII distribution are described in Chapter 4 and 5. Finally the postprandial effect of a fat-rich meal on factor VII was investigated in a randomized cross-over trial in a large group of

elderly women. This study is described in Chapters 6 and 7. A general discussion on factor VII, dietary fat and genetic disposition can be found in Chapter 8.

CHAPTER 2

Coagulation factor VII, dietary fat and blood lipids: a review

L.I. Mennen, E.G. Schouten, D.E. Grobbee, C. Kluft

Thromb Haemost 1996;76:492-499

Introduction

There is considerable, though inconclusive evidence, which suggests that coagulation factor VII is a risk indicator for cardiovascular disease. In the prospective Northwick Park Heart Study (NPHS) high levels of factor VII coagulant activity (FVII:C) were associated with an increased risk of ischaemic heart disease, which was stronger than the association between high levels of cholesterol and ischaemic heart disease[1]. Later this association was found to be confined to fatal events only[6]. Another prospective study (PROCAM) found a trend towards higher FVII:C values when only fatal events were taken into account[2]. Differences in measurement technique may explain the stronger association in the NPHS compared to the PROCAM study. In both studies a one-stage clotting assay was used, but with different factor VII-deficient substrate plasma's. When activated factor VII (FVIIa) concentrations were determined directly, the correlation between FVII:C and FVIIa was considerably greater for the NPHS assay than for the PROCAM assay. If the thrombotic response is determined by the circulating concentration of FVIIa, then the NPHS assay seems to be more specific for investigating the relation between factor VII and cardiovascular disease[44]. A second explanation for the stronger association in the NPHS might be the practice of the NPHS of obtaining blood samples in the non-fasting state, with the possibility of increases in FVIIa[28]. In the PROCAM study, samples were obtained from fasting individuals.

Also, some cross-sectional studies were carried out to investigate the association between factor VII and cardiovascular disease. In several studies patients with prior infarction had significantly elevated levels of FVII:C compared with patients without such a history[45-48]. The Atherosclerosis Risk in Communities Study (ARIC) showed significantly higher FVII:C in women with cardiovascular disease, but not in men[45]. Total factor VII (FVIIt) was elevated in first degree relatives of patients with premature ischemic heart disease compared to age-matched subjects without known risk factors[49]. Furthermore, patients with coronary artery disease and patients at high risk for this disease had higher levels of FVIIt compared to controls[50,51]. Finally, patients aged 45-64 years with thickened carotid arterial wall had a higher FVII:C[52], which was not observed in younger patients[53].

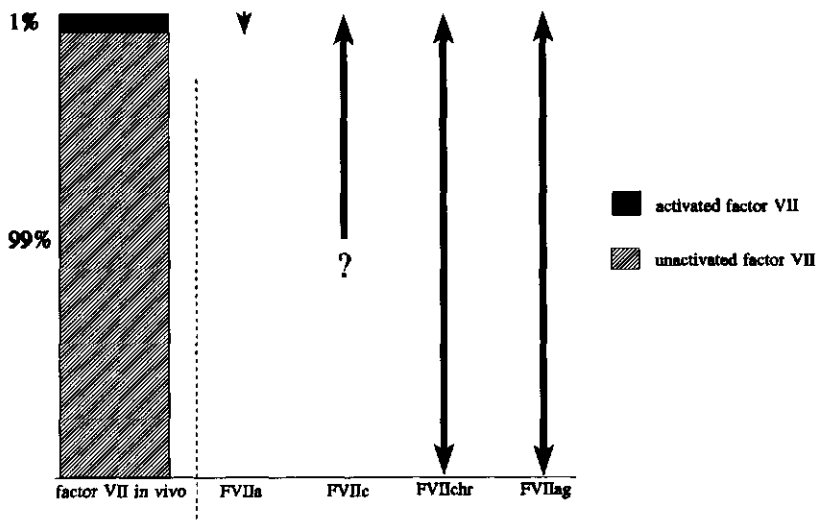
With a view to prevention of cardiovascular disease knowledge of the determinants that influence the known risk indicators is important. For factor VII the most important determinants seem to be genotype, age, gender, insulin resistance, body mass index, dietary fat and blood lipids[11,12,19,20,23,54-59]. Of these, dietary fat and blood lipids are subject to change and potential candidates for lowering the level of factor VII. To summarize current evidence with respect to dietary influence on factor VII, in this paper recent studies on factor VII, dietary fat and blood lipids will be reviewed. In order to make the

article more comprehensible, the available methods for measurement of factor VII will be described first, after which some attention will be paid to the mechanisms underlying the relation between factor VII and dietary fat and blood lipids. A distinction between acute and chronic effects of dietary fat is made, as the underlying mechanisms for these effects may be different. The acute effects are described for postprandial factor VII levels (measured in blood samples collected in the non-fasting state) and the chronic effects are described for the fasting factor VII levels (measured in blood samples collected in the fasting state).

Measurement of factor VII

In the circulation the main portion of factor VII occurs in an inactivated form (factor VII zymogen) and a small portion in the activated form. There are several methods for measurement of factor VII (Figure 2.1).
A recent technique is derived from the one stage-clotting assay, using a mutant thromboplastin, which does not activate factor VII during the measurement. With this technique the activated part of factor VII (FVIIa) can be measured directly[60]. FVII:C (measured by the one-stage-clotting assay) reflects the activated part of factor VII in the blood sample and some factor VII zymogen which is inadvertently activated by this technique.

Figure 2.1. Measurement of factor VII



FACTOR VII ASSAYS

For this analysis thromboplastin of several origins, i.e. rabbit, bovine and human, can be used, resulting in incomparable FVII:C levels[61,62]. Only bovine thromboplastin is hardly capable of activating factor VII zymogen and measurement of FVII:C with this thromboplastin could be seen as method to evaluate FVIIa[63,64]. Furthermore, the incubation time of the thromboplastin with factor VII and the factor VII-deficient plasma, will influence the outcome of the clotting assay[44,65]. Total factor VII can be determined by the chromogenic assay in which all factor VII zymogen is activated and the total activity is measured. Another method to assess the total amount of factor VII is by immunologic assay, which does not discriminate between factor VII zymogen and FVIIa[66]. Both last assays reflect the total amount of factor VII molecules (factor VII mass) present in the blood sample and should give similar results[61].

Mechanisms behind the relation between factor VII and dietary fat

Three different mechanisms are suggested by several authors to explain the relation between dietary fat and factor VII. Some of these mechanisms explain only the relation with FVIIt, others only with FVII:C.

Binding of factor VII

Carvalho et al[67] suggest that dietary fat has an effect on factor VII through prolonged half-life time of factor VII in the circulation, caused by binding of factor VII protein with triglyceride rich lipoproteins (TRLP). In an *in vitro* study binding of very low density lipoproteins (VLDL), low density proteins (LDL), high density proteins (HDL) and chylomicrons with FVII was observed[67]. The bond of FVII with LDL was less strong than with the other lipoproteins. No evidence has been obtained for binding *in vivo*, because no FVIIt was found in the isolated lipoproteins. However, the labile adsorption of FVIIt may have been abolished by the ultracentrifugal procedure, which was used for isolation of the lipoproteins. These data support only part of the theory; a binding between FVIIt and TRLP in vitro was found, whether this binding indeed prolongs the half-life time of factor VII protein has not been established.

Induced synthesis of vitamin K-dependent clotting factors

Another possibility for increased factor VII zymogen might be enhanced synthesis in the liver. It is suggested that activation of the extrinsic pathway by increased generation of prothrombin fragment 1+2 may lead to stimulation of the hepatic production of factor VII and other vitamin K dependent proteins[27,68]. This was shown in rabbits where intravenous injection of the fragment 1 region of prothrombin is associated with a transient increase in

the plasma concentration of factor II and factor X[69]. The positive association of vitamin K-dependent clotting factors with total cholesterol, LDL-cholesterol and triglycerides also supports this hypothesis[22]. In most studies on the effect of dietary fat on factor VII, however, other vitamin K-dependent clotting factors have not been measured, with the exception of factor X. The whole set of vitamin K-dependent clotting factors should be considered when this mechanism is further investigated.

Activation of factor VII by negatively charged surfaces

A third option for the mechanism behind the association of dietary fat with FVII:C is activation of factor XII, and thereby factor VII, by the negatively charged surface of the TRLP[70]. Several *in vitro* experiments showed that when stearic acid or vesicles, with a negative charge and size similar to TRLP, were added to plasma, FVII:C increased in a dose-dependent manner. Oleic acid and other unsaturated fatty acids had no effect on FVII:C[71,72]. Lipoprotein lipase (LPL) seems to be important in this reaction; when plasma of hypertriglyceridaemic patients with LPL deficiency was treated with LPL factor XII was promptly activated[73]. Thus, long-chain saturated fatty acids in the interface of lipoprotein remnants, produced by the interaction of TRLP with lipoprotein lipase (LPL), appear to provide the negatively charged surface that activates factor VII.

Chronic effects of dietary fat

Several experimental studies have recently been carried out to examine the effect of fat rich diets on fasting FVII:C or FVIIt (Table 2.1). Effects of high-fat and low-fat diets were compared and effects of diets rich in saturated or unsaturated fat on FVII:C or FVIIt were examined. Some studies focused on the effect of fish or fish-oil on FVII:C.

Effects of high-fat vs low-fat diets

Three randomized cross-over studies[36,74,75], one controlled[76] and one uncontrolled[25] intervention study in healthy adults compared high-fat with low-fat diets with respect to FVII:C. In all studies, except one[36], the diets were isocaloric and the ratio of polyunsaturated and saturated fat was higher in the low-fat diets compared to the high-fat diets. The differences in fat intake between the two experimental diets ranged in these studies from 7 en% to 50 en%. In two studies the change in fat was accompanied with a change in fibre intake, which must be taken in mind when comparing the results, since fibre intake might lower FVII:C[75,76]. FVII:C was significantly lower after the low-fat diet compared to the high-fat diet in four studies[25,36,75,76]. Marckmann et al[74] could not find an effect of either diet on FVII:C, but the difference between the high and

Table 2.1. Chronic effects of dietary fat

DIET	REF.	SUBJECTS	DESIGN	FVII:C	FVIIt
low-fat (A) vs high-fat (B)	74	6 males, 7 females, healthy, age 21-37	randomized cross-over	no difference	
	75	10 males, 11 females (2 using HRT), mean age 53 ± 4, mean BMI 23.9 ±2.4	randomized cross-over	A 8% lower	no difference
	36	6 healthy subjects, age 26-48	randomized cross-over	A 19% lower	
	76	32 healthy male students	controlled intervention	A 9% lower	
	25	17 females (some using contraceptives)	uncontrolled intervention	A 12% lower	no difference
	20	9 males, 2 females, age 21-31, BMI 18.7-27.2	randomized cross-over	A 13% lower	A 13% lower
	54	21 males, 17 females, age 21-30	randomized cross-over	no difference	
SAFA vs MUFA vs PUFA	20	9 males, 2 females, age 21-31, BMI 18.7-27.2	randomized cross-over	no difference	no difference
	54	21 males, 17 females, age 21-30	randomized cross-over	no difference	
	78	12 males, 16 females, age 38-66, BMI 18.3-31.9	randomized cross-over	no difference	
	80	103 males, age 52-54	controlled intervention	no difference	
stearic acid (A) vs other SAFA's (B)	27	15 healthy males, age 20-30	randomized cross-over	A 10% lower	A 10% lower
	26	15 healthy males, age 22-30, BMI 20.4-26.4	randomized cross-over	A 17% lower	
palmitic acid (A) vs myristic acid (B)	26	15 healthy males, age 20-30, BMI 20.4-26.4	randomized cross-over	no difference	
	79	12 healthy males, age 21-26, BMI 20.2 -25.7	randomized cross-over	A 2% lower	

low-fat diet was merely 8.3 en%. In the study of Brace et al[25] the effect was even smaller, but the low-fat diet lasted for 20 weeks compared to 2 weeks in the study of Marckmann et al[74]. However, most of the reduction in FVII:C in the study of Brace et al[25] occurred during the first four weeks of the low-fat period. The contradicting results might be due to a difference in ratio of the fatty acids of the diets used in both studies, or by the lack of a control group in the study of Brace et al[25].

One cross-sectional study in 198 rural Finnish men aged 70 to 89 showed a positive correlation of total fat intake and fasting FVII:C[24]. Whether this association indeed reflects chronic effects of total fat intake on FVII:C is, however, not clear.

There was no difference in FVIIt after a low or a high-fat diet[25,75], but in one study, where the participants were put on a two week low-fat diet after their habitual high- fat diet, FVIIt dropped significantly with 10-15% of initial values[20]. Furthermore Miller et al[77] reported a positive association between dietary fat intake and FVIIt.

Effects of high-saturated vs high-unsaturated fat diets

Diets with the same total fat content, but with a different fatty acid composition were tested in six randomized cross-over studies[10,26,27,54,78,79] and one study in which subjects received dietary counselling[80]. All included healthy subjects between 21 and 66 years and duration of the diets varied from three weeks to six months. No difference was found in FVII:C or FVIIt between diets containing mainly saturated fatty acids (safa), monounsaturated fatty acids (mufa) or polyunsaturated fatty acids (pufa)[20,54,78,80]. Three studies examined the effect of diets differing only in saturated fatty acid composition. FVII:C was significantly lower after a high stearic acid diet compared to a high palmitic or myristic/lauric acid diet[26,27]. The decrease in FVII:C after a high stearic acid diet was accompanied by a decrease in FVIIt[26,27]. Slightly higher FVII:C levels were found after a high myristic acid diet compared to a high palmitic acid diet[79]. Probably, the stearic acid content in saturated fat diets used in the studies mentioned above was not high enough to result in lower FVII:C levels after high safa diets. Also, some substances in the sheabutter (containing 7% unsaponifiable material), which was the origin of the stearic acid, might have influenced the factor VII metabolism.

One cross-sectional study showed a positive association of FVII:C with saturated and monounsaturated fat. No association was found between FVII:C and polyunsaturated fat[24]. This might be due to the low intake of polyunsaturated fat (mean=14.9 g/day, SD=8.6) in this study population.

Many studies[81-90] payed special attention to the effect of fish or fish-oil supplementation on fasting FVII:C levels, but only in two of them[81,90] an effect was observed. FVII:C increased significantly after supplementation with 10 g fish oil daily[81,90]. However, in one

of these studies[81] diabetes patients were included in stead of healthy subjects, and in both studies the fish-oil supplementation was relatively high[81,90].

Concluding remarks

Fasting FVII:C is likely to decrease after a low-fat diet compared to consumption of a high-fat diet, while the results on fasting FVIIt are not corresponding. There is no difference in effect on FVII:C or FVIIt after intake of mainly safa, mufa, pufa or fish (oil). Apparently the amount of fat is a more important determinant of FVII:C than the fat composition. Only stearic acid appears to behave differently with a remarkable lowering effect on FVII:C.

Acute effects of dietary fat

In several studies the effect of dietary fat on postprandial factor VII levels was examined (Table 2.2). Low-fat meals were compared to high-fat meals and FVII:C or FVIIt was measured. Others compared the effects of saturated and unsaturated fat meals on FVII:C and FVIIt. In patients with hyperlipidaemia or cardiovascular disease the effect of one fatty meal was investigated.

The effect of low-fat vs high-fat

The effect of a high-fat meal and a low-fat meal on postprandial factor VII levels has been investigated in a number of studies of different designs.

A randomized cross-over study of Marckmann et al[29] the effects of a high-fat (50 % of energy = en%) meal on FVII:C and FVIIt in six healthy males (21-30 years) was investigated. The meal resulted in a high postprandial FVII:C peak. FVIIt varied insignificantly during the 24-h period. A second study with similar design showed also an elevation of FVII:C after a high-fat meal, but postprandial FVIIt was lower compared to fasting levels[30]. Silveira et al[31] and Moor et al[91] conducted a fat-tolerance test among 33 patients who survived a first myocardial infarction and 10 healthy control subjects (mean age 48.8 ± 3.2 years). Levels of FVII:C increased in both groups, but more pronounced in control subjects. In contrast, FVIIt tended to be lower in the three-hour sample in patients and controls and gradually returned to baseline towards the end of the 12-hour period.

In two randomized cross-over studies FVII:C increased after two high-fat meals[32,92] compared to a fat-free meal. The difference between one of the high-fat meals and the fat-free meal after eight hours was 10.4%[32]. This difference is large enough to have biological significance, since the difference in FVII:C between people with no ischaemic heart

disease and those with (fatal) ischaemic heart disease is 4-10%[1,2,93]. FVIIt decreased after all meals in one study[32], while it increased only after a high saturated fat meal and did not change after the fat free meal in another study[92].
The effect of a low-fat meal (20 en%) was evaluated in two studies with similar design[29,30]. In one study FVII:C increased after the low-fat meal, but not as much as after a high-fat meal. Factor VIIt did not change. The other study showed a decrease in FVII:C and FVIIt after the low-fat meal.

The effect of high saturated vs high unsaturated fat
The response of FVII:C and FVIIt on a meal with mainly saturated or unsaturated fat was compared in different cross-over studies among small numbers of healthy middle-aged subjects[28,32,92]. Two studies included a low-fat control meal[32,92]. In the study of Salomaa et al[32] the post-prandial effect of a fat meal was tested, while the other studies evaluated the post-prandial effects after a one or four-week diet[28,92]. There was no difference in the post-prandial change of FVII:C or FVIIt between the high saturated and high unsaturated meal in two studies[28,32]. However, in one study FVII:C was highest on the saturated fat meal, intermediate on the unsaturated fat meal and lowest on the control meal. Factor VIIt levels did not differ significantly between the unsaturated fat and control meal, but were significantly increased on the saturated fat meal[92].

Concluding remarks
Some of the studies mentioned above comprised a diet-period of several days where blood samples were taken on the last day of each diet-period. In others the effect was just evaluated after one test-meal. Also, the composition of the meals, the number of participants and the time of blood sampling differed between the studies. Nevertheless, results were in general quite similar. Furthermore, in studies on acute effects as well as in studies on chronic effects of dietary fat on factor VII more or less the same conclusions can be drawn; an increase after a high-fat meal/diet and probably no different effects of several fatty acids.
There seems to be no difference in postprandial response of FVII:C or FVIIt between saturated fat consumption and unsaturated fat consumption, but this might be due to low numbers. FVIIt does not appear to increase after any type of meal. Probably factor VII protein only decreases due to endogenous fluctuations. FVII:C always

Table 2.2. Acute effects of dietary fat

MEAL TYPE	REF.	SUBJECTS	DESIGN	FVII:C	FVIIt
fat-free	32	10 healthy males, mean age 40.8 ±8.4, mean BMI 23.3 ±2.7	randomized cross-over	↓	↓
low-fat (20 en%)	29	6 healthy males, age 21-30, BMI 19-23	randomized cross-over	↑	-
	30	7 males, 10 females (1 pill user), healthy, age 20-34, BMI 19.4-28.2	randomized cross-over	↓	↓↓
	92	4 males, 1 female	randomized cross-over	-	-
high-fat (50 en%)	29	6 healthy males, age 21-30, BMI 19-23	randomized cross-over	↑↑	-
	30	7 males, 10 females (1 pill user), healthy, age 20-34, BMI 19.4-28.2	randomized cross-over	↑	↓
	31 + 91	33 patients who survived a first myocardial infarction, mean age 48.8 ±3.2 and 10 healthy control subjects, mean age 49.2 ±3.6	fat-tolerance test	↑	↓
high saturated fat	32	10 healthy males, mean age 40.8 ±8.4, mean BMI 23.3 ±2.7	randomized cross-over	↑	↓
	92	4 males, 1 female	randomized cross-over	↑↑	↑
	28	5 males, 4 females, healthy	randomized cross-over	↑	-
high unsaturated fat	32	10 healthy males, mean age 40.8 ±8.4, mean BMI 23.3 ±2.7	randomized cross-over	↑	↓
	92	4 males, 1 female	randomized cross-over	↑	-
	28	5 males, 4 females, healthy	randomized cross-over	↑	-

responds with an increase after high-fat meals, but it is not yet clear whether this response also occurs after a low-fat meal.

