Relation of Plasma Coagulation Factor VII and Fibrinogen to Carotid Artery Intima-Media Thickness

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Summary

Plasma clotting factor VII and plasma fibrinogen have been claimed as independent risk factors for occlusive cardiovascular disease The aim of this study was to investigate whether these coagulation parameters affect early atherosclerosis, additional to their possible effect on arterial thrombosis

We used high-resolution quantitative ultrasonography to measure carotid intima-media thickness in 121 healthy volunteers, aged 18 to 56 years It has previously been demonstrated that an increased artery wall thickness is seen in advanced atherosclerosis To validate our methodology for relatively young individuals, we assessed the association of intima-media thickness with the risk-factor status of our subjects, by including classical cardiovascular risk factors, e g age, sex, serum cholesterol, smoking habits and blood pressure Thereafter, we studied the effect of factor VII and fibrinogen plasma levels on carotid intima-media thickness, as well as that of polymorphisms of the factor VII and fibrinogen genes

All classical risk factors except smoking and family history were associated with intima media thickness When adjusted for by multivariate linear regression analysis, age, blood pressure and cholesterol appeared to be independent determinants of intima-media thickness Factor VII and fibrinogen levels showed no association in multivariate analysis with intima-media thickness We conclude that artery wall thickness measurement by ultrasound is a useful tool to investigate the role of clotting factors in early atherosclerosis Factor VII and fibrinogen levels in young and middle-aged volunteers have no association with early atherosclerotic vessel wall changes

Introduction

A relation between high levels of coagulation factors as factor VII and fibrinogen and the risk of ischaemic heart disease has been shown in several studies Therefore, coagulation parameters have been suggested to be independent risk factors for occlusive cardiovascular disease (1–4) Occlusive cardiovascular disease generally originates from thrombus formation on an atherosclerotic plaque it is the result of a decades-long chronic atherosclerotic process, combined with the acute phenomenon of arterial thrombosis (5) The clotting factor level may have an effect on the progression of atherosclerosis, on arterial thrombosis, or on both

The aim of this study was to assess recent methodology in the evaluation of the role of fibrinogen and clotting factor VII in the very early stage of atherosclerosis, before progression to atherosclerotic plaques or thrombus formation Specifically, we sought for a non-invasive, easy-to-use method to assess the role of clotting factors in atherosclerosis We used high-resolution ultrasonography to measure carotid artery intima-media thickness with the computer-based Cardiovascular Measurement System (CMS) Intima-media thickness measured with ultrasound has been shown to be a reliable indicator of the atherosclerotic process (6–8) with a high concordance of the ultrasound measurement and pathologic evaluation (9) Most studies, however, have been performed in older subjects who either suffered from arterial disease or belonged to a specific risk group (e g hypercholesterolemic patients) We were interested in the use of this method in the earliest stage of atherosclerotic vessel wall changes Therefore, as a validation, we assessed the association between classic risk factors and intima-media thickness in young and middle-aged adults Only after this yielded satisfactory results, did we proceed to investigate the role of clotting factor levels in these subjects

First, plasma levels of clotting factor VII and of fibrinogen were determined to study their relation with carotid intima-media thickness These clotting factor levels, however, may have been affected by extraneous factors that also enhance atherosclerosis, i e age, smoking habits and lipids Therefore, we adjusted for these factors by multivariate analysis Of course, this only allows adjustment for extraneous influences that are known and can be measured accurately

A second option to adjust for confounding factors lies in the determination of polymorphisms of the factor VII and fibrinogen genes As has been reported recently, several of these polymorphisms are associated with the plasma levels of the clotting factors These include a HaeIII polymorphism (alleles H1 and H2) for the β-chain of fibrinogen (10) and a MspI polymorphism (alleles M1 and M2) in the factor VII gene (11) The H2-allele was found to be associated with higher than average fibrinogen levels, and the M2-allele with lower than average levels of factor VII Obviously, these polymorphisms cannot be influenced by extraneous factors, and therefore they offer the possibility to study the effect of clotting factor levels, unaffected by external factors as smoking, lipid levels or even the atherosclerotic process itself

Materials and Methods

Study Design

We studied 121 healthy volunteers, aged 19–56 years They were all free of clinical signs of cardiovascular disease, diabetes mellitus or any other chronic disorder, and did not use anticoagulants or lipid-lowering drugs Use of oral

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contraceptives was not an exclusion criterion We measured intima media thickness (IMT) of the carotid arteries, classical cardiovascular risk factors and plasma levels of factor VII and fibrinogen In addition, we determined polymorphisms of the factor VII and fibrinogen genes

Assessment of Carotid Intima-media Thickness

Ultrasonographic scanning of the carotid arteries was performed with the Aloka Echo Camera SSD 650 equipped with a high density linear array probe with 7 5 MHz transducer frequency in B mode The axial resolution was at least 0 3 mm The subjects were lying in a supine position with the head slightly extended and rotated 45 degrees away from the side which was scanned Scanning was performed in the anteroposterior plane, imaging the carotid bifurcation and the common carotid artery Three B mode images of the left and right common carotid artery were frozen at peak diastole on sight, and recorded on a SVHS video cassette tape Images were coded to ensure that later intima media thickness (IMT) measurements were performed blinded for subjects' identity, coagulation factor levels or risk factor status