Blood lipids

Blood lipids may have an important role in the relation between dietary fat and factor VII. Many researchers have investigated this by relating blood lipids to (fasting or postprandial) factor VII in blood samples taken at the same time. In these studies triglycerides and FVII:C or FVIIt were analyzed. Also, total cholesterol, HDL cholesterol or sometimes LDL cholesterol were measured. Furthermore, the association of triglycerides and cholesterol with factor VII was evaluated in intervention studies on chronic effects of a diet on factor VII. Since the design of these intervention studies is described above, we will not go into detail on this here. Finally the relation between blood lipids and FVII:C and FVIIt was investigated in patients with primary hyperlipidaemia.

Factor VII and triglycerides

All cross-sectional studies which included measurements of triglycerides showed a positive correlation between triglycerides and FVII:C[24,37-39,94-97], or FVIIt[16,37,40-42,95,96]. Four of the studies did not comprise women[24,38,39,95] and one study, in which FVII:C and FVIIt measurements were done among men and women, showed only a correlation between triglycerides and FVIIt in men[41]. The participants in this study were relatively young (mean age men 23 and women 24 years) compared to other studies which were carried out among middle-aged or elderly people, so the studies might not be completely comparable. In most of the studies factor VII was measured after an overnight fast, the authors of two articles, however, did not mention the state of the participants when blood samples were drawn[37,42].

In eight intervention studies triglycerides and FVII:C were measured. None of these studies showed a positive association between triglycerides and FVII:C[20,25,27,74-76,79]. In five studies FVII:C decreased after a low-fat diet, while triglycerides did not change or even increased[25,74-76].

Factor VII and cholesterol

Findings on the relation of serum cholesterol concentration with factor VII in healthy subjects are much less consistent. Eight out of 13 cross-sectional studies showed a positive association between total cholesterol and FVII:C[24,37-39,41,95,98], or FVIIt[37,41,42,95] which was stronger in men than in women[37,41,98].

HDL and LDL cholesterol positively correlated with FVII:C in one study[94] while they

were positively associated with FVIIt only in men in another study[41]. In several studies a correlation between those parameters could not be found at all[16,37,38,40,41,94,97]. Finally, Yang et al[99] reported a positive correlation between total cholesterol/HDL cholesterol ratio and FVIIt in 48 healthy men.
In two intervention studies a positive association between total cholesterol and FVII:C was found[20,36]. In six other studies the change in total cholesterol was coherent with the change in factor VII, but nothing was mentioned on the association between these variables[25,27,74-76,79].

Factor VII in hyperlipidaemic patients
Patients with primary hyperlipidaemia, type IIa, IIb or IV of the Fredrickson classification[100] participated in several studies in which fasting factor VII and blood lipids were measured[55,101,102]. FVII:C and FVIIt were higher in middle-aged patients with type IIa, IIb or IV hyperlipidaemia compared to healthy controls of the same age[55,101,102]. For FVIIt this difference was not significant[55,101]. Another study among elderly patients, showed elevated FVII:C and FVIIt levels in patients with type IIa and IIb but not in type IV hyperlipidaemia compared to controls[103]. Nordoy et al[104] found opposite results in a study where patients with type IV but not type IIa and IIb had higher FVIIt levels. One explanation for these contradictory results might be that in the latter study a different method was used to measure factor VII (factor VII normotest, which measures FVII-phospholipid complex) and that blood samples were not taken after an overnight fast[104]. Other differences between studies might further have complicated the results; some patients received treatment, but according to the authors this treatment did not interfere with coagulation parameters. Some controls used lipid-lowering diets, but this does not explain the different results found for type II and IV hyperlipidaemic patients. In view of the evident relation between triglycerides and factor VII and the weak association between cholesterol and factor VII, it could have been expected that factor VII was higher in patients with increased levels of triglycerides, i.e. type IIb and IV hyperlipidaemia. Still, this was not confirmed in the studies mentioned above.

Concluding remarks
There is convincing data from cross-sectional studies which show a relation between triglycerides and FVII:C or FVIIt. This is not confirmed by results from intervention studies. The cross-sectional association might only reflect the association of FVII:C and triglyceride after a fat-rich (evening) meal. This indicates that the change in factor VII after a long-term diet is independent of triglycerides, while post-prandial change in factor VII does depend on triglycerides.

The association between different subfractions of cholesterol and FVII:C or FVIIt is not quite clear. This may be due to different types of participants in studies performed thus far; diabetes patients, hypertensive patients, or healthy participants were included. Also, differences in age distribution of the participants may have accounted for the discrepancy in results. More likely however, the relation of cholesterol with FVII:C and FVIIt is due to the associations between FVII:C and large lipoprotein particles, chylomicrons, VLDL and IDL[70]. The interrelation between triglycerides and cholesterol might explain the association of factor VII with cholesterol. Unfortunately in the reported studies, the associations between cholesterol and factor VII were not adjusted for triglyceride levels.

Gene-environment interaction: factor VII polymorphism and triglycerides

FVII polymorphism

Recently a polymorphism for the factor VII gene was identified, which is strongly associated with plasma FVII:C levels[23]. The base change causing the polymorphism is a G to A substitution in the second position of the codon for amino acid 353, leading to replacement of arginine (R) by glutamine (Q) in the protein product of the allele (R/Q353). The Q allele is associated with relatively low FVII:C levels while, the R allele is associated with relatively high FVII:C levels[23,57,105-107]. In caucasians Q allele frequencies of 0.06, 0.09, 0.10 and 0.13 have been reported[23,57,106,107]. In Dravidian Indians, Gujaratis and Afro-Caribbeans the frequency of this allele was 0.29, 0.25 and 0.08 respectively[57,105].

Interaction with dietary fat and triglycerides

Lane et al[57] reported that the association between triglycerides and FVII:C among Caucasians, Afro-Caribbeans and Gujarati Indians depended on the R/Q353 genotype. In the Gujaratis no correlation between triglycerides and FVII:C was found among those with the Q allele, while a correlation was present in persons with the RR genotype. The same results were found in a study by Humphries et al[58], who examined FVII:C and FVIIt in 364 caucasian men. However, in a study of Saha et al[105], where fasting FVII:C levels were compared with triglyceride levels in Dravidian Indians, the opposite was true; the relation between triglycerides and FVII:C was stronger in persons with the RQ or QQ genotype than in persons with the RR genotype[105]. In a study of Silveira et al[106] 31 male post-infarct patients underwent a fat-tolerance test and FVIIa, FVIIt and triglycerides were measured. The association between postprandial triglycerides response and fasting FVIIt was not significant in participants with the RQ genotype, while it was significant in those with the RR genotype. In both the RQ group and the RR group a significant association between triglyceride and FVIIa response on the test meal was found. It is likely that the

polymorphism considerably modifies the association between fasting factor VII levels and triglycerides. It is, however, from these studies not clear whether this association is stronger in persons who carry the Q allele or in those with the RR genotype. This might be due to the fact that the R/Q353 polymorphism itself is not a direct determinant of the factor VII response, but may be in strong allelic association with some causative polymorphic sequence somewhere else on the factor VII gene.
There are so far little data which suggest that the genotype influences the postprandial response of FVIIa on a fat load. In the study of Silveira et al[106] FVIIa increased in both genotype groups in response to fat intake and the differences between RR and RQ individuals in the fasting state were maintained in the postprandial state[106]. The absolute postprandial rise in FVIIa was higher in the RR group than in the RQ group. However, so far this study is the only postprandial study in which genotype has been taken into account. Considering this, and the contradicting results of studies on the effect of genotype on the association between triglycerides and factor VII, more research is needed to document the interrelations between factor VII, triglycerides, dietary fat, R/Q353 and other polymorphisms.

Discussion and conclusion

It is not surprising that results of studies investigating factor VII are not always consistent. Several methods are presently used to measure factor VII, not all of which are comparable. The method most widely used, i.e. one-stage clotting assay, is the one with most uncertainty on what is actually measured. As the activated form of factor VII has a priming function in triggering the clotting cascade, measurement of FVIIa seems the most relevant way to study factor VII. So far this method has rarely been used in studies on factor VII and dietary fat.
Another complication is the difference in timing of blood sampling; results of studies on fasting and postprandial levels are in fact incomparable. More importantly, different mechanisms might account for effects of dietary fat and blood lipids on fasting and postprandial factor VII. For example, after a long-term high-fat diet, fasting levels might be higher, but also the response of factor VII on a fat load might be more pronounced than after a long-term low-fat diet. It is known, for example, that the response of lipoproteins on a fat load is not only dependent on the type of fat given but also on the type of fat which is habitually consumed[108]. Furthermore, the R/Q353 polymorphism probably modifies this reaction.
Factor VII is a risk indicator for cardiovascular disease, the association being stronger between factor VII and fatal events than non-fatal events. Trace amounts of FVIIa

represent the very first active protease in the extrinsic pathway of coagulation and elevated plasma levels of FVIIa may give rise to an extensive initiation of the clotting cascade after rupture of atherosclerotic plaques. Factor VIIa may in particular be important in the outcome of a thrombotic event, perhaps through the eventual size of the occlusive thrombus.

For the purpose of prevention of cardiovascular disease through a change in dietary habits, it is necessary to know if and how the level of FVIIa can be influenced. In this context, it is good to reflect on the mechanism that might explain the relation between dietary fat and factor VII. Data of studies on this topic are insufficient to throw light on which of the mechanisms that are proposed is really relevant. There are still many questions to be solved. For example, why do we not find an increase in FVII:C after saturated fat intake and a decrease after consumption of fish(oil), since the half life time of chylomicron remnants is longer after a saturated fat meal and shorter after intake of fish oil[108,109]. Furthermore, in the fasting state almost no large lipoprotein particles are present in the circulation; no activation by lipoproteins can occur, so how does that explain the higher fasting FVII:C levels found after a high-fat diet in some studies? Answers to these questions could support or refine the presently proposed mechanisms or reveal a new theory.

Many studies have been performed suggesting that factor VII, dietary fat and blood lipids are somehow related, independent from other factors. It seems quite clear that the amount of fat intake is important; high-fat meals increase factor VII. Whether fat composition is of any importance remains to be clarified. Intervention and biochemical studies are needed to investigate this and to find the explaining mechanism behind this relation. For further research it is advisable that total factor VII and FVIIa is measured with one standard technique for each method, and that blood is collected at fasting and postprandial conditions. Also, the polymorphisms of the gene coding for factor VII should be taken into account while studying the relation between factor VII, dietary fat and blood lipids.

CHAPTER 3

The association of dietary fat and fibre with coagulation factor VII in the elderly: The Rotterdam Study

Abstract

Considerable evidence suggests that a high concentration of coagulation factor VII is a risk indicator for ischaemic heart disease. Factor VII is known to be influenced by dietary fat and probably by dietary fibre in young and middle-aged people. There are no data available in elderly people and the effects of different types of fat are unclear. This study examines the relation of factor VII coagulant activity (FVII:C) with dietary fat and fibre in The Rotterdam Study. The Rotterdam Study is a population-based study among 7983 men and women, aged 55 years and over. FVII:C was measured in 3007 subjects (1730 women and 1277 men, mean age 67.3 ± 7.8 and 66.3 ± 7.0 years respectively). Measurements included cardiovascular risk factors and habitual diet was assessed by a semiquantitative food-frequency questionnaire. Associations that were statistically significant or nearly significant differed for some nutrients between men and women. Total fat intake showed a direct association with FVII:C only in women (ß=0.1 %/g, 95%CI:0.01,0.20). Saturated fat intake was associated with FVII:C in women (ß=0.18 %/g, 95%CI: -0.001,0.36) and in men (ß=0.11 %/g, 95%CI:-0.06,0.27). Monounsaturated fat was positively related to FVII:C in women (ß=0.17 %/g, 95%CI:-0.05,0.39 and polyunsaturated fat was inversely associated with FVII:C in men (ß=-0.15 %/g, 95%CI:-0.33,0.03). Fibre intake was inversely associated with FVII:C in both men (ß=-0.31 %/g, 95%CI:-0.57,-0.06) and women (ß=-0.36 %/g, 95%CI:-0.63,-0.09). No associations were found for energy intake. In elderly people FVII:C is associated with fat and fibre intake. This suggests that FVII:C is influenced by nutritional factors, even at old age.

L.I. Mennen. J.C.M. Witteman, J.H. den Breeijen, E.G. Schouten, P.T.V.M. de Jong, A. Hofman, D.E. Grobbee

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Introduction

Factor VII is one of the coagulation factors of the extrinsic pathway of the coagulation cascade. This pathway is activated during vascular injury, which results in the rapid formation of a blood clot. A high concentration of factor VII contributes to a high thrombotic tendency. In the presence of atherosclerosis, this tendency may be a trigger for a cardiovascular event. Atherosclerosis is common in elderly people, reflecting a development over many years. The effects of thrombosis, however, are acute and subject to change even at older ages. Increased thrombotic tendency in elderly people constitutes an elevated risk for cardiovascular disease and considerable evidence indeed suggests that factor VII coagulant activity (FVII:C) is a major contributor[1,2]. In the Northwick Park Heart Study (n=1511) factor VII was found to be a risk indicator for ischaemic heart disease[1] and in the PROCAM study (n=10581) this seemed to be the case when only fatal events were taken into account[2].

Two nutritional factors, dietary fat and fibre, are considered major environmental determinants of FVII:C. From several intervention studies among young adults it is known that FVII:C depends on dietary fat intake[10,30-32,36,92]. A high-fat diet results in high FVII:C levels and after a fatty meal post-prandial FVII:C rises concomitantly with the rise in triglycerides[30-32,92]. These changes in FVII:C are believed to be independent of the type of fat consumed[20,36]. The results from three observational studies among middle-aged men however, are inconsistent[34,36,110]. Connely et al[110] and Miller et al[36] observed an association between FVII:C and dietary fat whereas Rankinen et al[34] did not.

Three cross-over trials in young and middle-aged subjects showed a decrease in FVII:C after a high-fibre diet[35,75,76]. An association between dietary fibre and FVII:C was also observed in one cross-sectional study[34]. No other nutrients have consistently been found to be associated with FVII:C[30,36].

To our knowledge, there are no published data on FVII:C and dietary factors in elderly people. It is important to know whether the association of FVII:C with dietary factors also exists at an age when most cardiovascular events occur. Because the level of FVII:C directly influences the thrombotic tendency, in the presence of atherosclerosis it may determine whether a cardiovascular event is triggered. We studied the relation between FVII:C and dietary fat and fibre in the Rotterdam Study.

Subjects and methods

Population

The Rotterdam Study is a single-center population-based prospective cohort study of 7983 persons. All inhabitants of 55 years and over from a suburb of Rotterdam were asked to participate in the study, which resulted in a response rate of 78%. In short, the objective of the study is to clarify determinants of occurrence of chronic disabling cardiovascular, neurogeriatric, locomotor and ophthalmologic diseases. The rationale and design of the Rotterdam Study have been published elsewhere[111].

Dietary data were available for 5435 persons and study subjects were excluded for the following reasons: myocardial infarction in the year before the investigation (n=574), diabetes mellitus (n=779), use of anticoagulants (n=1349), stasis during venepuncture (n=1513) and no blood sample available (n=933). Because some subjects were excluded for multiple reasons, a total of 4976 were not eligible for this study. The present analysis is thus based on cross-sectionally obtained data for 3007 subjects.

Examination procedures

The participants came to the research center at varying times during the day. At the research center a brief clinical examination was performed and height and weight were measured. Body mass index was determined by dividing the weight by the square of height (kg/m^2). Blood pressure was measured, with the subject in a sitting position, at the right arm by using a random zero sphygmomanometer. The participants were not asked to refrain from smoking or physical exercise and non-fasting blood samples were taken using a 21 gauge butterfly needle after no or minimal stasis.

Dietary assessment

The habitual diet was assessed by a semiquantitative food-frequency questionnaire. The questionnaire was a modification of a validated self-administered mailed semiquantitative food-frequency questionnaire that was used previously in a large-scale prospective cohort study, involving a younger population. Measures of the validity and repeatability of the original questionnaire for several nutrients have been reported[112,113]. The questionnaire was adapted to allow an easy and time-efficient dietary assessment in an older population. Modification consisted mainly of inclusion of additional items (icecream, cornflakes and linseed); collection of more detailed information on vegetable, fruit, and meat consumption; and a different mode of administration, which was undertaken in two consecutive phases. The modified questionnaire contains 170 food items in 13 food groups and general questions about dietary habits. Its aim is to assess habitual food intake during

the past year.
In the first phase a self-administered questionnaire was handed out and explained to each participant by trained research assistants during a home visit. Participants had to mark the foods that they consumed at least once a month. Also, the amounts of several foods they used were weighed and the content of cups and bowls was measured. In the second phase a dietary interview was conducted by trained dietitians on the basis of the already completed dietary questionnaire. During the dietary interview the dieticians concentrated on obtaining accurate information on amount and frequency of food items noted by participants as consumed at least once a month. General questions to check the consistency of the completed dietary questionnaire were asked. The modified questionnaire was validated by comparison with multiple food records. The correlation coefficients were corrected for within-person variation in food records and adjusted for age, sex and energy intake. For the different types of fat and fibre they varied between 0.50 to 0.65 (Klipstein et al, unpublished observations, 1994).
All data were entered into a self-made interactive computer program, which included automatic checks for inconsistencies in the answers of the participants. A computer application was developed to calculate the frequency and amounts of foods consumed. Questionnaires were checked for completeness by an interactive dataentry program and automatically coded for later conversion into nutrients. The program checked further for consistency, range and other response errors. The conversion from foods to energy and nutrient intake was done with a computerized version of the Dutch Food Composition Table[114] and percentages of total energy (en%) delivered by macronutrients were calculated.

Laboratory measurements
Samples were collected into siliconized vacutainer tubes containing 0.129 mol/L sodium citrate (Becton Dickinson, Meylan, France). Samples were centrifuged for 10 minutes at 1600 x g and 4°C. Citrated plasma was snap frozen and stored at -80°C until analyzed. FVII:C was measured with a one-stage clotting assay by using human thromboplastin (Tromborel S, Behringwerke, Germany) and factor VII-deficient plasma (Ortho Diagnostic System, Beerse, Belgium). This assay measures the ability of the sample to correct the clotting time of human factor VII-deficient plasma. The plasma levels were then expressed as percentage activity by relating the clotting time to a calibration curve constructed of a standardized control plasma. As a control, the pooled plasma of 50 healthy middle-aged people was used and three control samples were run with each batch of study samples. The coefficient of variation is 7.3% for this method. Serum total cholesterol was determined using an automated enzymatic procedure[115]. HDL-cholesterol concentration was

measured similarly, after precipitation.

Data analysis

The association of FVII:C with dietary factors was examined by multiple linear regression analysis, using the BMDP-statistical package[116]. In the analysis, both total weight and en% of macronutrients were used. Adjustments were made for age and when nutrients in grams were used in the analysis, adjustment for energy intake was made by including total kilojoules as an independent variable in the regression model. In some models we included additional nutrients when appropriate.

Table 3.1. General characteristics of the study population (means ± SD)

	Women (n=1730)	Men (n=1277)
Age (years)	67.3 ± 7.8	66.3 ± 7.0
Total cholesterol (mmol/L)	6.9 ± 1.2	6.4 ± 1.2
HDL-cholesterol (mmol/L)	1.5 ± 0.4	1.2 ± 0.3
Systolic blood pressure (mmHg)	137.6 ± 21.6	137.5 ± 21.1
Diastolic blood pressure (mmhg)	72.9 ± 10.8	75.3 ± 11.3
Body mass index (kg/m^2)	26.6 ± 4.0	25.8 ± 2.8
Factor VII:C (% of standard)	120.4 ± 24.3	108.0 ± 22.5
Smoking (%): current	19.1	24.6
former	28.8	58.6
never	52.1	16.8

Results

General characteristics of the study population are presented in Table 3.1. Mean age was 66.3 years in men and 67.3 years in women. Mean FVII:C was 108.0% in men and 120.4% in women. Energy and nutrient intakes from the subjects' habitual diet are shown in Table 3.2. Mean energy intake was 9546 kJ in men and 7515 Kj in women.
The coefficients and 95% confidence intervals of regression analysis with FVII:C as dependent variable and nutrient intake as independent variable are shown in Table 3.3. Total fat intake showed a direct association with FVII:C only in women. Saturated fat intake was associated with FVII:C in women; in men the association was smaller.

Monounsaturated fat was positively related to FVII:C in women and polyunsaturated fat was inversely associated with FVII:C in men. In general, the results were the same when calculated for the percentage of energy delivered by the nutrient as for the absolute intake.

Table 3.2. Calculated daily energy and nutrient intake (means ± SD)

	Women (n=1730)		Men (n=1277)	
Energy (kJ)	7515 ± 1687.0		9546 ± 2119.6	
	g	en%	g	en%
Protein	75.9 ± 16.6	17.2 ± 3.1	89.6 ± 21.2	15.9 ± 2.7
Carbohydrates	197.2 ± 53.5	43.9 ± 6.7	241.7 ± 65.1	42.5 ± 7.0
Total fat	73.1 ± 23.0	36.2 ± 6.2	93.6 ± 29.1	37.0 ± 6.0
Saturated fat	29.2 ± 10.2	14.5 ±3.3	36.5 ± 12.4	14.3 ± 3.0
Monounsaturated fat	24.8 ± 8.5	12.3 ± 2.7	32.2 ± 11.3	12.6 ± 2.7
polyunsaturated fat	13.5 ± 6.8	6.7 ± 2.8	18.0 ± 8.3	7.1 ± 2.7
Cholesterol[†]	216.8 ± 70.3		263.5 ± 90.5	
Fibre	16.1 ± 4.8		18.2 ± 5.4	

[†] the percentage energy delivered by the nutrient

[#] in mg

Furthermore, total cholesterol intake was associated with FVII:C in men and women, but when saturated fat was included in the regression model the regression coefficients of cholesterol were smaller. Fibre intake was inversely associated with FVII:C in both men and women. When this association was adjusted for total, saturated, or polyunsaturated fat the results did not change. No associations were found with energy intake.

Table 3.3. Regression coefficients and 95% confidence (95% CI) intervals of several dietary components and factor VII:C*

		Women (n=1730)		Men (n=1277)	
		ß	95% CI	ß	95% CI
Energy (1000 Kj)		0.3	-0.4,1.0	-0.3	-0.9,0.3
Total fat	g	0.10	0.005,0.20	0.11	-0.06,0.27
	en%	0.23	0.04,0.41	0.25	-0.16,0.66
Saturated fat	g	0.18	0.001,0.36	0.11	-0.06,0.27
	en%	0.40	0.05,0.75	0.25	-0.16,0.66
Monounsaturated fat	g	0.17	-0.05,0.39	0.03	-0.15,0.20
	en%	0.40	-0.03,0.83	0.06	-0.39,0.51
Polyunsaturated fat	g	0.04	-0.16,0.24	-0.15	-0.33,0.03
	en%	0.14	-0.27,0.55	-0.36	-0.81,0.09
Cholesterol	mg	0.02	-0.0003,0.04	0.02	0.0002,0.04
Fibre	g	-0.36	-0.63,-0.09	-0.31	-0.57,-0.06

* All models adjusted for age. When intake in grams was used in the model, the model was also adjusted for total energy intake. The regression coefficients denote the change in factor VII:C as a percentages per unit change in nutrient intake.

Discussion

We investigated associations of several nutrients with FVII:C among 3007 elderly people. Associations that were statistically significant or nearly significant differed for some nutrients between men and women. Plasma FVII:C levels were associated with cholesterol and with fibre intake in both women and men. Also, an association was observed between FVII:C and saturated fat intake, which was stronger in women than in men. Furthermore FVII:C was related to total and monounsaturated fat in women and to polyunsaturated fat in men. Energy intake was not associated with FVII:C.

Factor VII activation was determined by a one-stage clotting assay, a method that is commonly used in other studies[1,2]. Although determination of factor VII with an improved method (factor VIIa)[60] may have been preferable, this measurement was not in use at the start of our study in 1991.

Furthermore, in this study blood samples were collected from subjects in a nonfasting state. However, when we adjusted the associations for the time since the last meal, the results did not change.

There have been several intervention studies that compared the effect of saturated and unsaturated fat on FVII:C[20,28,32,54]. In a cross-over trial among 21 male and 17 female subjects a diet rich in mono- or polyunsaturated fat was consumed for 23 days, and no change in FVII:C was found on either diet[54]. Another cross-over trial among 11 young adults, in which two low-fat diets were given, showed a similar decrease in FVII:C after a diet with mainly saturated fat compared with a diet with mainly unsaturated fat[20]. Also, there was no difference in post-prandial levels of FVII:C after a meal with high saturated fat or a meal with high polyunsaturated[28,32]. The results of these studies suggest that FVII:C decreases with a low-fat diet, irrespective of the type of fat.

In two cross-sectional studies among middle-aged men, a positive association of FVII:C with dietary fat was also observed[30,36,110], but nothing was reported for associations of FVII:C with different types of fat.

The results of the trials mentioned above showed positive associations of FVII:C with all fat types. This is not in line with the results of our study. In those trials however, saturated and unsaturated fats were fully exchanged in the different diets to get a high contrast between the fat types. This results in an unnaturally high intake of unsaturated fat; the intake of unsaturated fat in an ordinary Western diet is much lower[20,54]. Because data on habitual diet were used in our study, the intake of unsaturated fat may have been too low to uncover a positive association with FVII:C.

The results of our study showed some differences between men and women, but this was mainly restricted to mono- and polyunsaturated fat. The lack of association between FVII:C and total fat in men may be a consequence of the inverse association of polyunsaturated fat with FVII:C in men, whereas in women monounsaturated fat was positively associated with FVII:C. We have no other explanations for the differences in men and women.

In agreement with three intervention studies and one cross-sectional study in middle-aged subjects[34,35,75,76], our cross-sectional study showed an inverse association of FVII:C with fibre intake in elderly people; an increase of fibre intake of 10 g would result in a decrease of FVII:C of 3.6% in women and 3.1% in men. Such an increase would raise the mean fibre intake towards that recommended by the Dutch Nutrition Council[117].

The study conducted here was a cross-sectional study. The results show an association between FVII:C and dietary factors, but no definite conclusions can be made on a causal relation. Two mechanisms have been proposed to explain a causal relation between FVII:C and dietary fat[56,71]. One mechanism is based on decreased catabolism of factor VII through binding of factor VII protein to triglyceride rich lipoproteins[56]. The second is based on activation of factor VII by negatively charged surfaces which occurs during lipolysis of these lipoproteins[71].

The mechanism to explain an association of FVII:C with fibre is unknown so far. A high-fibre intake may be a marker of a healthy diet or even of a healthy lifestyle. Another option is the lowering effect of fibre on blood cholesterol[118], which in turn might have an effect on factor VII[41]. Intervention studies that confirm the specific effect of fibre intake on factor VII are needed to disentangle direct and indirect effects.

A high factor VII concentration increases the thrombotic tendency, which may be a trigger for cardiovascular events. If factor VII can be lowered, a cardiovascular event might be postponed or even prevented. Because factor VII influences the thrombotic tendency directly, even at older ages, lowering of FVII:C may be of public health importance. The results from this study suggest that FVII:C may be affected by a low-fat or a high-fibre diet, or both at old age. Intervention studies are needed to further support the preventive potential of changes in diet through this mechanism.

CHAPTER 4

Dietary effects on coagulation factor VII vary across genotypes of the R/Q353 polymorphism

Abstract

Coagulation factor VII clotting activity (FVII:C) is a risk indicator for cardiovascular disease. FVII:C is influenced by diet and by the R/Q353 polymorphism. We evaluated the association of factor VII with dietary factors taking the R/Q353 polymorphism into account.

In 3005 elderly subjects FVII:C was measured and the extreme quintiles of the FVII:C distribution were selected for measurement of the R/Q353 genotype and total factor VII (FVIIt). In these 1158 subjects, habitual diet was assessed with a semiquantitative food-frequency questionnaire. The frequency of the Q353 allele was 0.24 in the lowest quintile and 0.09 in the highest quintile. The quintiles were combined for performance of linear regression analyses.

FVII:C was inversely associated with fibre (ß=-0.64 %PP/g, CI:-1.07,-0.21) and protein intake (ß=-0.16 %PP/g, CI:-0.31,-0.01) and positively with saturated fat intake (ß=0.19 %PP/g, CI:-0.10,0.48). FVIIt was inversely associated with fibre (ß=-0.38 %PP/g, CI: -0.71,-0.05). No important other associations with diet were observed.

The inverse association of FVII:C with fibre was clearly stronger in subjects with the RR genotype (ß=-0.76 %PP/g, CI:-1.23,-0.29), than in those with the RQ/QQ genotypes (ß=-0.19 %PP/g, CI:-0.97,0.59). The same was found for FVIIt. The association of FVII:C with saturated fat was positive in those homozygous for the R allele and inverse in those carrying the Q allele.

These findings suggest that the strength of the association between coagulation factor VII and diet varies across the genotypes of the R/Q353 polymorphism.

L.I. Mennen, M.P.M. de Maat, E.G. Schouten, C. Kluft, J.C.M. Witteman, A. Hofman, D.E. Grobbee

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Introduction

Factor VII is a vitamin K-dependent coagulation factor, which together with tissue factor is a very potent coagulant[5]. In the gene coding for factor VII the R/Q353 polymorphism is the result of a single base change in the codon for amino acid 353, which leads to the replacement of arginine (R) by glutamine (Q)[23]. The Q353 allele is associated with lower levels of factor VII clotting activity (FVII:C) and total factor VII[23]. In the Northwick Park Heart Study and the Prospective Cardiovascular Münster Study FVII:C was shown to be positively associated with the incidence of (fatal) ischaemic heart disease[1,2,6,119]. Furthermore, the HVR4 polymorphism, another polymorphism in the factor VII gene of which the A1 allele is related with lower factor VII levels, was associated with familial acute myocardial infarction (Iacoviello et al, unpublished observations, 1997).
FVII:C and total factor VII were found to be positively associated with intake of dietary fat in a cross-sectional study[77]. Results from several experimental studies showed an increase of FVII:C on a high-fat diet, while it decreased on a low-fat diet[20,36]. The effects of a high-fat diet on total factor VII are less clear[120]. From one intervention study and two cross-sectional studies an inverse association of FVII:C with dietary fibre was reported[33-35]. Finally, a change in protein intake was associated with a change in FVII:C and total factor VII[36].
As the R/Q353 polymorphism substantially influences the level of both FVII:C and total factor VII[121], the association of factor VII with dietary factors may differ between genotype groups. This was evaluated in cross-sectional data from the Rotterdam Study.

Subjects and methods

Population
The Rotterdam Study is a single-centre population-based prospective cohort study of 7983 persons. All inhabitants of 55 years and over from a suburb of Rotterdam were asked to participate in the study, with a response rate of 78%. In short, the objective of the study is to clarify determinants of occurrence of chronic disabling cardiovascular, neurogeriatric, locomotor and ophthalmologic diseases. The rationale and design of the Rotterdam Study have been published elsewhere[111].
Dietary data and blood samples were not available for 2548 and 993 persons respectively. The following characteristics may affect the factor VII level and were therefore used as exclusion criteria: myocardial infarction in the year before the investigation (n=574 persons), diabetes mellitus (n=779 persons), use of anticoagulants (n=1349 persons), stasis during venepuncture (n=1513 persons). None of the women used hormone replacement

therapy. As some subjects were excluded for multiple reasons a total of 4978 was not eligible for this study. In blood samples of the 3005 people who were left after exclusions, FVII:C was measured. Individuals in the two extreme quintiles of the distribution of FVII:C were selected, expecting an enrichment for the Q353 allele in the lowest quintile. The present analysis is thus based on cross-sectionally obtained data for 1158 subjects.

Examination procedures

The participants came to the research centre between 08.00 h and 16.30 h. At the research centre a brief clinical examination was performed and height and weight were measured. Body mass index was calculated by dividing weight by the square of height (kg/m^2). Non-fasting blood samples were taken using a 21 gauge butterfly needle after minimal stasis. Time between last meal and blood sampling was recorded.

Dietary assessment

The habitual diet was assessed by a semiquantitative food-frequency questionnaire. The questionnaire was a modification of a validated self-administered semiquantitative food-frequency questionnaire that was previously used in a large-scale prospective cohort study, involving a younger population. Measures of the validity and repeatability of the original questionnaire for several nutrients have been reported[112,113]. The questionnaire was adapted to allow an easy and time efficient dietary assessment in a population of older persons. The modified questionnaire contained 170 food items in 13 food groups and general questions about dietary habits. The aim was to assess habitual food intake during the past year.

Dietary assessment was undertaken in two consecutive phases. First a self-administered questionnaire was handed out and explained to each participant during a home visit by trained research assistants. Participants had to mark the foods that they consumed at least once a month. Also, the amounts of several foods they used were weighed and the content of cups and bowls was measured. Secondly a dietary interview was conducted by trained dietitians on the basis of the already completed dietary questionnaire. During the dietary interview the dieticians concentrated on obtaining accurate information on amount and frequency of food items noted by participants as consumed at least once a month.

The conversion from foods to energy and nutrient intake was established with a computerized version of the Dutch Food Composition Table[114] and percentages of total energy (en%) delivered by macronutrients were calculated.

Laboratory measurements

Blood was collected in siliconized vacutainer tubes containing 0.129 mol/L sodium citrate

(Becton Dickinson). Samples were centrifuged for 10 minutes at 1600 x g and 4°C. Citrated plasma was snap frozen and stored at -80°C until laboratory analysis. FVII:C was measured with a one-stage-clotting assay using human thromboplastin (Tromborel S, Behringwerke, Germany) and factor VII-deficient plasma (Ortho Diagnostic System, Beerse, Belgium). The results are expressed as percentages of pooled plasma (%PP). The total concentration of factor VII (FVIIt) was estimated by a two-stage amidolytic micro-titre assay (Chromogenix, Mölndal, Sweden)[122]. For the measurements of FVII:C and FVIIt different pooled plasma's were used. Serum total cholesterol was determined using an automated enzymatic procedure[115].

DNA was extracted from buffy-coats by a small scale method[123]. Each polymerase chain reaction (PCR) reaction (25 µL) contained 50-100 ng of the DNA extract, 50 ng of each appropriate primer, 10 mmol/L Tris/HCL (pH 9.0), 50 mmol/L KCL, 0.01 % gelatin, 0.1 % Triton X-100, 1 mmol/L $MgCl_2$, 0.2 mmol/L dNTP and 0.1 unit of SuperTaq polymerase (HT Biotechnology LTD, Cambridge, UK).

Oligonucleotid primers were as described by Lane et al[57]. The reaction components were incubated at 94°C for 4 minutes, followed by 32 cycles of 94°C for 1 minute, 59°C for 1.5 minutes and 72°C for 2 minutes.

Ten µL of PCR product were digested with 5 units of MspI (Gibco BRL, Life Technologies Inc., Gaithersburg, USA) at 37°C overnight. The digestion products were separated by electrophoresis through a 2% agarose gel in 44 mmol/L Tris/borate, 1 mmol/L EDTA, containing 0.4 µg/mL etidiumbromide and visualized using UV light. MspI digestion yielded a constant band of 40 base pair (bp). The common R allele gave bands of 205 bp and 67 bp and the Q353 allele gave a band of 282 bp as described previously[23].

Data analysis

The association of FVII:C and FVIIt with dietary factors was examined by means of multiple linear regression analysis using the BMDP-statistical package[116]. For these analyses the quintile groups were combined. Adjustments were made for age and energy intake by including them as an independent variable in the regression model.

To investigate whether the association of FVII:C and FVIIt with dietary factors varies across the genotypes, the same analyses were carried out separately in those with RR genotype and those with RQ/QQ genotypes. As there were only 32 persons with the QQ genotype, they were combined with those with the RQ genotype for this analysis.

Table 4.1. Characteristics and dietary intake of the study population (mean ± SD)

	Lowest quintile (n=538)	Highest quintile (n=575)
Age (years)	66.9 ± 7.8	66.2 ± 7.1
% Women	60.0	55.2
Body mass index (kg/m^2)	25.7 ± 3.7	26.6 ± 3.3
FVII:C (%PP)	85.0 ± 12.4	149.4 ± 17.1
FVIIt (%PP)	69.1 ± 15.6	107.4 ± 21.0
Frequency Q allele	0.24 (0.22,0.27)	0.09 (0.07,0.11)*
Energy (MJ)	8.3 ± 2.1	8.4 ± 2.2
Protein (g)	81.0 ± 19.7	81.1 ± 19.0
Carbohydrates (g)	213.3 ± 60.9	214.0 ± 61.6
Total fat (g)	80.9 ± 27.7	82.4 ± 28.4
saturated fat (g)	31.7 ± 11.7	32.4 ± 11.9
monounsaturated fat (g)	27.6 ± 10.8	28.2 ± 10.8
polyunsaturated fat (g)	15.5 ± 7.8	15.5 ± 7.7
Fibre (g)	17.3 ± 5.8	16.7 ± 4.8

* 95% confidence interval

Results

General characteristics and dietary intake of the study population are presented in Table 4.1, for the lowest and the highest quintile separately. The characteristics that were also measured in the source population of 3005 people (i.e. age, body mass index and FVII:C) were very similar to those of the study population of 1158 subjects (data not shown). In the lowest quintile 249 individuals (frequency 0.24) carried the Q353 allele and in the highest quintile 51 (frequency 0.05). In the total group FVII:C was 35% lower (80.6 %PP versus 125.3 %PP) and FVIIt 48% (49.6 %PP versus 95.7 %PP) in individuals with the QQ genotype compared to those with the RR genotype (Table 4.2). The FVII:C and FVIIt levels for those with the RQ genotype were intermediate.

Table 4.3 shows the regression coefficients of factor VII and dietary factors for the total population. FVII:C was inversely related to fibre intake (ß=-0.64 %/g, CI:-1.07,-0.21) and protein intake (ß=-0.16 %/g, CI:-0.31,-0.01). Adjustment for fat intake, did not change these results. Saturated fat intake was positively associated with FVII:C (ß=0.19 %/g, CI:-

0.10,0.48). FVII:C was not associated with total energy intake, total fat intake and mono- or polyunsaturated fat intake. FVIIt was inversely related to fibre intake (ß=-0.38 %/g, CI:-0.71,-0.05). No associations with any of the other dietary factors were found.

Table 4.2. FVII:C and FVIIt levels according to genotype (mean (SE))

		RR	RQ	QQ
FVII:C (% PP*)	total group	125.3 (1.2)	94.2 (1.7)	80.6 (5.1)
		n=858	n=268	n=32
	lowest quintile	87.0 (0.6)	82.8 (0.9)	76.2 (2.7)
		n=334	n=218	n=31
	highest quintile	149.6 (0.7)	144.0 (2.1)	215.0
		n=524	n=50	n=1
FVIIt (%PP*)	total group	95.7 (0.9)	68.2 (1.1)	49.6 (1.9)
		n=829	n=262	n=31
	lowest quintile	75.0 (0.8)	62.7 (0.9)	49.4 (1.9)
		n=325	n=213	n=30
	highest quintile	109.0 (0.9)	92.1 (12.6)	55.5
		n=504	n=49	n=1

* FVII:C and FVIIt were measured with different pooled plasma's

To explore these relations further we examined the associations of the three dietary factors with factor VII separately for the genotype groups. As the association of diet with factor VII was in similar direction in individuals with the RQ and QQ genotype (data not shown) and because the group of individuals with the QQ genotype was too small for meaningful regression analysis, the group with the QQ genotype was combined with the group with the RQ genotype. The results are presented in Table 4.4. In individuals with the RQ/QQ genotype the associations of FVII:C with the dietary factors were weaker compared to those with the RR genotype. For fibre and protein there was no association in individuals with the Q353 allele and for saturated fat the association was reversed.

Table 4.3. Regression coefficients and 95% confidence intervals (95% CI) of several dietary factors and factor VII*

	FVII:C		FVIIt	
	ß	95% CI	ß	95% CI
Energy (%PP/MJ)	0.20	-0.80,1.00	-0.20	-0.98,0.58
Protein (%PP/g)	-0.16	-0.31,-0.01	-0.004	-0.12,0.11
Total fat (%PP/g)	0.07	-0.08,0.22	0.05	-0.07,0.17
Saturated fat (%PP/g)	0.19	-0.10,0.48	0.09	-0.13,0.31
Monounsaturated fat (%PP/g)	0.09	-0.22,0.40	0.003	-0.25,0.26
Polyunsaturated fat (%PP/g)	-0.04	-0.35,0.27	0.09	-0.16,0.34
Fibre (%PP/g)	-0.64	-1.07,-0.21	-0.38	-0.71,-0.05

* Adjusted for age and sex. When intake in grams was used in the model, also adjustments were made for total energy intake. The regression coefficients indicate the change in factor VII in percent pooled plasma per unit change in nutrient intake.

Table 4.4. Regression coefficients and 95% confidence intervals (95% CI) of protein, saturated fat and fibre intake and factor VII according to genotype*

		FVII:C		FVIIt	
		ß	95% CI	ß	95% CI
Protein (%PP/g)	RR	-0.22	-0.40,-0.04	-0.01	-0.15,0.13
	RQ/QQ	-0.01	-0.25,0.23	-0.0004	-0.14,0.14
Saturated fat (%PP/g)	RR	0.35	0.02,0.68	0.23	-0.005,0.47
	RQ/QQ	-0.40	-0.87,-0.07	-0.28	-0.59,0.03
Fibre (%PP/g)	RR	-0.76	-1.23,-0.29	-0.46	-0.63,-0.11
	RQ/QQ	-0.19	-0.97,0.59	-0.15	-0.66,0.36

* All models adjusted for age, sex and total energy intake. The regression coefficients denote the change in factor VII in percent pooled plasma per unit change in nutrient intake.

For FVIIt there was a positive association with fibre in subjects homozygous for the R allele and no association in those with the RQ/QQ genotype. Although there was a negligible association between saturated fat and FVIIt in the total group, a positive association was observed in individuals with the RR genotype, while an inverse association was found in individuals with the RQ/QQ genotype. When we adjusted the regression models for belonging to the lowest or highest quintile, the results were similar.

Discussion

In this study FVII:C was in the total group inversely associated with fibre and protein intake and positively with saturated fat. In subjects with the RQ/QQ genotype these associations were weaker and for saturated fat the association was inverse. FVIIt was inversely associated with fibre intake and, in subjects with the RR genotype, also positively with saturated fat.

In an unselected population the frequency of the Q353 allele is expected to be 0.10[14,123]. If there is a direct relation between the R/Q353 polymorphism and the level of FVII:C, a similar frequency would be expected in our population of extreme quintiles of the distribution of FVII:C. The frequency in total population of 1158 subjects was, however, 0.14. This suggests that another factor, linked to the R/Q353 polymorphism, is associated with FVII:C.

There have been several intervention studies in which the effect of diet on factor VII was studied. Marckmann and colleagues[75,76] have reported a reduction in FVII:C after a low-fat/high-fibre diet compared to a high-fat/low-fibre diet in young and middle-aged subjects. No difference in FVIIt between both diets was observed. In one intervention study among diabetic patients, FVII:C was almost 23% lower on a high-fibre diet compared to a low-fibre diet. The results from our study suggest that dietary fibre in older men and women is related to FVII:C as well as FVIIt; an increase in fibre intake of 10 g (an increase that would raise the mean fibre intake towards the level of the current Dutch Guidelines for Healthy Eating[117]) would result in a decrease of FVII:C by 6.4 %PP and of FVIIt by 3.8 %PP. It is however important to notice that the regression coefficient may be overestimated as we included only the extreme quintiles of the distribution of factor VIIc.

In several studies FVII:C decreased after a low-fat diet compared to a high-fat diet[20,25,36]. No difference was observed between diets with different fatty acid composition[20,54,78,80]. In our study only an association with saturated fat was found. One explanation may be that in intervention studies the intake of different fatty acids is usually much higher than it would be in a habitual diet. Another difference is that in our study blood samples were collected in a non-fasting state at a non-standardized moment, while in intervention studies samples

were collected in the fasting state. However, when we adjusted the associations for the time since the last meal before blood sampling, the results were similar.

A possible mechanism behind the relation of dietary fat and fibre with FVII:C may be found in the positive association of FVII:C with serum triglycerides as reported previously[120]; serum triglycerides are lowered by fibre intake and directly increased by fat intake[28,125]. The associations of factor VII with the three dietary factors all varied between the genotype groups of the R/Q353 polymorphism. To our knowledge no other studies on diet and factor VII have been published which included assessment of the R/Q353 polymorphism. Some evidence is available that the association between factor VII and serum-triglycerides is influenced by the R/Q353 polymorphism. In two studies a positive association between factor VII and triglycerides was found in individuals with the RR genotype but no association in those with the Q allele[57,58]. As the association of factor VII with dietary fat and with dietary fibre may both be mediated through serum-triglycerides, it could be expected that the influence of the R/Q353 polymorphism is also found in the association of factor VII with diet. It is, however, important to note that the individuals with the RQ genotype were combined with those with the QQ genotype, because of small numbers in the latter group. Theoretically the association of factor VII with dietary factors may be different if analyzed in each genotype group separately. Furthermore the R/Q353 polymorphism is strongly linked to the -323Ins10 polymorphism, which is suggested to be the functional polymorphism[107,124].

In combination with tissue factor, factor VII is a very potent procoagulant, and high levels may increase the risk of a thrombotic event. The RR genotype of the R/Q353 polymorphism of factor VII is a risk indicator for myocardial infarction through increased factor VII levels. This risk may be especially important in an environment of a high saturated fat/low-fibre and protein diet.

CHAPTER 5

Coagulation factor VII, serum-triglycerides and the R/Q353 polymorphism: differences between older men and women

Abstract

Coagulation factor VII activity (FVII:C) is a risk indicator for cardiovascular disease. It is related to serum-triglycerides, and the R/Q353 polymorphism (alleles R and Q) in the gene coding for factor VII is strongly associated with factor VII. The association of serum-triglycerides with factor VII may differ between the genotypes, but the results of earlier studies were inconsistent and did not include older people. We studied FVII, triglycerides and the R/Q353 polymorphism in the Rotterdam Study.

In 1158 older subjects (489 men and 669 women) FVII:C, factor VIIt, serum-triglycerides and the R/Q353 genotype were determined.

In women triglycerides were positively associated with FVIIt and FVII:C (FVIIt: ß=12.4 %PP/mmol/L, CI: 10.3-14.5; FVII:C: ß=13.1 %PP/mmol/L, CI:10.4-15.8). These associations varied by genotype (FVIIt: RR: ß=11.7, CI: 9.6-13.8, RQ/QQ: ß=7.9, CI: 4.6-11.2; FVII:C: RR: ß=12.5, CI:9.5-15.5, RQ/QQ: ß=6.4, CI:1.4-11.4).

In men the associations of FVIIt and FVII:C with triglycerides were weaker (FVIIt: ß=5.9, CI:4.1-7.7; FVII:C: ß=8.7, CI:6.2-11.2). There was no difference between the genotype groups.

These results suggest that only in older women the strength of the association of factor VII with serum-triglycerides varies according to genotype of the R/Q353 polymorphism.

L.I. Mennen, M.P.M. de Maat, E.G. Schouten, C. Kluft, P.T.V.M. de Jong, A. Hofman, D.E. Grobbee

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Introduction

Increased factor VII coagulant activity (FVII:C) is associated with an increased risk of heart disease[1,119]. Therefore, interest in determinants of factor VII has grown. The R/Q353 polymorphism in the gene coding for FVII is the result of a single base change in the codon for amino acid 353, leading to the replacement of arginine (R) by glutamine (Q)[23]. Presence of the Q allele is related to lower levels of FVII:C and total factor VII[23].
In several studies serum-triglyceride concentration was positively associated with FVII:C[37,38,94,97] and with total factor VII[16,40]. As the R/Q353 polymorphism has a substantial influence on the level of both FVII:C and total factor VII[121], the association of triglycerides with factor VII may differ between the genotypes. In a few studies this was investigated, however results are difficult to interpret, since subjects differed in ethnic background, disease status and gender[57,58,105,126,127]. Furthermore, sample sizes were too small to account for the low frequency of the Q allele (0.099)[124]. Although the thrombogenic potential of factor VII may be especially important in older people among whom atherosclerosis is common, none of these studies included elderly subjects. The Rotterdam Study, a population-based study, gave the opportunity to evaluate factor VII, serum-triglycerides and the R/Q353 polymorphism in a large cohort of Caucasian men and women, where adequate representation of the Q allele could be ensured by selecting the upper and lower quintile of the FVII:C distribution.

Subjects and methods

The Rotterdam Study is a population-based study among 7983 subjects of 55 years and over[111]. Subjects with myocardial infarction in the year before the investigation, diabetes mellitus, use of anticoagulants or stasis during venepuncture were excluded, leaving 3005 subjects. None of the female subjects used hormone replacement therapy.
FVII:C was measured in non-fasting blood samples with a one-stage-clotting assay using human thromboplastin (Tromborel S, Behringwerke, Germany) and factor VII-deficient plasma (Ortho Diagnostic System, Beerse, Belgium). The results are expressed as percentages of pooled plasma (%PP). Individuals in the extreme quintiles of the FVII:C distribution were selected (n=1158), expecting an enrichment for the Q allele (i.e. subjects with the RQ or QQ genotype) in the lowest quintile. In plasma of these persons total factor VII (FVIIt) was estimated by a two-stage amidolytic micro-titre assay (Chromogenix, Mölndal, Sweden)[122]. Different pooled plasma's were used for the measurement of FVII:C and FVIIt. Serum-triglycerides were measured with a colorimetric assay using a Kodak Ektachem 250 Analyzer.

DNA polymorphisms were determined as described previously[124]. Briefly, genomic DNA was amplified using PCR with oligonucleotid primers as described by Lane et al[57]. The reaction components were incubated at 94°C for 4 minutes, followed by 32 cycles of 94°C for 1 minute, 59°C for 1.5 minutes and 72°C for 2 minutes. Ten µl PCR product were digested with 5 units of MspI (Gibco BRL) at 37°C. MspI digestion yielded a constant band of 40 base pair (bp). 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[23].
Complete data were available for 669 women and 489 men. Differences between the lower and the upper quintile, and between genotype groups were compared with the Student T-test. The relation of FVII:C and FVIIt with serum-triglycerides was examined using linear regression analyses, separately for men and women, with age and body mass index as possible confounders. Since adjustment of the regression models for belonging to the lowest or highest quintile did not change the results, the quintiles were combined. The same analyses were carried out separately for genotype groups. As the association of triglycerides with factor VII was in the same direction in individuals with the RQ and QQ genotype and because the group of individuals with the QQ genotype was too small (n=32) for meaningful analyses, all individuals carrying the Q allele were combined in one group.

Results and discussion

As the results for men and women were different and combined interpretation may not be appropriate, they are given separately.

Men

There was no difference between the lowest and the highest quintile of FVII:C in age (mean (SD): 67.3 (7.2) vs 65.8 (6.7) years). In the lowest quintile the frequency of the Q allele was 0.25 and triglyceride concentration was 1.8 (0.8) mmol/L, in the highest quintile this was 0.06 and 2.5 (1.4) mmol/L. In the total group FVIIt was 48 %PP and 88 %PP for men with the QQ and RR genotype respectively, and FVII:C was 85 %PP and 119 %PP (Table 5.1A). FVIIt and FVII:C were both positively associated with serum-triglycerides (Table 5.2A). For FVIIt there was no difference between the genotype groups. For FVII:C there was a trend towards a stronger association with triglycerides in men carrying the Q allele.

Table 5.1. FVII:C and FVIIt (mean ± SE) per genotype for men (A) and women (B).

A. Men

		Total group	Lowest quintile	Highest quintile
FVIIt*	RR	87.8 ± 1.1 (n=337)	68.8 ± 1.2 (n=122)	98.6 ± 1.1 (n=215)
	RQ	64.3 ± 1.6 (n=121)	57.6 ± 1.2 (n=93)	86.6 ± 2.8 (n=28)
	QQ	48.2 ± 1.8 (n=12)	47.5 ± 1.9 (n=11)	55.5 (n=1)
FVII:C*	RR	119.0 ± 1.7 (n=352)	79.7 ± 0.7 (n=127)	140.5 ± 1.0 (n=225)
	RQ	90.6 ± 2.5 (n=125)	76.7 ± 1.0 (n=96)	136.6 ± 2.5 (n=29)
	QQ	85.4 ± 1.2 (n=12)	73.6 ± 2.4 (n=11)	215.0 (n=1)

B. Women

		Total group	Lowest quintile	Highest quintile
FVIIt*	RR	101.1 ± 1.2 (n=492)	78.8 ± 1.0 (n=203)	116.7 ± 1.2 (n=289)
	RQ	71.5 ± 1.5 (n=141)	66.6 ± 1.1 (n=120)	99.5 ± 1.9 (n=21)
	QQ	50.5 ± 2.8 (n=19)	50.5 ± 2.8 (n=19)	
FVII:C*	RR	129.9 ± 1.5 (n=506)	91.5 ± 0.8 (n=207)	156.3 ± 0.8 (n=299)
	RQ	97.4 ± 2.3 (n=143)	87.6 ± 1.2 (n=122)	154.4 ± 2.2 (n=21)
	QQ	77.7 ± 4.0 (n=20)	77.7 ± 4.0 (n=20)	

* FVII:C and FVIIt are presented in %PP, different pooled plasma's were used

Humphries et al[58] reported in a group of Caucasian males a positive association between FVIIt and triglycerides in men homozygous for the R allele and a weaker association for men carrying the Q allele. Saha et al[105] studied Dravidian Indians and observed a positive association in both genotype groups, the association being stronger in subjects carrying the Q allele. Several differences between these study populations, like ethnic background, diet and inclusion of women, may explain the difference in results. In our study among older men the association of FVIIt with triglycerides was confirmed both in those with the RR genotype and in those carrying the Q allele. We conclude that no major differences between the genotypes of the R/Q353 polymorphism in the association of FVIIt and triglycerides are present in Dutch elderly men.

Table 5.2. Regression coefficients and 95% confidence intervals (95% CI) of triglycerides on FVII:C and FVIIt by genotype for men (A) and women (B)*.

A. Men

	FVIIt		FVII:C	
	ß	95% CI	ß	95% CI
Total group	5.9	4.1,7.7	8.7	6.2,11.2
RR	5.5	3.7,7.3	7.3	4.6,10.0
RQ/QQ	5.7	2.7,8.7	10.8	5.8,15.7

B. Women

	FVIIt		FVII:C	
	ß	95% CI	ß	95% CI
Total group	12.4	10.3,14.5	13.1	10.4,15.8
RR	11.7	9.6,13.8	12.5	9.5,15.5
RQ/QQ	7.9	4.6,11.2	6.4	1.4,11.4

* All models adjusted for age and body mass index. The regression coefficients give the change in FVIIt and FVII:C in percent pooled plasma per unit change in triglycerides (%PP/mmol/L).

Interpretation of results on FVII:C are more complicated, since it is increased immediately after fat intake and samples can be taken in fasting and postprandial state[107]. In our study samples were taken throughout the morning and afternoon, but adjustment for time since last meal did not change the results, suggesting that postprandial effects of the Dutch light breakfast and lunch are limited. This may be different in other populations with different ethnic background and dietary habits. The relation between FVII:C and triglycerides did not differ between the genotype groups in our study. The same was observed in a study among Caucasian patients with non-insulin-dependent-diabetes mellitus, where fasting blood samples were taken[126]. Humphries et al[58] observed a positive association between non-fasting FVII:C and triglycerides in Caucasian males with the RR genotype, but not in those carrying the Q allele. Similar results were observed in Gujarati Indians by Lane et al[57]. In Dravidian Indians, however, the association between fasting FVII:C and triglycerides was only found in those carrying the Q allele[105]. The discrepancies in these results may be ascribed to the different prandial states and the differences in dietary habits

between the study populations. Furthermore, if the relation between FVII:C and triglycerides depends on the amount of activated factor VII in the sample, different results will be expected between essays for FVII:C which differ in sensitivity towards activated factor VII[44]. This could thus be another explanation for the discrepancy in findings between the studies described. Further analysis in larger groups and with specific attention to the prandial state and the essay used to measure factor VII is required.

Women

There was no difference between the lowest and the highest quintile of FVII:C in age (mean (SD): 66.8 (8.2) vs 66.6 (7.3) years). In the lowest quintile the frequency of the Q allele was 0.23 and triglyceride 1.7 (0.7) mmol/L, in the highest quintile this was 0.03 and 2.4 (1.1) mmol/L. In the total group FVIIt was 51 %PP and 101 %PP for women with the QQ and RR genotype respectively, and FVII:C was 78 %PP and 130 %PP (Table 5.1B). The triglyceride concentration was positively associated with FVIIt and FVII:C (Table 5.2B). The regression coefficients were almost twice as high in women with the RR genotype, compared to women carrying the Q allele ($p<0.05$ for interaction) and they were larger compared to those found in men.

No substantial data on the relation of triglycerides with factor VII between genotype groups in women are available. In our study we observed a stronger relation of both FVIIt and FVII:C with triglycerides in RR homozygotes, while these associations in Q allele carriers was similar to that in men. These results suggest that the regulation of the factor VII metabolism by the R allele is different in women compared to men. Support for this view is given by Meilahn et al[127], who observed a post-menopausal increase in FVII:C only in women homozygous for the R allele and not in those carrying the Q allele. This may explain why they did not find a genotype-triglycerides interaction in the total group of pre- and postmenopausal women. In conclusion, our findings suggest, that at least in postmenopausal women the triglyceride status may contribute in those with the RR genotype to an increased risk for myocardial infarction, provided that the risk observed in epidemiological studies in men also applies to women.

General

Humphries et al[58] previously discussed the impact of the interaction of triglyceride status and the R/Q353 polymorphism in relation to the risk of myocardial infarction. Our present data strongly suggest to pay specific attention to gender differences. The impact of genetic variation in factor VII may obviously be quite distinct in populations which differ in life style. The possibility that both genetically susceptible groups and life style elements can be identified as risk determinants may open possibilities for targeted prevention.

CHAPTER 6

Factor VIIa response to a fat-rich meal does not depend on fatty acid composition: A randomized controlled trial

Abstract

A fat-rich meal increases 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 cross-over 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 % of fat) and a control breakfast (1.5 en% fat) (cross-over). The fat-rich breakfasts differed in fatty acid composition: one rich in palmitic acid (21.7 g), one in stearic acid (18.6 g), the other two in linoleic and linolenic acid one with a ratio 3:1 (12.5/3.9 g) and another with a ratio of 15:1 (18.8/1.2 g). At 8.00 AM before the breakfast (fasting) and at 1.00 and 3.00 PM blood samples were taken, in which FVIIa and serum-triglycerides were measured.
FVIIa response to the fat-rich meals ranged from 11.6 IU/mL (95% confidence interval: 8.3,14.9) on the stearic meal to 15.9 IU/mL (12.0,19.8) on the linoleic/linolenic 15:1 meal at 1.00 PM and from 14.9 IU/mL (10.6,19.2) to 21.1 IU/mL (16.6,25.6) for the same meals at 3.00 PM. The responses did not differ between the fat-rich meals. After the control breakfast FVIIa decreased with 6.3 IU/mL (3.9,8.7) at 1.00 PM and with 8.7 IU/mL (6.3,11.1) at 3.00 PM. The triglyceride response was lower after both linoleic/linolenic rich breakfasts compared to the palmitic and stearic breakfast ($p<0.05$) 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.

L. Mennen, M. de Maat, G. Meijer, P. Zock, D. Grobbee, F. Kok, C. Kluft, E. Schouten

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Introduction

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, where the prevalence of atherosclerosis is high.

FVIIa can be measured directly, but also the coagulant activity of factor VII (FVII:C) can be measured. Since the direct measurement of FVIIa became only recently available, FVII:C was often used in previous studies. This measurement, however, does not only reflect 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 ischaemic heart disease was observed[2,6]. It is by now accepted that FVII:C increases postprandially after intake of dietary fat[120]. It is however not yet clear whether this increase is dependent on fatty acid composition of the fat consumed. Five studies have evaluated this for FVII:C, but the results were contradicting and sample sizes were small (n=4-10)[28,32,92,128,129]. In a few experimental studies, also a post-prandial increase in FVIIa has been observed[106,130-132], but none of these studies investigated different fatty acids. Furthermore, FVIIa is higher in post-menopausal women compared to men of the same age[13], but (elderly) women have rarely been included in studies on FVIIa and dietary fat.

One of the mechanisms which may explain an effect of dietary fat on factor VII is based on activation of factor VII during lipolysis of triglyceride rich lipoproteins[70,120]. 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[28,106].

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

Subjects and 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 fulfil the following inclusion criteria: older then 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, no medical problems after fat consumption. For purpose of a separate research question subjects were selected on the genotype of the R/Q353 polymorphism of the gene coding for factor VII, in order to have comparable numbers of subjects in each genotype group. In total 96 women entered the study. One participant dropped out because of illness and four because not enough blood could be sampled. At the end of the study complete data were available of 91 participants (35 with the RQ or QQ genotype and 56 with RR). The study was approved by the Medical Ethics Committee of the Agricultural University Wageningen and written informed consent was obtained from all participants.

Height and weight were measured and body mass index was calculated by dividing weight by the square of height (kg/m^2). Blood pressure was measured with the subject in recumbent position, three times at five minutes intervals. The mean of the last two measurements was taken as the mean blood pressure. The habitual fat intake was estimated with a validated food-frequency questionnaire[133]. The general characteristics of the study population are presented in Table 6.1.

Table 6.1. General characteristics of the female study population (n=91)

	MEAN	SD
Age (years)	75.7	5.2
Body mass index (kg/m^2)	27.7	4.1
Systolic blood pressure (mmHg)	141.6	19.4
Diastolic blood pressure (mmHg)	72.5	9.5
Total cholesterol (mmol/L)*	6.03	0.86
Serum-triglycerides (mmol/L)*	1.7	0.67
Factor VIIa (IU/mL)*	75.2	32.1
Current smoking (%)	7.7	

* the levels are from fasting blood samples

Intervention Procedure

Every participant received each of the five different breakfasts with at least one week in between, in different order (cross-over). 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 8.00 AM after an overnight fast. Within half an hour after taking blood samples with minimal stasis, they ate their breakfast. At 1.00 PM and 3.00 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 10.00 AM and 2.00 PM coffee or tea with a fat free snack were served and at noon a fat free lunch. 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.

Table 6.2. Analyzed fat composition of the breakfasts

	Palmitic	Stearic	Linol 3:1*	Linol 15:1#	Control
kJ+	3966	3721	3798	3815	3657
Fat g	55.7	49.3	51.4	51.5	1.5
Fat en%+	53	50	51	51	1.5
C16:0§	21.7	4.9	5.8	5.3	0.3
C18:0§	3.2	18.6	3.9	3.9	0.06
C18:1§	20.4	18.6	22.1	20.0	0.2
C18:2§	7.6	5.2	12.5	18.8	0.7
C18:3§	0.2	0.1	3.9	1.2	0.05

The underlined numbers show the fat exchange between the breakfasts

* Linol 3:1 = linoleic/linolenic acid in ratio 3 to 1

\# Linol 15:1 = linoleic/linolenic acid in ratio 15 to 1

\+ calculated

§ In grams per breakfast, C16:0 = palmitic acid, C18:0 = stearic acid, C18:1 = oleic acid, C18:2 = linoleic acid, C18:3 = linolenic acid

Breakfast

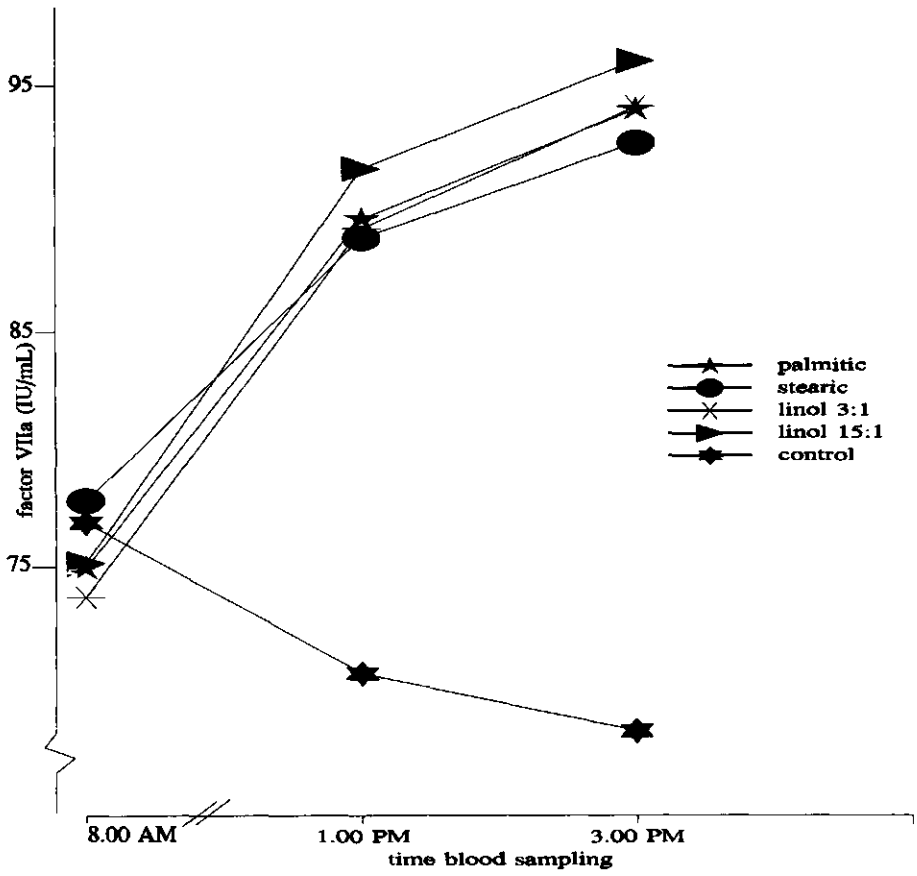
At each day the participants received a different breakfast with similar total energy content. Four breakfasts contained 50 energy percent (en%) of fat (fat-rich breakfast), the control breakfast only 1.5 en% of fat (Table 6.2). In the control breakfast fat was

exchanged for carbohydrates (46 en% vs 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), the other two in linoleic (C18:2) and linolenic acid (C18:3), one with a ratio 3:1 (12.5/3.9 g) (linol 3:1) and another with a ratio of 15:1 (18.8/1.2 g) (linol 15:1).
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 yoghurt 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 (nutrical, Nutricia Zoetermeer). Each participant received the breakfasts in a different order.

Laboratory measurements
Blood was collected in siliconized vacutainer tubes containing 0.129 mol/L sodium citrate (Becton Dickinson). The first 5 mL were used for measurement of blood lipids. Samples were centrifuged for 30 minutes at 1500 x g 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, Germany), with a clotting assay using soluble recombinant tissue factor (Staclot, Diagnostica Stago, France)[60]. The recorded clotting time is inversely related to the FVIIa level (expressed in IU/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[115].

Data analysis
For every participant the five fasting measurements were combined to calculate 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 fasting FVIIa level from the levels at 1.00 PM and 3.00 PM. To determine whether average response on a type of breakfast differed from zero, 95% confidence intervals (CI) were computed. Multiple comparisons were made with the Tukey test to investigate whether the FVIIa response differed between the fat-rich breakfasts.

Figure 6.1. Response of FVIIa for each breakfast separately



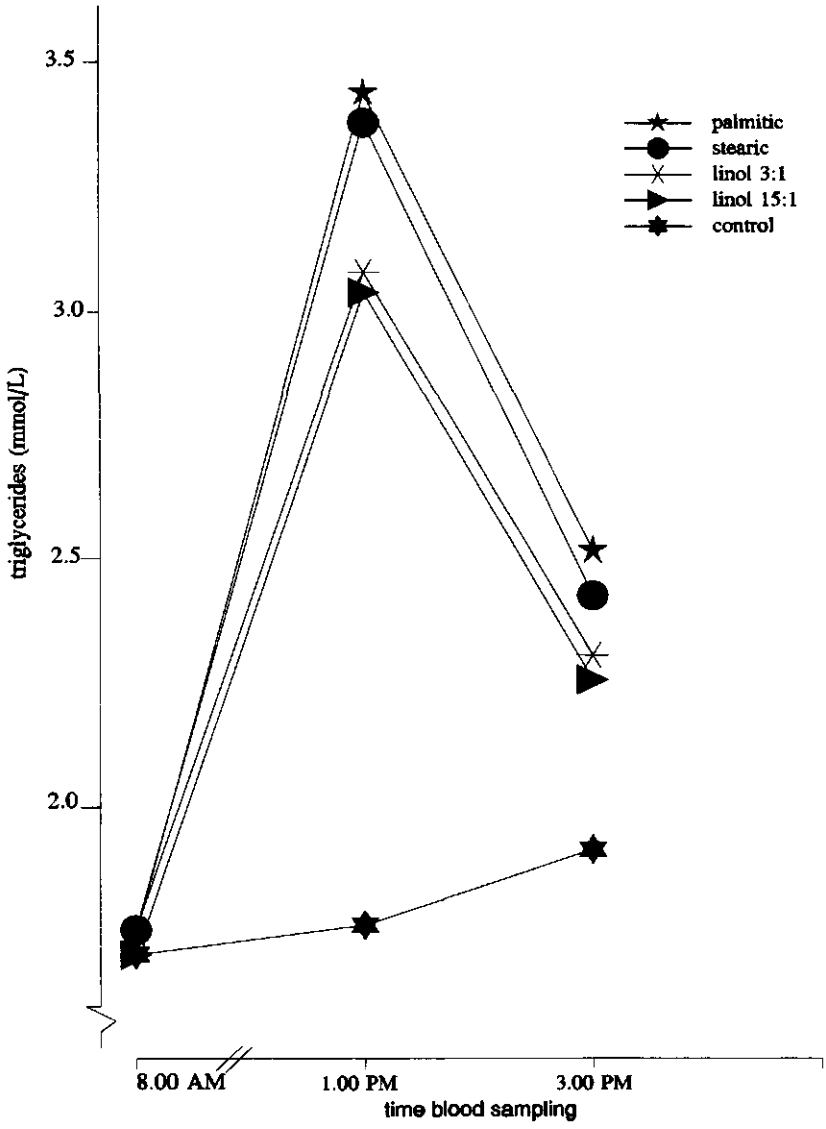
Results

The FVIIa responses did not differ between the fat-rich breakfasts (Figure 6.1). FVIIa response to the fat-rich meals ranged from 11.6 IU/mL (95% confidence interval: 8.3,14.9) on the stearic meal to 15.9 IU/mL (12.0,19.8) on the linoleic/linolenic 15:1 meal at 1.00 PM and from 14.9 IU/mL (10.6,19.2) to 21.1 IU/mL (16.6,25.6) for the same meals at 3.00 PM. When the results on the fat-rich breakfast were combined, FVIIa increased from 74.9 IU/mL (SD: 32.3) to 88.4 IU/mL (SD: 38.6) at 1.00 PM and to 93.0 IU/mL (SD: 41.0) at 3.00 PM (mean response at 3.00 PM: 19.5 IU/mL (15.8,23.2)). After the control breakfast FVIIa decreased with 6.3 IU/mL (3.9,8.7) at 1.00 PM and with 8.7 IU/mL (6.3,11.1) at 3.00 PM. The mean difference between the FVIIa response to the four fat-rich breakfasts combined and the response to the control breakfast in the 3.00 PM sample was 28.0 IU/mL (CI: 24.1,31.9).
After the control breakfast triglycerides concentration increased slightly up to 1.77 mmol/L (SD:0.89) in the 3.00 PM sample. The triglycerides responses on the fat-rich breakfasts were highest in the 1.00 PM sample, but were still elevated in the 3.00 PM sample (Figure 6.2). The triglyceride level at 1.00 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 1.00 PM between the palmitic and stearic breakfast compared to linol 3:1 and 15:1 breakfast ranged from 0.28 mmol/L for stearic vs linol 15:1 to 0.39 mmol/L for palmitic vs linol 3:1 ($p<0.05$ for differences between breakfasts). There was no association between the response of FVIIa and the response of triglycerides at any of the blood sampling occasions.

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 to what elderly people are used to eat at breakfast (approximately 15 g fat[134]), in order to maximize the chance of a FVIIa response. In fact, on a normal day FVII:C would probably hardly start to rise until after lunch[29]. For feasibility reasons we decided to put the amount of fat usually eaten at breakfast and lunch together in one fat-rich breakfast. Therefore, this does not necessarily reflect the FVIIa change at these hours in a normal situation.

Figure 6.2. Response of serum-triglycerides for each breakfast separately



The participants in our study may have been a somewhat healthy selection of the general elderly population, because of the inclusion criteria we used in order 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[23], while in our study population 38% carried this allele. Since the FVIIa response to a fat-rich meal is lower in subjects carrying the Q allele than in those with the RR genotype[135], the increase in FVIIa after a fat-rich meal as observed in our study, probably underestimates the true increase. Furthermore, only women were included in our study. Post-menopausal women have higher values of FVIIa compared to men of the same age[13]. It is, however, not yet known whether the response of FVIIa to a fat-rich meal in post-menopausal women is also higher than in men.
For practical reasons it was not possible to obtain more than three blood samples per day in these elderly women. Although Figure 6.1 suggests that after the fat-rich breakfast, FVIIa is still rising at 3.00 PM, it is expected from the literature that the peak in the FVIIa response occurred at some point between 1.00 PM and 3.00 PM[106,128,130-132].

Table 6.3. Previous studies on the response of FVII:C to a fat-rich meal

REF	DIET*	SUBJECTS	FVII:C
28	SAFA vs PUFA	5 men, 4 women, healthy	--#
32	SAFA vs PUFA	10 men, middle-aged	--
92	SAFA vs PUFA	4 men, 1 women, healthy	SAFA ↑†
128	olive oil vs MCT fat	4 men, 2 women, age 22-43	olive oil ↑ MCT --
129	C18:0 vs C14:0	10 men, age 21-28	C18:0 ↑ (ns§) C14:0 --

* SAFA = saturated fat, PUFA = polyunsaturated fat, MCT = medium chain triglycerides, C18:0 = stearic acid, C14:0 = myristic acid

\# '--' = no difference

† '↑' = increased

§ ns = non significant

Five studies have been published in which the effect of fatty acid composition on FVII:C was investigated[28,32,92,128,129] (Table 6.3). In three studies the effect of FVII:C to a meal rich

in saturated fat was compared to a meal rich in unsaturated fat[28,32,92], but only in one of them a difference between the meals was found[92]. Sanders et al[128] compared a meal rich in olive oil with one rich in medium chain triglycerides and observed an increase in FVII:C only after the olive oil meal. Stearic and myristic acid, both saturated fats, were compared in a study by Tholstrup et al[129]. Their results showed no increase after myristic acid and a minor increase after stearic acid, though this was not significant.

A major difference between the studies is that in some of them FVII:C was studied directly after a fat-load[32,128,129], while in the others postprandial blood samples were taken on the final day of a one or four week diet[28,92]. The diet consumed in the period before the post-prandial test may influence the response of factor VII. However, in our study we did not find an association between nutrient intake of the habitual diet and the postprandial response of FVIIa. 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 which could explain the effect of dietary fat on factor VII involves triglyceride rich lipoproteins (TRLP)[70]. During lipolysis of TRLP factor VII becomes activated. In our study we measured serum-triglycerides as an estimate of circulating postprandial TRLP, but the concentration of serum-triglycerides was not associated with FVIIa. This was also observed in one previous study[132]. Silveira et al[106], however, did observe a positive association between FVIIa and serum-triglycerides, measured six hours after a fat load. This discrepancy in results may indicate that either serum-triglycerides do not reflect TRLP or that triglycerides are not involved in the mechanism behind the relation between factor VII and dietary fat.

FVIIa is a very potent coagulant, which in complex with tissue factor may induce thromboembolic 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 post-prandial increase in triglycerides.

CHAPTER 7

Postprandial response of coagulation factor VIIa varies across R/Q353 genotypes

Abstract

Activated factor VII (FVIIa) is, together with tissue factor, a very potent coagulant. The amount of circulating FVIIa at the time of a cardiovascular event, may be a key determinant of its outcome. The level of FVIIa is influenced by dietary fat intake and genetic factors. We have investigated whether the response of FVIIa to a fat-rich breakfast varies across genotypes of the R/Q353 polymorphism in the factor VII gene.
In 321 elderly women (>60 years) the genotype for the R/Q353 polymorphism was assessed. Fiftyfour randomly selected women with the RR genotype and 37 women with the RQ or QQ genotype participated in the intervention study. Subjects received five breakfasts each on a separate day: one control breakfast (1,5 energy percent of fat (en%)) and four high-fat test breakfasts (50 en%). At 8.00 AM before each breakfast (fasting) and at 1.00 PM and 3.00 PM blood samples were taken for measurement of FVIIa.
The mean fasting FVIIa level was 93.7 IU/mL (SD:26.5) in women with the RR genotype and 38.3 (SD:17.8) in those with the QQ genotype. The FVIIa response to the test breakfasts at 1.00 PM and 3.00 PM differed from the response to the control breakfast in both genotype groups. The mean absolute total response to all four test breakfasts was 37.0 IU/mL in those with the RR genotype and 16.1 IU/mL in those carrying the Q allele ($p<0.001$ for difference). Likewise, the FVIIa response relative to the fasting FVIIa level was significantly higher in women homozygous for the R allele.
FVIIa increases profoundly after a fat-rich meal in elderly women with the RR genotype for the R/Q353 polymorphism. This observation may indicate a considerable difference in cardiovascular risk between genotype groups concerning a fat-rich diet.

L. Mennen, M. de Maat, G. Meijer, P. Zock, D. Grobbee, F. Kok, C. Kluft, E. Schouten

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Introduction

Coagulation factor VII circulates in plasma mainly as an inactive zymogen, and about 1% of this 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. Until recently FVIIa could not be measured. Instead a functional clotting assay was used (factor VII:C), which reflects FVIIa and an unknown part of factor VII zymogen. In two longitudinal studies a positive association between factor VII:C and the risk of fatal ischaemic heart disease was observed[2,6].

In the gene coding for factor VII the R/Q353 polymorphism is the result of a single base change in the codon for amino acid 353 and leads to the replacement of arginine (R) by glutamine (Q)[23]. Presence of the Q allele is associated with low levels of factor VII[23].

Another major determinant of FVIIa is dietary fat intake. Several intervention studies, comprising mostly small numbers of middle-aged male subjects, have shown an increase of FVIIa after a fat-rich meal[106,128,130-132]. It is not yet clear whether this response of FVIIa to fat intake is affected by the R/Q353 polymorphism.

We studied the FVIIa response to fat-rich meals in a large number of apparently healthy elderly women, who were selected on their genotype for the R/Q353 polymorphism.

Subjects and methods

Elderly women living independently in retirement communities were asked to participate in the study. The women had to fulfil 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-antagonist, no use of hormone replacement therapy, no medical problems after fat consumption. In 321 women the genotype for the R/Q353 polymorphism was assessed. Thirtyfive out of the 55 women found to carry the Q allele were able to participate and fiftysix women with the RR genotype were randomly selected. Thus a total of 91 women were included in the intervention study. The study has been approved by the Medical Ethics Committee of the Wageningen Agricultural University and written informed consent was obtained from all participants.

Participants received five breakfasts, each at a different day. At 8.00 AM (after an overnight fast) the first blood sample was taken and within half an hour the subjects ate their breakfast. At 1.00 PM and 3.00 PM additional blood samples were taken. The participants stayed in the same room during the whole procedure and received after the breakfast only fat free foods if wanted, and were not allowed to eat anything else.

All breakfasts had the same total energy content, but four breakfasts contained 50 energy percent (en%) of fat (test breakfast), while the control breakfast contained only 1.5 en% of 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 yoghurt mixed with 65 g energy rich powder (caloreen, Clintec Utrecht), 20 g sugar, one cup of tea and 100 mL energy rich drink (nutrical, Nutricia Zoetermeer). For purpose of a separate research question the test-breakfasts differed in fatty acid composition.
Blood was collected in siliconized vacutainer tubes containing 0.129 mol/L sodium citrate (Becton Dickinson). Samples were centrifuged for 30 minutes at 1500 x g and 20°C. Citrated plasma was snap frozen and stored at -80°C until laboratory analysis. FVIIa was measured on the STA instrument (Boehringer, Germany) with a clotting assay using soluble recombinant tissue factor (Staclot, Diagnostica Stago France)[60]. The clotting time recorded is inversely related to the FVIIa level (expressed in IU/mL).
DNA was extracted from mouth swabs according to Meulenbelt et al[136]. DNA polymorphisms were determined as described previously[124]. Briefly, genomic DNA was amplified using PCR with oligonucleotide primers as described by Lane et al[57]. The reaction components were incubated at 94°C for 4 minutes, followed by 32 cycles of 94°C for 1 minute, 59°C for 1.5 minutes and 72°C for 2 minutes. Ten µl PCR product were digested with 5 units of MspI (Gibco BRL) at 37°C. MspI digestion yielded a constant band of 40 base pair (bp). 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[23].
The response of FVIIa to each meal was calculated by subtraction of the fasting FVIIa level from the levels at 1.00 PM and 3.00 PM. Since the number of persons with the QQ genotype was too small for meaningful analysis, they were combined with subjects having the RQ genotype. Furthermore, the mean of the responses of FVIIa to the four test-breakfasts was used in this study, as there was no difference in response of FVIIa between the test-breakfasts[135]. The mean absolute total response was calculated as the mean response to the test breakfasts at 3.00 PM minus the response to the control breakfast at 3.00 PM. The mean relative total response is the mean absolute total response as a percentage of the fasting FVIIa level. With the Student T-test it was evaluated whether the response to the test breakfasts differed from the response to the control breakfast, and whether the mean absolute and relative total response differed between the genotype groups. To allow further adjustment for fasting level of FVIIa a linear regression analysis was performed with the mean absolute total response as dependent variable and the genotype group and fasting FVIIa level as independent variables.

Table 7.1. General characteristics according to genotype of the female study population (mean ± SD)

	Total (n=91)	RR (n=56)	RQ (n=32)	QQ (n=3)
Age (years)	75.7 ± 5.2	75.6 ± 5.2	76.0 ± 5.2	76.0 ± 6.1
Body mass index (kg/m^2)	27.7 ± 4.1	28.0 ± 3.9	27.4 ± 4.5	26.1 ± 4.5
Systolic blood pressure	142 ± 19.4	142 ± 21.9	142 ± 15.2	133 ± 18.6
Diastolic blood pressure	72 ± 9.5	73 ± 9.5	71 ± 9.0	68 ± 17.8
Total cholesterol*	6.03 ± 0.86	6.12 ± 0.86	5.90 ± 0.87	5.87 ± 0.34
Serum-triglycerides*	1.70 ± 0.67	1.72 ± 0.63	1.69 ± 0.76	1.47 ± 0.51
Factor VIIa (IU/mL)*	75.2 ± 32.1	93.3 ± 26.7	49.3 ± 19.1	39.5 ± 17.2
Frequency Q allele#	0.09			

* fasting levels, lipids in mmol/L

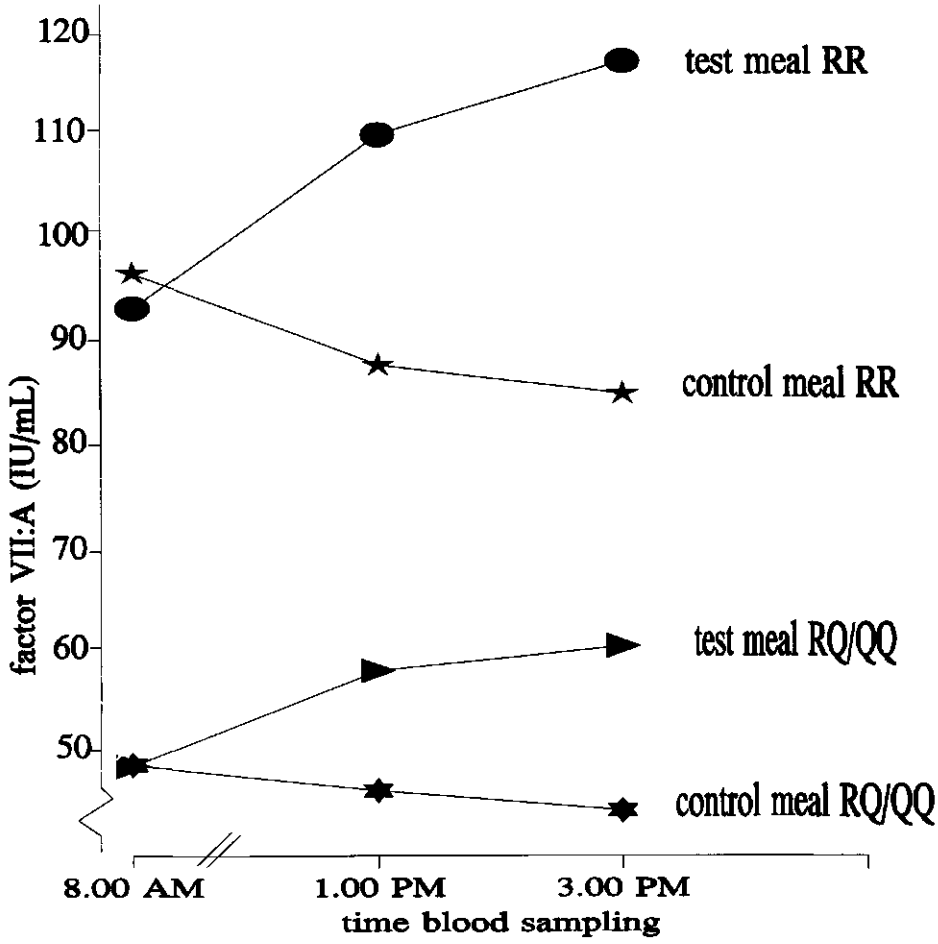
\# frequency in source population of 321 women

Results

General characteristics of the study population according to genotype are presented in Table 7.1. The frequency of the Q allele in the source population of 321 women was 0.09. Compared to women homozygous for the R allele, fasting FVIIa was 47% lower (49.3 IU/mL vs 93.3 IU/mL) in women with the RQ genotype and 58% lower (39.5 IU/mL versus 93.3 IU/mL) in women homozygous for the R allele.

The response of FVIIa to the test-breakfasts at 1.00 PM and at 3.00 PM differed from the response to the control breakfast, both for those with the RR genotype and those with the RQ or QQ genotype (Figure 7.1). The mean absolute total response of FVIIa was 37.0 IU/mL in the group with the RR genotype and 16.1 IU/mL ($p<0.001$) in those carrying the Q allele. Notably, the R/Q353 polymorphism was an independent predictor of the FVIIa response even after adjustment for fasting FVIIa level in the linear regression model (ß=-11.8 (95% confidence interval: -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 those carrying the Q allele respectively.

Figure 7.1. Mean FVIIa levels after test and control breakfast according to genotype



Discussion

The results of this study in elderly women clearly demonstrate that the absolute as well as the relative response to a fat-rich meal depend on the genotype of the R/Q353 polymorphism.

The subjects in this study were selected on genotype to get similar numbers of women with the RR and RQ or QQ genotype. The frequency of the Q allele in the source population of 321 women, however, was similar to the frequency previously reported in white Europeans[57,58].

In five earlier studies an increase in FVIIa was found after a fat-rich meal[106,128,130-132]. Miller et al[130] observed a 19% increase in FVIIa six and a half hour after a high-fat (64 en% of fat) meal and no response to a low-fat (9 en% of fat) meal. In a study by Sanders et al[128], it was found that FVIIa increased in response to 90 g and 120 g of fat, with a peak at 7 hours, but not in response to 15 or 60 g of fat. Kapur and colleagues[132] demonstrated an increase in FVIIa six hours after a fat rich meal (30 g/m^2). Two studies by Silveira et al[106,131], among 31 male post-infarction patients and 24 healthy males, showed an increase of FVIIa after six hours, during a fat tolerance test. The absolute increase in FVIIa was higher in post-myocardial infarction patients with the RR genotype than in patients carrying the Q allele. However, in contrast to our study, the percentual increase in FVIIa did not differ between the genotypes[106]. This discrepancy may be explained by different type of subjects; men with myocardial infarction in the study of Silveira et al[106] and healthy elderly women in our study. However, we think it is more likely that Silveira et al[106] did not find a difference in percentual response between the genotypes because they studied a much smaller number of subjects (31 of whom 8 with the RQ genotype).

One of the mechanisms which may explain an effect of dietary fat on factor VII, is based on activation of factor VII by negatively charged surfaces during lipolysis of triglyceride rich lipoproteins (TRLP)[70]. The fact that our data show differences in activation for the R and Q allele, independent of the fasting level of FVIIa, suggests a molecular difference between the two types of FVIIa in this respect. The results of our study do not clarify how the R/Q353 polymorphism could influence the activation of factor VII, but it may be that the charge change ensuing from the substitution of the positively charged arginine with a neutral glutamine affects the interaction of factor VII with lipid surfaces[137].

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 R/Q353 polymorphism. Since 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 recommendable for these subjects.

CHAPTER 8

General discussion

In this thesis a cross-sectional study among 489 elderly men and 669 elderly women (>55 years) and an intervention study among 91 elderly women (>60 years) are described. These studies were designed to investigate (1) the magnitude of the association of FVII:C and FVIIt with diet and (2) the response of FVIIa to fat-rich meals with different fatty acid composition. For both objectives the modifying effect of the R/Q353 polymorphism was taken into account. A complete overview of the main findings of the cross-sectional and intervention study described in this thesis is given in Table 8.1 and the most important results are mentioned below.

In the cross-sectional study FVII:C and FVIIt were inversely associated with dietary fibre. An increase of dietary fibre intake with 10 g per day would lead to a decrease in FVII:C with 6.4 %PP and in FVIIt with 3.8 %PP. These associations were even higher in subjects with the RR genotype, while they were absent in subjects carrying the Q allele. Furthermore, in elderly women the association of FVII:C with serum-triglycerides was twice as strong in women homozygous for the R allele, compared to women carrying the Q allele (Table 8.1).

The results from the intervention study showed that FVIIa increased after a fat-rich breakfast. The absolute response of FVIIa on a fat-rich meal was twice as high in elderly women homozygous for the R allele than in those carrying the Q allele. Also the response relative to the fasting FVIIa level was 10% higher in women with the RR genotype. There was no difference in FVIIa response between fat-rich breakfasts rich in either palmitic acid, stearic acid, or linoleic/linolenic acid with a ratio 3:1 or 15:1. Furthermore, we found no evidence that serum-triglyceride concentration mediates the effect of dietary fat on FVIIa (Table 8.1).

The methodological aspects of the cross-sectional and intervention study are described in the discussion sections of the former chapters. In this chapter, the specific findings will be discussed in a broader perspective, with special focus on the biological plausibility. Moreover, practical implications and suggestions for future research will be addressed.

Factor VII and coronary heart disease

Epidemiologic evidence

In at least 13 studies the association of factor VII with coronary heart disease has been investigated. These studies mainly focused on myocardial infarction as endpoint of interest, while in prospective follow up studies total coronary heart disease is taken instead. A summary of these studies is given in Table 8.2.

Two major prospective follow up studies have been performed among male subjects, in

Table 8.1. Main findings of the cross-sectional and intervention study described in this thesis

Independent variable	Total group*	RR*	RR/QQ*
Cross-sectional, men and women, 858 RR and 300 RQ/QQ			
Dietary fibre	FVII:C ↓	FVII:C ↓↓	FVII:C --
	FVIIt ↓	FVIIt ↓↓	FVIIt --
Saturated fat	FVII:C ↑	FVII:C ↑↑	FVII:C ↓↓
	FVIIt --	FVIIt: ↑	FVIIt ↓
Protein	FVII:C ↓	FVII:C ↓	FVII:C --
	FVIIt --	FVIIt --	FVIIt --
Triglycerides	men:	men:	men:
	FVII:C ↑	FVII:C ↑	FVII:C ↑
	FVIIt ↑	FVIIt ↑	FVIIt ↑
	women:	women:	women:
	FVII:C ↑↑	FVII:C ↑↑	FVII:C ↑
	FVIIt ↑↑	FVIIt ↑↑	FVIIt ↑
Intervention, women, 56 RR and 35 RQ/QQ			
fat-rich breakfast:			
palmitic	FVIIa ↑↑	FVIIa ↑↑↑	FVIIa ↑
stearic	FVIIa ↑↑	FVIIa ↑↑↑	FVIIa ↑
linoleic/linolenic 3:1	FVIIa ↑↑	FVIIa ↑↑↑	FVIIa ↑
linoleic/linolenic 15:1	FVIIa ↑↑	FVIIa ↑↑↑	FVIIa ↑
fat-free breakfast	FVIIa ↓	FVIIa ↓↓	FVIIa ↓

* *cross-sectional*: ↑ = positive association, ↓ = inverse association, -- = no association, more arrows indicate a stronger association
intervention: ↑ = increase, ↓ = decrease, more arrows indicate a stronger effect

Table 8.2. Studies on factor VII and coronary heart disease

Ref	Design	Population (age)	Endpoint*	FVII:C#	FVIIt	FVIIa
2	follow up, 6 years	2116 men (49)	23 fatal CHD	8.4% ↑		
6	follow up, 19 years	1511 men (40-60)	88 fatal CHD	9.4% ↑		
3	follow up, 2 years	335 subjects (55)	19 fatal MI	15.2% ↑		
46	case-control	men, 36 case (57) 7 control (59)	prior MI	28% ↑		
47	case-control	66 case (62) 16 control (43)	MI	22.1% ↑	--	
48	case-control	15 case (50) 15 control (32)	CHD	7% ↑ (ns)	21% ↑	
138	case-control	51 case (60) 31 control (58)	MI	--		
139	case-control	men, 94 case (40) 99 control (40)	MI	7% ↑	17% ↑	--
140	cross-sectional	2365 subject (54)	CHD	--	--	
141	case-control	68 case (69) 37 control (57)	CHD	--	--	
142	case-control	12 case	MI		--	
143	case-control	24 case 32 control (20-80)	MI	--		
144	case-control	men, 461 case	MI	6.2% ↓		

* CHD = coronary heart disease, MI = myocardial infarction

↑ = higher in patients compared to healthy control subjects, ↓ = lower in patients compared to healthy control subjects, -- = no difference between groups

which the relation between factor VII and coronary heart disease was evaluated. Both studies showed that FVII:C was almost 10% higher in men who died from coronary heart disease compared to those who remained free of coronary heart disease[2,6]. A difference of 1 SD in FVII:C was associated with a difference of nearly 50% in the probability of dying of coronary heart disease, but with no difference for non-fatal myocardial infarction[6]. In

another prospective study, which was relatively small, similar results were obtained[3].
In four case-control studies higher levels of FVII:C[46-48,139] or FVIIt[48,139] in patients with coronary heart disease compared to healthy control subjects were found. However, in other studies no differences in FVII:C[138,140,141,143], FVIIt[47,140-142] or FVIIa[139] between persons with and without coronary heart disease were observed. The results of one study even showed lower FVII:C levels in patients with myocardial infarction compared to healthy control subjects[144].
The results from the prospective follow up studies show that factor VII is a risk indicator for coronary heart disease, while the results from the case-control studies are conflicting. This may be explained by the possibility hat the level of factor VII is changed as a consequence of a coronary event. In that case, the case-control design may not be suitable for studying factor VII. From all these studies it is, however, not clear whether factor VII is indeed causally linked to coronary heart disease.

Biological explanations

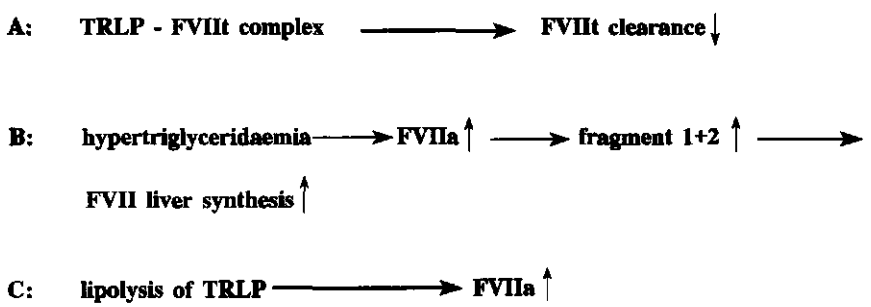
The question remains whether it is FVIIt or FVIIa which is important for coronary heart disease. Total factor VII is present in excess and one may think that a small increase in the total amount would probably not affect the thrombotic tendency. The amount of FVIIa in the circulation, however, is limited and it is therefore conceivable that when tissue factor is present, even a small increase in FVIIa may lead to an extreme formation of thrombin. This would lead to an imbalance in the haemostatic process and thereby to thrombosis. This is supported by the observation that active site-inhibited FVIIa competes with normal FVIIa for tissue factor and is a useful competitive inhibitor of thrombus formation initiated by acute arterial injury[145].
Coronary thrombosis occurs almost exclusively in atherosclerotic arteries[146] as a result of plaque rupture[147,148]. FVIIa becomes important at the moment of plaque rupture, when large amounts of tissue factor present in the plaque are released[149,150]. The amount of activated factor VII in the circulation at that time may determine the size of the resulting blood clot and therefore the outcome of the event. This may also explain why factor VII is more clearly associated with fatal than with non-fatal coronary heart disease. Possibly, fatality is a consequence of more extensive clot formation in larger coronary vessels. Furthermore, the chance of a plaque rupture may be larger when more atherosclerosis is present and when the plaque is rich in cholesterol crystals[151]. In elderly, the prevalence of atherosclerosis is high and the type of plaque may be more susceptible to rupture compared with younger individuals. Occurrence of thrombosis on top of the existing atherosclerosis will probably increase the risk of a coronary event in elderly people. As factor VII has a key position in the formation of a thrombus at the moment of plaque

rupture, it may have more public health importance in elderly people than in young or middle-aged people.
Although there seems to be evidence that FVIIa is important in coronary heart disease, we should not forget that FVIIa is but one factor in the whole haemostatic system. The thrombotic-thrombolytic equilibrium at the time of plaque rupture plays a decisive role in the thrombotic response and hence in the outcome of the event[152]. At this time we do not know exactly how this equilibrium is regulated and no method is available to measure the state of this balance. Therefore, as a surrogate, measurements of factors which we think affect this balance are used in studies to investigate the onset and development of coronary heart disease. Examples of such factors associated with coronary heart disease in prospective follow up studies are fibrinogen, factor VIII and plasminogen activator inhibitor[3,4,153]. Future research including measurement of FVIIa in stead of FVII:C, may contribute to elucidating the thrombotic processes taking place during a cardiovascular event.

Factor VII and dietary fat

It is well known that factor VII is influenced by dietary fat[120]. A biological explanation for this relationship may be founded on the association between serum-triglycerides and factor VII. Several mechanisms are suggested which describe the causal pathway from intake of dietary fat to an increase in factor VII. These possible mechanisms are extensively described in Chapter 2 and are summarized in Figure 8.1.
There are, however, some weaknesses in these hypothesized mechanisms. Mechanism A and B both explain the association of FVIIt with dietary fat[27,67-69]. As evidence for mechanism A indeed binding between FVIIt and triglyceride rich lipoproteins (TRLP) in vitro was found, but it is not clear whether this actually reduces the clearance in vivo. The evidence for mechanism B is quite scarce. Stimulation of the hepatic production of factor VII by fragment 1+2 has been shown in rabbits[69], but it is not known whether this occurs in humans as well. Mechanism C is most extensively investigated. It explains the postprandial activation of factor VII after fat consumption. In a study among patients with lipoprotein lipase deficiency no activation of factor VII after a fat-rich meal was seen[73].

Figure 8.1. Possible mechanisms explaining the association of dietary fat with factor VII
TRLP = triglyceride rich lipoproteins



This means that factor VII is activated during lipolysis of TRLP. As the half life time of chylomicron remnants is longest after a saturated fat meal[108], the postprandial increase in FVIIa is expected to be larger after saturated fat compared to unsaturated fat. The results of our study do not confirm this, since the response of FVIIa was similar for meals rich in unsaturated and saturated fat. Furthermore, when 1 g of fish oil a day is consumed, the amount of TRLP decreases and the length and level of the postprandial hyperlipidaemia are lower[109]. There are no studies which investigated the postprandial response of FVIIa after a meal with fish. However, in studies which evaluated FVII:C in relation to a long-term fish diet, no change in FVII:C was observed[81-90]. In two of those studies which did show an effect, an increase in FVII:C after the fish diet rather than a decrease was found[81,90]. This suggests that the differences in postprandial lipaemia between different fat types may be too small to be reflected in the activation of factor VII. Also the lack of association between postprandial FVIIa and serum-triglycerides as observed in our intervention study may indicate that it is not the amount of TRLP which is involved in the process, but rather the way the TRLP are metablised. These discrepancies between what would be expected according to the proposed mechanisms and what is observed in experimental studies may rely on the shortage of knowledge of the exact metabolism of fatty acids and the involvement of other factors in this process.
Another aspect of mechanism C is the involvement of other coagulation factors. First it was thought that the negatively charged surfaces of the TRLP, existing during lipolysis, would activate factor XII and thereby factor VII[70]. Lately it became evident that factor IX and not factor XII plays a role in this process. This was based on the observation that patients who were deficient of factor XII showed a normal postprandial increase in FVIIa,

while patients deficient in factor IX did not[130,131]. This may be explained by the inhibition of FVIIa by tissue factor pathway inhibitor (TFPI). FVIIa in complex with factor X is quickly inhibited by TFPI, while re-activation of factor VII through factor IX can not occur, since factor IX is not present. Therefore, no postprandial increase in FVIIa will be seen.
In conclusion mechanism A and B could explain the increase in fasting FVII:C seen after a high-fat diet. If these mechanisms are true, one would only expect an increase in fasting FVIIt, and not in fasting FVIIa after a long-term high-fat diet. Future research should clarify whether this indeed is true. Mechanism C could explain the postprandial increase of FVIIa, although the exact pathway for the activation of factor VII by TRLP is still obscure.

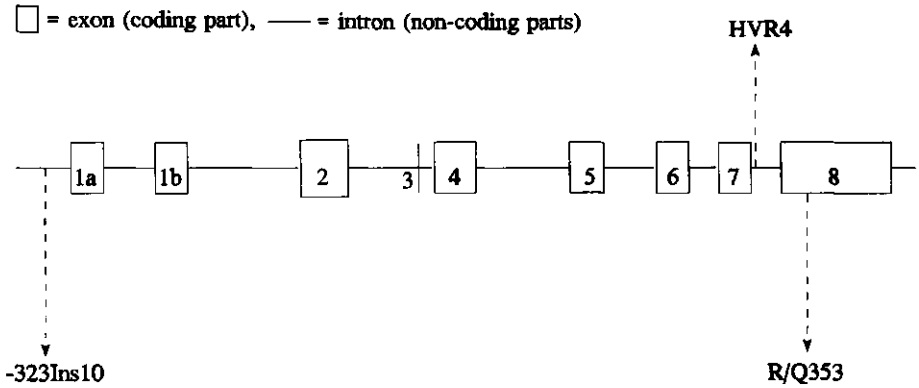
The question remains whether an increase in FVIIa after a fat-rich meal indeed leads to an increased formation of thrombin, and thereby to a higher risk of a thrombotic event. Kapur et al[132] were not able to show a postprandial increase in fragment 1+2, a marker of thrombin generation, concomitant with an increase in FVIIa after a fat-rich meal. However, in a non-disease situation there will be no release of tissue factor, nor triggering of the coagulation cascade and consequently no increase in fragment 1+2. It would be interesting to know whether an increase in FVIIa leads to an increase in clot formation at the instant tissue factor is released, for example during plaque rupture. For now, however, there is no reliable method available to determine the potential blood clot formation at a certain FVIIa level.

Factor VII and genetics

The gene coding for factor VII shows strong sequence and organizational homology with the other vitamin K-dependent clotting factors and is located close to the gene encoding coagulation factor X on chromosome 13[154]. Figure 8.2 shows the gene coding for factor VII, which has eight introns and nine exons.
Three polymorphisms of the factor VII locus have been associated with plasma levels of FVII:C, FVIIt and FVIIa[23,121,155,156]. About 30% of the variation in plasma FVII:C can be explained by these polymorphisms of the factor VII locus[121]. In the promoter region of the gene the -323Ins10 polymorphism is a consequence of an insertion of the sequence CCTATATCCT[157]. It has two alleles, P0 and P10, of which the latter is associated with lower levels of factor VII[121]. In intron 7 the HVR4 polymorphism consists of a variable number of tandem repeats of 37 bp[158]. The alleles of the HVR4 polymorphism contain between 4 and 8 repeats and are named H5 to H8. Since the HVR4 polymorphism is

situated in an intron, it is supposed to be a marker of another polymorphism which influences the level of factor VII. The R/Q353 polymorphism is the most extensively studied polymorphism of the factor VII gene at this moment. It is located in exon 8 and is

Figure 8.2. The factor VII gene and the three known polymorphisms
□ = exon (coding part), —— = intron (non-coding parts)



caused by a substitution of guanine by adenine in the codon for amino acid 353. This leads to a replacement of arginine (R) by glutamine (Q) in the factor VII protein. The R/Q353 and the -323Ins polymorphism are closely linked, and the Q and the P10 allele are both associated with lower levels of FVIIa, FVIIt and FVII:C[156].

As the position of the -323Ins10 polymorphism is upstream from the start of translation of the gene, it seems the best candidate to have a functional effect on the level of factor VII. Indeed Humphries et al[107] showed a stronger association of FVII:C and FVIIt with this polymorphism compared to the R/Q353 polymorphism. In contrast Heywood et al[159] showed a greater predictive value for FVII:C levels of the R/Q353 polymorphism than of the -323Ins10 polymorphism. This suggests that both polymorphisms may be linked to one or more as yet unidentified polymorphisms, which may have different functional effects on either FVIIa or FVIIt. It seems conceivable that the -323Ins10 polymorphism (located in the promoter region) would only affect the synthesis of factor VII, while the R/Q353 polymorphism (located in an exon) would affect the activity of factor VII. Differences in susceptibility to the level of FVIIa and FVIIt in the measurement of FVII:C between the studies of Heywood et al[159] and Humphries et al[107], may therefore also explain their contradicting results.

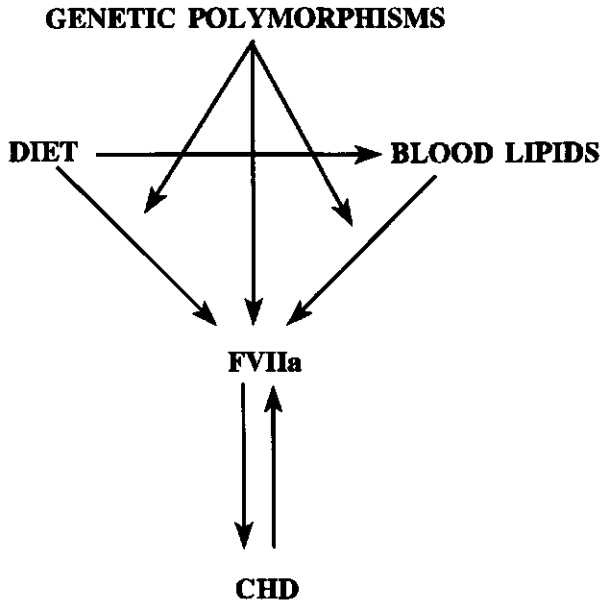
Lane et al studied the association between the R/Q353 polymorphism and the presence of myocardial infarction[144]. There was no difference in the distribution of the RR or RQ/QQ genotypes between the case and control subjects. This may be explained by the fact that this study included males only. In our studies we have shown that there is a remarkable difference between men and women concerning the effects of the R/Q353 polymorphism on the associations of factor VII with serum-triglycerides. It may be that the R/Q353 polymorphism (or the functional polymorphism responsible for the differences in FVIIa levels) has a larger effect in women than in men. The results of Lane et al[144] may also reflect the view that factor VII is more strongly related to fatal coronary heart disease. Their case-control study did not include fatal events, which may have caused an underestimation of the presence of the R-allele in the cases.
In our cross-sectional study it was observed that the R/Q353 polymorphism affects the association of FVII:C and FVIIt with dietary factors and serum-triglycerides. In our intervention trial it was shown that also the response of FVIIa to a fat-rich meal depends on the genotype for the R/Q353 polymorphism. The charge change ensuing from the substitution of the positively charged arginine with a neutral glutamine may affect the interaction of factor VII with lipid surfaces[137], which could explain our observations. This does not account for the differences between genotype groups in the association of FVII:C and FVIIt with dietary fibre and protein. Moreover, if the R/Q353 polymorphism is not a functional one, the explanation may be completely different.

In conclusion circulating levels of FVIIa may be important for the outcome of a coronary event. These levels are influenced by diet, blood lipids and by genetic polymorphisms (Figure 8.3). Besides a direct influence of genetic polymorphisms on FVIIa, they also affect the level indirectly through a modifying effect on the relation of FVIIa with diet and blood lipids. It is conceivable that an individual with a specific genetic variant may have high fasting levels of FVIIa compared to individuals with another variant. Subsequently, consumption of a high-fat meal by this susceptible subject may result in a dramatic increase in FVIIa. These extremely increased levels are caused by a synergy between the intake of dietary fat and the genetic variant. This synergy between diet and genetic factors, which will lead to profoundly elevated FVIIa levels after a high-fat dinner, may be one of the explanations of the small peak in thrombotic events in the evening[160,161].

Practical implications

During the last years nutrition education has been focused on a reduction of total fat intake and an exchange of saturated fat for unsaturated fat. These recommendations intended to achieve a reduction of LDL cholesterol level and thereby a reduction in the incidence of cardiovascular disease. Lately a debate has started on the value of lowering the total fat content in the diet[162]. A replacement of fat by carbohydrates in the diet will indeed lead to a decrease in LDL cholesterol, but also to a decrease in HDL cholesterol[163].

Figure 8.3. Interrelations of FVIIa with genetic factors, diet, triglyceride rich lipoproteins (TRLP), and coronary heart disease (CHD)



This means that the lipoprotein profile (ratio LDL/HDL) will not improve and a low-fat diet will not lead to a decreased risk of cardiovascular disease. Therefore, some argue that

recommendations should only address the fat composition and not the total amount of fat. However, as we have shown in this thesis, total fat intake does affect the level of FVIIa. Since the risk of coronary events may be higher at peak levels of FVIIa, reduction of total fat intake may indeed be worthwhile. In the discussion on the efficacy of low-fat diets in prevention of cardiovascular disease, the reducing effect of low total fat intake on FVIIa should be included.

The results from our cross-sectional study on dietary fibre need to be confirmed in intervention studies. If indeed an increase in the intake of dietary fibre results in a decrease of FVIIa, this would be a valuable scientific basis for the recommendation to increase the consumption of dietary fibre. Furthermore, if confirmed by others, our results indicate that a change in the diet of elderly people may lead to a improvement of their risk profile for thrombotic events.

From the studies described in this thesis it seems evident that the R/Q353 polymorphism modifies the response of factor VII to a change in the diet. Recommendations for screening of the population on polymorphisms of the factor VII gene seem rather premature. There are several aspects to consider before we can decide if screening of the population for a factor VII polymorphism is useful. First we should know whether FVIIa is indeed a risk factor for coronary heart disease. Secondly, it must be clear which polymorphism(s) have a functional effect on the level of FVIIa. Finally, we should know if and how the polymorphism in the factor VII gene interacts with other polymorphisms important for coronary heart disease. However, it is conceivable that in the distant future people at high risk for cardiovascular disease will be screened for their susceptibility to a certain dietary change. A polymorphism in the factor VII gene may be one of the candidates for such preventive susceptibility screening.

Factor VII and future research

In this thesis only the R/Q353 polymorphism of the factor VII gene is studied. Several other polymorphisms which are associated with the factor VII level have been identified, and it is likely that in the future more will be discovered. It is, however, not clear which polymorphism is in fact responsible for the difference in FVIIa levels between people, i.e. which is the functional polymorphism. Of course a certain haplotype could also be especially important for the level of FVIIa. Molecular and biochemical research is needed to clarify this and to clarify the mechanism by which the genetic factors affect the association of factor VII with diet.

Prospective studies on factor VII and coronary heart disease have so far been focused on FVII:C. Although FVIIa seems more relevant than FVII:C in this case, it has not been

included in prospective studies. Epidemiological research is needed to find out whether FVIIa is really implicated in the causal pathway of coronary heart disease, or whether it is merely a risk indicator. An answer to this question may be easy to find in a case-control setting using the functional polymorphism that determines the level of FVIIa. Including a genetic factor as an independent variable eliminates bias caused by a change in FVIIa level as a result of the coronary event. In the case-control study fatal cases of coronary heart disease events should be taken as an endpoint and the polymorphism should be determined. If possible blood samples can be obtained for measurement of FVIIa. If the FVIIa levels correlate well with the polymorphism, results of the study should be able to show whether FVIIa is indeed a risk factor.

Many intervention studies on factor VII and dietary fat have so far been performed, but not many have included measurement of FVIIa. The fluctuation of FVIIa during a day with habitual meals could be studied, to identify at which time a peak level of FVIIa occurs in normal life. At the same time it can be studied whether lower amounts of fat as compared to high-fat meals also increase postprandial FVIIa, i.e. if there is a dose-response relation. In order to further clarify the mechanism behind the relation of factor VII and dietary fat it may be interesting to look at polymorphisms which affect lipoprotein lipase activity or the lipoprotein metabolism. An example of such a polymorphism could be the different Apo E variants. Furthermore intervention studies on FVIIa and a long-term diet rich in dietary fibre are essential, as FVIIa measurement was not included in studies on this subject before. Not only different amounts of dietary fibre but also different types of fibre, soluble or non-soluble, should be evaluated.

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SUMMARY

Factor VII is a vitamin K-dependent coagulation factor present in the plasma mainly as inactive factor VII zymogen. About 1% of factor VII circulates in the activated form: FVIIa. When the vessel wall is damaged, tissue factor is released and the inactive factor VII zymogen will become rapidly activated. This will eventually lead to the formation of fibrin, which together with activated platelets will form a blood clot. The coagulation mechanism exists to prevent extensive blood loss during vessel damage. However, when the coagulation mechanism is activated in narrowed arteries, this may result in a harmful occlusion of a blood vessel. Indeed, several coagulation factors are found to be risk indicators for cardiovascular disease. In longitudinal studies it was observed that factor VII is a risk indicator for coronary heart disease, and more specifically for fatal events.
In view of prevention of coronary heart disease knowledge of the determinants of factor VII levels is needed. Genetic factors are very important predictors of the factor VII level. The Q allele of the R/Q353 polymorphism in the gene coding for factor VII is associated with lower levels of total factor VII (FVIIt) as well as activated factor VII (FVIIa). This polymorphism is a result of a single base change in the codon for amino acid 353 and leads to the replacement of arginine (R) by glutamine (Q). Furthermore, it is known that in young and middle-aged people factor VII increases with an increase in dietary fat intake. Although the incidence of coronary heart disease increases with increasing age, factor VII has not been studied extensively in elderly people. Therefore we investigated the relation of diet with factor VII in elderly men and women and evaluated whether this relation differs between the genotypes of the R/Q353 polymorphism.
In **Chapter 2** a review is given of all recent studies on the relation of factor VII with dietary fat and blood lipids. The studies presented included mostly young and middle-aged males. First the different methods to measure factor VII are described and examples of mechanisms behind the relation of factor VII and dietary fat are given. Factor VII coagulant activity (FVII:C) seems to increase after a fat-rich diet as well as postprandially after a fat-rich meal. The chronic effects of a fat-rich diet on fasting FVIIt are less clear, while FVIIt does not respond to a fat-rich meal. For both FVII:C and FVIIt it seems that the type of fat has no influence on the response. Results from cross-sectional studies show that serum-triglycerides and total-cholesterol are associated with FVII:C and with FVIIt. This association is, however, not always seen in studies on the chronic effects of dietary fat on factor VII. Finally the association of factor VII with blood lipids may vary across genotypes of the R/Q353 polymorphism. This was also shown for the association of factor VII with dietary fat in a small study among male post-infarction patients.
It seems quite clear that the amount of fat intake is important; high-fat meals increase factor VII. Whether fat composition is of any importance remains to be clarified.

We used the cross-sectional data from the Rotterdam Study, a population-based study among 7,983 people older than 55 years, to study the association of FVII:C and FVIIt with diet (**Chapter 3**). Subjects were excluded when they used anticoagulants, had diabetes mellitus, had a myocardial infarction in the year before the investigation and when no dietary data or blood samples taken with minimal stasis were available. In total 1730 women and 1277 men were included in the cross-sectional data analysis.
In this population FVII:C was positively associated with total fat intake in women only (ß=0.1 %/g, 95%confidence interval (CI):0.01,0.20). Saturated fat intake was associated with FVII:C in women (ß=0.18 %/g, CI:-0.001,0.36) and in men (ß=0.11 %/g, CI:-0.06,0.27). Monounsaturated fat was positively related to FVII:C in women (ß=0.17 %/g, CI:-0.05,0.39), while polyunsaturated fat was inversely associated with FVII:C in men (ß=-0.15 %/g, CI:-0.33,0.03). Fibre intake was inversely associated with FVII:C in both women (ß=-0.36 %/g, CI:-0.63,-0.09) and men (ß=-0.31 %/g, CI:-0.57,-0.06). This means for example that if the fibre intake in women would increase with 10 g, FVII:C would decrease with 3.6%. The results of this cross-sectional study suggest that FVII:C may be affected by a low-fat and/or a high-fibre diet at old age.
To look further into these relations the study population was divided into quintiles of the FVII:C distribution (**Chapter 4**). For the subjects in the extreme quintiles (upper and lower) FVIIt, serum-triglycerides and the genotype for the R/Q353 polymorphism were determined. The lowest quintile comprised 538 subjects and the highest 575. Of these subjects 858 were homozygous for the R allele, 268 were heterozygous and 32 were homozygous for the Q allele. FVII:C was 35% lower (80.6 % pooled plasma (PP) vs 125.3 %PP) and FVIIt 48% (49.6 %PP vs 95.7 %PP) in individuals with the QQ genotype compared to those with the RR genotype. The FVII:C and FVIIt levels for those with the RQ genotype were intermediate.
In this subpopulation of the two extreme quintiles FVII:C was again positively associated with saturated fat intake and inversely with fibre and protein, while FVIIt was only inversely associated with fibre. As the group of subjects homozygous for the Q allele was too small for further meaningful analyses, it was combined with the heterozygotes. When subjects were analyzed by genotype group separately, the association of dietary fibre with FVII:C was clearly stronger in subjects homozygous for the R allele (ß=-0.76 %PP, CI:-1,23,-0.29), compared to those carrying the Q allele (ß=-0.19 %PP, CI:-0.97,0.59). Similar results were found for FVIIt. The association of FVII:C with protein was only present in those with the RR genotype and not in those with the RQ or QQ genotype. The same was true for the association of FVIIt with saturated fat. The association of FVII:C with saturated fat however was positive in those homozygous for the R allele, while it was inverse in those carrying the Q allele. In elderly people a high saturated fat/low-fibre diet

may increase the risk of a coronary event through increased factor VII levels. This may be especially important in subjects with the RR genotype.
Similar analyses were carried out for the association of FVII:C and FVIIt with serum-triglycerides **Chapter 5**). In women triglycerides were positively associated with FVIIt and FVII:C. These associations were stronger in women homozygous for the R allele (FVIIt: ß=11.7, CI: 9.6-13.8; FVII:C: ß=12.5, CI:9.5-15.5) compared to women carrying the Q allele (FVIIt: ß=7.9, CI: 4.6-11.2; FVII:C ß=6.4, CI:1.4-11.4). In men the associations of FVIIt and FVII:C with serum-triglycerides were weaker (FVIIt: ß=5.9, CI:4.1-7.7; FVII:C: ß=8.7, CI:6.2-11.2). There was no difference in this association between the genotype groups. The R/Q353 polymorphism modifies the association between factor VII and triglyceride status, especially in elderly women. This indicates that both genetic factors and life style elements are important for determining a persons coagulable state.
The results of the cross-sectional study indicate that the influence of dietary fat on factor VII may be different between elderly women homozygous for the R allele and women carrying the Q allele and that serum-triglycerides may be an intermediate factor. Therefore we hypothesized that the response of factor VII after a fat-rich meal is higher in elderly women with the RR genotype and that this response is associated with a postprandial increase in serum-triglycerides. We tested this hypothesis in an intervention trial among 91 elderly women who were selected on their genotype for the R/Q353 polymorphism. Furthermore it was tested whether the factor VII response depended on the fatty acid composition of the fat-rich meal.
Fiftysix women with the RR genotype and 35 women with the RQ or QQ genotype were included in the study (**Chapter 6**). The women received on separate days four different fat-rich breakfasts (50 energy % of fat) and a control breakfast (1.5 en% fat) (cross-over). The fat-rich breakfasts differed in fatty acid composition: one rich in palmitic acid, one in stearic acid, the other two in linoleic and linolenic acid one with a ratio 3:1 and another with a ratio of 15:1. At 8.00 AM before the breakfast (fasting) and at 1.00 and 3.00 PM blood samples were taken, in which FVIIa and serum-triglycerides were measured.
When the data of all subjects were combined and analyzed as a total group the FVIIa response to the fat-rich meals ranged from 14.9 IU/mL (CI: 10.6,19.2) on the stearic meal to 21.1 IU/mL (CI: 16.6,25.6) on the linoleic/linolenic 15:1 meal at 3.00 PM. The average responses on the fat-rich meals were not clearly different from each other. After the control breakfast FVIIa decreased with 6.3 IU/mL (CI: 3.9,8.7) at 1.00 PM and with 8.7 IU/mL (CI: 6.3,11.1) at 3.00 PM. The triglyceride response was lower after both linoleic/linolenic rich breakfasts compared to the palmitic and stearic breakfast ($p<0.05$). The response of serum-triglycerides was not associated with the FVIIa response at any of the blood sampling occasions. This suggests 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.

When the data were analyzed for the two genotype groups separately again no difference in FVIIa response between the different fat-rich meals could be observed (**Chapter 7**). Therefore the mean FVIIa response on the four fat-rich meals was taken to evaluate the differences in FVIIa response between the genotype groups. The mean absolute combined response to all four test breakfasts was 37.0 IU/mL in those with the RR genotype and 16.1 IU/mL in those carrying the Q allele ($p<0.001$ for difference). Likewise, the FVIIa response relative to the fasting FVIIa level was significantly higher in women homozygous for the R allele (42%) compared to the women carrying the Q allele (32%). In elderly women the response of FVIIa to a fat-rich meal clearly depends on the genotype for the R/Q353 polymorphism, but not on the fatty acid composition of the meal. One mechanism to explain these results is that the charge change ensuing from the substitution of the positively charged arginine by the neutral glutamine affects the interaction of factor VII with lipid surfaces.

In conclusion the results from our cross-sectional study show associations of dietary fibre, saturated fat and protein with FVII:C and of fibre with FVIIt. These associations were weaker or absent in elderly men and women carrying the Q allele compared to those homozygous for the R allele. Also, the association of serum-triglycerides with FVII:C and FVIIt varied across genotypes of the R/Q353 polymorphism. In our intervention study we observed a profound increase of FVIIa after a fat-rich meal, which was twice as high in elderly women with the RR genotype, compared to women with the RQ or QQ genotype. This increase did not depend on the type of fat consumed and was not associated with an increase in serum-triglycerides. This indicates that in view of prevention of a coronary event, a diet with a low to moderate fat intake may be recommendable, particularly in elderly women homozygous for the R allele.

In **Chapter 8** studies on factor VII and coronary heart disease are summarized. From prospective studies it seems evident that factor VII:C is associated with fatal coronary events, but results from case-control studies are inconclusive. It seems likely that not FVIIt, but FVIIa is important in the outcome of a coronary event. Additionally the hypotheses explaining the association of factor VII with dietary fat are discussed. The exact mechanisms behind the effect of dietary fat on factor VII remain to be clarified. Three polymorphisms of the factor VII locus, which are associated with factor VII levels are described. It seems clear that polymorphisms in the gene coding for factor VII influence factor VII directly but also indirectly through effects on the relation of diet and blood lipids with factor VII. Finally suggestions for practical implications and future research are made. The fact that total fat intake is responsible for changes in factor VII rather than fat type, should be included in the discussion concerning the validity of the

total fat intake recommendations to lower the risk of cardiovascular disease. In future studies on the relation of factor VII and coronary heart disease measurement of FVIIa and the genotype of the functional polymorphism should be included. Polymorphisms which influence the fatty acid metabolism should be investigated in intervention studies on factor VII and dietary fat and intervention studies are needed to confirm the inverse association between factor VII and dietary fibre.

SAMENVATTING

Factor VII is een vitamine K-afhankelijke stollingsfactor die in het plasma voornamelijk aanwezig is in de vorm van het inactieve factor VII zymogeen. Ongeveer 1% van het in het plasma aanwezige factor VII komt voor in de geactiveerde vorm: FVIIa. Tijdens beschadiging van een bloedvat komt tissue factor vrij uit de vaatwand, dat in staat is om factor VII snel te activeren. Activatie van factor VII zal dan uiteindelijk leiden tot de vorming van fibrine, dat tezamen met geactiveerde bloedplaatjes een stolsel vormt. Het doel van dit stollingsmechanisme is het voorkómen van extreem bloedverlies tijdens schade aan de vaatwand. Als dit mechanisme echter te sterk geactiveerd wordt in vernauwde bloedvaten kunnen stolsels optreden die het vaatlumen kunnen afsluiten. Van verschillende stollingsfactoren is bekend dat ze een risicoindicator zijn voor hart- en vaatziekten. Zo is uit longitudinale studies gebleken dat factor VII een risicoindicator vormt voor coronaire hartziekten en met name voor fatale gevallen daarvan.
In het kader van preventie van hart- en vaatziekten is het nodig meer te weten van de determinanten van het niveau van factor VII. Genetisch factoren zijn zeer belangrijke voorspellers van het factor VII niveau. Zo houdt het Q allel van het R/Q353 polymorfisme in het gen dat codeert voor factor VII verband met lagere niveaus van totaal factor VII (FVIIt) en geactiveerd factor VII (FVIIa). Dit polymorfisme is een gevolg van een verwisseling van één basepaar in het codon voor aminozuur 353 en leidt tot een vervanging van arginine (R) door glutamine (Q). Het is bekend dat bij mensen van jonge en middelbare leeftijd factor VII sterk stijgt bij een verhoging van de vetinneming. Alhoewel de kans op het krijgen van symptomatische coronaire hartziekte groter wordt naar mate men ouder wordt, is factor VII nog niet uitgebreid onderzocht bij oudere mensen. Daarom hebben wij de relatie tussen factor VII en voeding onderzocht bij oudere mannen en vrouwen, en hebben we gekeken of deze relatie beïnvloed wordt door het R/Q353 polymorfisme.
In **hoofdstuk 2** worden alle recente onderzoeken naar factor VII en voedingsvet en bloedlipiden beschreven. De deelnemers in deze onderzoeken waren met name mannen van jonge en middelbare leeftijd. Als eerste worden de verschillende methoden om factor VII te meten weergegeven en worden mogelijke mechanismen die de relatie tussen factor VII en voedingsvet kunnen verklaren besproken. Uit de studies blijkt dat factor VII coagulant activiteit (FVII:C) zowel na een vetrijk dieet verhoogd is als direct na een vetrijke maaltijd. De chronische effecten van een vetrijk dieet op FVIIt zijn minder eenduidig, maar FVIIt reageert in ieder geval niet acuut op een vetrijke maaltijd. Voor zowel FVII:C als FVIIt geldt dat het soort vet geen invloed heeft op de respons op lange en korte termijn. Uit cross-sectionele onderzoeken blijkt dat serum-triglyceriden en totaal cholesterol samenhangen met FVII:C en FVIIt. Dit verband wordt echter niet

waargenomen in de meeste interventie-onderzoeken naar lange termijn effecten van vetinneming op factor VII. Tenslotte lijkt de associatie van factor VII met bloedlipiden te variëren tussen de genotypen van het R/Q353 polymorfisme. Een klein onderzoek bij mannelijke post-infarct patiënten liet dezelfde variatie zien voor het acute effect van een vetrijke maaltijd op factor VII.

De cross-sectionele data van de Rotterdam Study, een prospectief onderzoek onder 7.983 mensen ouder dan 55 jaar, zijn gebruikt om het verband van FVII:C en FVIIt met voeding te onderzoeken (**hoofdstuk 3**). Deelnemers werden uitgesloten als ze anticoagulantia gebruikten, een hartinfarct hadden gehad in het jaar voorafgaande aan het onderzoek, diabetes mellitus hadden of als geen bloedmonster of voedingsdata beschikbaar waren. In totaal werden 1730 vrouwen en 1277 mannen geselecteerd voor het onderzoek.

Alleen bij vrouwen was FVII:C positief geassocieerd met totale vetinneming (ß=0.1 %/g, 95%betrouwbaarheidsinterval (BI):0.01,0.20). Inneming van verzadigd vet hield zowel bij vrouwen (ß=0.18 %/g, BI:-0.001,0.36) als bij mannen (ß=0.11 %/g, BI:-0.06,0.27) verband met FVII:C. Enkelvoudig onverzadigd vet was positief gerelateerd aan FVII:C in vrouwen (ß=0.17 %/g, BI:-0.05,0.39), terwijl meervoudig onverzadigd vet invers gerelateerd was aan FVII:C in mannen (ß=-0.15 %/g, BI:-0.33,0.03). Inneming van voedingsvezel was invers geassocieerd met FVII:C in vrouwen (ß=-0.36 %/g, BI:-0.63,-0.09) en in mannen (ß=-0.31 %/g, BI:-0.57,-0.06). Dit betekent bijvoorbeeld dat een verhoging van de vezelinneming met 10 g bij oudere vrouwen zou kunnen leiden tot een verlaging van FVII:C met 3.6%. De resultaten van dit cross-sectionele onderzoek geven aan dat FVII:C mogelijk ook op oudere leeftijd beïnvloed kan worden door een vetarme en/of vezelrijke voeding.

Om het effect van het genotype op deze relaties nader te onderzoeken is de studiepopulatie opgesplitst in quintielen van de FVII:C verdeling (**hoofdstuk 4**). In bloedmonsters van de deelnemers uit de twee uiterste quintielen (de hoogste en de laagste) zijn FVIIt, serum-triglyceriden en het genotype voor het R/Q353 polymorfisme bepaald. In het laagste quintiel bevonden zich 538 deelnemers en in het hoogste 575. In de totale groep waren 858 deelnemers homozygoot voor het R allel, 268 heterozygoot, en 32 homozygoot voor het Q allel. FVII:C was 35% lager (80.6 % pooled plasma (PP) vs 125.3 %PP) en FVIIt was 48% later (49.6 %PP vs 95.7 %PP) in personen met het QQ genotype in vergelijking met personen die het RR genotype hadden. Het niveau van FVII:C en FVIIt van de heterozygote personen lag daar tussenin.

In deze subpopulatie van extreme quintielen hield FVII:C eveneens positief verband met de inneming van verzadigd vet en invers met voedingsvezel en eiwit, terwijl FVIIt alleen invers geassocieerd was met voedingsvezel. Omdat er voor verdere zinvolle data-analyse te weinig mensen waren met het QQ genotype is deze groep samengevoegd met de groep

heterozygoten. Wanneer de gegevens apart per genotypegroep werden geanalyseerd bleek het verband van FVII:C met voedingsvezel duidelijk sterker te zijn in personen die homozygoot zijn voor het R allel (ß=-0.76 %PP, BI:-1,23,-0.29), in vergelijking met de dragers van het Q allel (ß=-0.19 %PP, BI:-0.97,0.59). Vergelijkbare resultaten werden gevonden voor FVIIt. Het verband van FVII:C met eiwit was alleen aanwezig onder de homozygoten voor het R allel, en niet onder de dragers van het Q allel. Hetzelfde gold voor de relatie tussen FVIIt en verzadigd vet. FVII:C was echter positief gecorreleerd aan verzadigd vet in personen met het RR genotype en invers in personen met het RQ of QQ genotype. Een voeding rijk aan verzadigd vet en arm aan vezel kan in oudere mensen het risico op coronaire hartziekten vergroten via een verhoging van het factor VII niveau. Dit zal vooral van belang zijn in personen met het RR genotype.

Dezelfde data-analyse is in deze subpopulatie uitgevoerd voor het verband van FVII:C en FVIIt met serum-triglyceriden (**hoofdstuk 5**). FVIIt en FVII:C waren positief gerelateerd aan serum-triglyceriden bij vrouwen. Dit verband was sterker bij vrouwen die homozygoot waren voor het R allel (FVIIt: ß=11.7, BI: 9.6-13.8; FVII:C: ß=12.5, BI:9.5-15.5) in vergelijking met vrouwen die het Q allel dragen (FVIIt: ß=7.9, BI: 4.6-11.2; FVII:C ß=6.4, BI:1.4-11.4). De associatie van FVII:t en FVII:C met serum-triglyceriden bij mannen was zwakker (FVIIt: ß=5.9, BI:4.1-7.7; FVII:C: ß=8.7, BI:6.2-11.2). Er was bij mannen geen verschil tussen de genotypegroepen in het verband van FVIIt en FVII:C met serum-triglyceriden. Het blijkt dus dat het R/Q353 polymorfisme de associatie tussen factor VII en triglyceriden modificeert. Dit geeft aan dat zowel genetische factoren als omgevingsfactoren belangrijk zijn bij de bepaling van iemands stollingsstatus.

De resultaten van het cross-sectionele onderzoek geven aan dat de invloed van voedingsvet op factor VII verschillend kan zijn voor oudere vrouwen die homozygoot zijn voor het R allel en vrouwen die het Q allel dragen en dat het serum-triglyceriden niveau een intermediair kan zijn. Naar aanleiding hiervan is de hypothese opgesteld dat de respons van factor VII op een vetrijke maaltijd hoger is bij vrouwen met het RR genotype en dat deze respons verband houdt met een postprandiale verhoging in serum-triglyceriden. We hebben deze hypothese getoetst in een interventie-onderzoek bij 91 oudere vrouwen die geselecteerd waren op het genotype van het R/Q353 polymorfisme. Bovendien is onderzocht of de factor VII respons afhankelijk is van de vetzuursamenstelling van de maaltijd.

Vijfenzestig vrouwen met het RR genotype en 35 vrouwen met het RQ of QQ genotype zijn voor dit onderzoek geselecteerd (**hoofdstuk 6**). De vrouwen kregen vier vet-rijke ontbijten (50 energie% vet) en één controle-ontbijt (1.5 en%) op verschillende dagen (cross-over). De vetrijke ontbijten verschilden in vetzuursamenstelling: 1 was rijk aan palmitinezuur, 1 rijk in stearinezuur, de andere twee waren rijk aan linol- en linoleenzuur, 1 met een ratio 3:1 en 1 met een ratio 15:1. Om 08.00 uur werd voor het ontbijt nuchter

bloed afgenomen en om 13.00 en om 15.00 uur werd opnieuw bloed afgenomen. In deze bloedmonsters werd FVIIa en serum-triglyceriden bepaald.
In eerste instantie zijn de gegevens van alle deelnemers gezamenlijk geanalyseerd. De FVIIa respons op de vetrijke ontbijten liep van 14.9 IU/mL (BI: 10.6,19.2) na het stearinerijke ontbijt tot 21.1 IU/mL (BI: 16.6,25.6) na het linol/linoleenzuur rijke ontbijt met ratio 15:1 om 15.00 uur. De effecten van de vetrijke ontbijten waren niet verschillend van elkaar. Na het controle ontbijt was FVIIa met 6.3 IU/mL (BI: 3.9,8.7) gedaald om 13.00 uur en met 8.7 IU/mL (BI: 6.3,11.1) om 15.00 uur. De triglyceridenrespons was lager na beide linol/linoleenzuurrijke ontbijten dan na de palmitine- en stearinezuurrijke ontbijten ($p<0.05$). De respons van serum-triglyceriden hield op geen enkel moment van bloedafname verband met de respons van FVIIa. Deze resultaten suggereren dat de FVIIa respons op een vetrijke maaltijd in oudere vrouwen niet afhankelijk is van de vetzuursamenstelling van de maaltijd en dat deze respons niet gemedieerd wordt door een postprandiale stijging in serum-triglyceriden.
Deze gegevens zijn opnieuw geanalyseerd, maar nu voor de beide genotypegroepen apart (**hoofdstuk 7**). Er was in geen van de groepen een duidelijk verschil te zien in de respons tussen de vier vetrijke ontbijten. Daarom zijn de resultaten van de vetrijke ontbijten gecombineerd om de verschillen in FVIIa respons tussen de genotypegroepen te vergelijken. De gemiddelde absolute respons op de vier vetrijke ontbijten was 37.0 IU/mL voor deelnemers met het RR genotype en 16.1 IU/mL voor deelnemers met het RQ of QQ genotype ($p<0.001$ voor verschil). Bovendien was de relatieve FVIIa respons (gecorrigeerd voor nuchter FVIIa niveau) significant hoger bij vrouwen homozygoot voor het R allel (42%) dan bij dragers van het Q allel (32%). Het lijkt duidelijk dat de respons van FVIIa op een vetrijke maaltijd in oudere vrouwen wel afhangt van het genotype voor het R/Q353 polymorfisme, maar niet van de vetzuursamenstelling van het ontbijt. Het kan zijn dat de verandering van lading, als gevolg van de uitwisseling van het positief geladen arginine met het neutrale glutamine, invloed heeft op de interactie tussen factor VII en vetoppervlakken (hoofdstuk 7).
Concluderend lijken de resultaten van het cross-sectionele onderzoek erop te wijzen dat voedingsvezel, verzadigd vet en eiwit verband houden met FVII:C en FVIIt en dat deze verbanden zwakker of zelfs afwezig zijn bij oudere mensen die het Q allel dragen in vergelijking met ouderen homozygoot voor het R allel. Ditzelfde geldt voor het verband van serum-triglyceriden met FVII:C en FVIIt. Uit ons interventie-onderzoek is gebleken dat FVIIa sterk verhoogd is na een vetrijke maaltijd, maar dat deze verhoging twee keer zo hoog is bij oudere vrouwen met het RR genotype in vergelijking met vrouwen die het Q allel dragen. Het type vet in de maaltijd lijkt geen invloed te hebben op deze verhoging. Dit geeft aan dat in het kader van preventie van coronaire hartziekten een voeding met een

laag tot gemiddeld vetgehalte is mogelijk aan te raden voor oudere vrouwen die homozygoot zijn voor het R allel.

In **hoofdstuk 8** worden studies naar de relatie tussen factor VII en coronaire hartziekten samengevat. Uit prospectieve studies lijkt het duidelijk dat FVII:C geassocieerd is met coronaire hartziekten, maar resultaten van case-control studies zijn niet eenduidig. Het lijkt erop dat niet FVIIt, maar FVIIa belangrijk is bij coronaire hartziekten. Tevens worden de mechanismen die het effect van voedingsvet op factor VII zouden kunnen verklaren besproken. De exacte werking van de mechanismen achter het effect van voedingsvet op factor VII moet nog nader onderzocht worden. De drie polymorfismen in het factor VII gen die verband houden met factor VII niveaus worden kort beschreven. Het lijkt evident dat de polymorfismen in het factor VII gen factor VII niet alleen direct beïnvloeden, maar ook indirect via effecten op de relatie van voeding en bloedlipiden met factor VII. Tenslotte worden implicaties en aanbevelingen voor vervolgonderzoek gegeven. Het feit dat factor VII met name beïnvloed wordt door de totale vetinneming en niet door het type vet, zou betrokken moeten worden in de discussie rond de vraag of de aanbevelingen voor totaal vet nog wel valide zijn. In toekomstig onderzoek naar de relatie tussen factor VII en coronaire hartziekten zouden FVIIa en het genotype van het functionele polymorfisme gemeten moeten worden. Polymorfismen die betrokken zijn bij het vetmetabolisme zouden betrokken moeten worden bij interventiestudies naar factor VII en voedingsvet. Als laatste zijn interventiestudies nodig die de relatie tussen factor VII en voedingsvezel bevestigen.

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CURRICULUM VITAE

Louise Ingeborg Mennen was born in Mijdrecht, the Netherlands, on April 5th, 1969. She completed secondary school at the Alkwin Kollege in Uithoorn in 1987. Next she started studying Human Nutrition at the Wageningen Agricultural University. Her majors were in epidemiology and public health, and in 1992 she spend 6 months at the Department of Clinical Nutrition at the University of Kuopio, Finland. She graduated from University in June 1993. From July 1993 until May 1994 she worked as a research assistant for the project entitled 'Effects of Regular Physical Activity on Risk Factors for Cardiovascular Disease' at the Department of Epidemiology and Public Health, Wageningen Agricultural University. Thereafter she started with the PhD-project described in this thesis, which partly took place at the Department of Epidemiology and Biostatistics, Erasmus University Rotterdam and partly at the Division of Human Nutrition and Epidemiology, Wageningen Agricultural University in collaboration with Gaubius Laboratory, TNO-PG Leiden and with Unilever Nutrition Centre, Unilever Research Laboratory Vlaardingen. In the summer of 1995 she attended the Annual New England Epidemiology Summer Program at Tufts University in Boston, USA. She was a member of the organizing committee of the foreign excursion for PhD-students to Scandinavia in 1997. In October she will start working at the National Institute for Health and Medical Research, Unit 21, in Paris, France.