Intima media thickness was measured later on in one session for all subjects with the Cardiovascular Measurement System (CMS) (12) Frozen images were digitized at a resolution of 512 × 512 pixels with 8 bits of grey levels Calibration of the images was performed by manually identifying a 4 cm distance on a cm scale in the image, typical pixel size in the nonmagnified mode was 0 1 mm A one centimeter traject of the posterior carotid wall one centimeter proximal to the carotid bifurcation was enlarged four times by cubic spline interpolation Next six intima-media thickness measurements were performed over this one centimeter range at 6 measurement sites in each recording The thickness of the intima-media complex as defined by Pignoli (6) was measured as the distance between the lumen intima interface and the media adventitia interface on the B-mode image The actual measurement at each of these six sites was carried out with the digital caliper on the CMS The intima-media thickness at each of the six measurement sites was determined by manually defining one pair of points at each measurement site and by averaging these six distance measures This multiple measurement approach results in a high degree of accuracy and precision In this way in each subject 36 intima media thickness measurements (6 measurements × 6 images) were performed, the average value of these 36 measurements was defined as the intima media thickness (IMT) in each subject

Other Measurements

Information on subject's smoking habits (current smoking, package years of cigarette smoking) and presence of cardiovascular disease in first-grade relatives before the age of 60 years (history of myocardial infarction, intermittent claudication) were obtained by means of a questionnaire Family history was coded as either negative or positive Body mass index was calculated as Quetelet index (wt/ht^2 in kg/m^2) Systolic and diastolic blood pressure were measured three times in each subject before venepuncture with a Hawksley random zero mercury sphygmomanometer after a minimum of ten minutes of rest The mean was used in the analysis

In fresh blood samples total serum cholesterol and HDL serum cholesterol concentration were determined by standard enzymatic assays, against the WHO standard Plasma fibrinogen concentration and plasma clotting factor VII activity were determined by the methods according to Clauss (13) (fibrinogen, g/l) and Owren (14) (factor VII, % of standard) Polymorphisms of the β-chain of fibrinogen and of factor VII were determined with the use of HaeIII and MspI restriction enzymes as reported by Thomas (10) and Green (11) The alleles with the restriction site were designated H1 (fibrinogen) and M1 (factor VII), and the non-cleavable alleles were designated H2 and M2

Statistical Analysis

We analysed the relation of the factors of interest with carotid wall thickness by linear regression techniques The regression coefficient obtained by this method, indicates the increase (or decrease, dependent on the sign of the coefficient) in intima media thickness per unit increase in the factor studied All continuous variables (e g blood pressure, cholesterol, age) were entered into the regression equation as such, and therefore the coefficients indicate the change in wall thickness (in μm) per one mmHg increase in blood pressure one mmol/l increase in cholesterol and one year increase in age To facilitate interpretation and comparison between variables we calculated the change in intima media thickness for meaningful increments of the independent variables, i e 10 mmHg for blood pressure 1 mM for cholesterol, 10 years for age and 10 package-years for smoking The original regression coefficients can easily be derived by dividing the change in IMT by 10 for blood pressure, age and package-years Discrete variables were coded as 0 or 1 In this instance the regression coefficient indicates the difference in intima media thickness between the two categories

Since several of the variables may be associated, we subsequently set up a multivariate model which yields mutually adjusted regression coefficients

Results

Baseline Values and Relations between Determinants

Baseline characteristics in our study showed the expected mean values for healthy subjects, with a broad range that facilitated the evaluation of possible effects of risk factors (Table 1)

The average carotid intima media thickness was 519 μm (SD 72, range 397–781 μm) Left and right intima media thickness showed a Pearson correlation coefficient of r = 0 83, with average values of 521 μm and 517 μm, respectively

Several of the determinants were interrelated Factor VII plasma level was associated with sex (mean 99 4% in men, and 111 4% in women), and was positively correlated with the total cholesterol level (regression coefficient 6 4% FVII per mmol/l cholesterol) and fibrinogen level (regression coefficient 15 8% FVII per g/l fibrinogen) Fibrinogen showed, in addition to its association with the factor VII level, increasing values with age (regression coefficient 0 13 g/l per 10 yr) Systolic and diastolic blood pressure were higher in men than in women (mean SBP 124 mmHg in men, and 117 mmHg in women, mean DBP 81 mmHg in men, and 76 mmHg in women), and in the obese (regression coefficient 1 8 mmHg systolic and 1 6 mmHg diastolic per kg/m^2)

Clotting Factor Gene Polymorphism and Clotting Factor Plasma Levels

The MspI polymorphism of factor VII had an allele-frequency for the M2 allele of ten percent, 18 percent of the individuals were carriers

Table 1 General characteristics of 121 healthy volunteers

Variable	n	Mean	(SD)	Range
Sex male	64			
female	57			
Age (yrs)		34 5	(9 2)	19–56
Plasma fibrinogen (g/l)		2 9	(0 6)	1 8–4 4
Plasma factor VII act (%)		105 1	(26 3)	51–196
Systolic BP (mmHg)		120 3	(14 4)	89–167
Diastolic BP (mmHg)		78 6	(10 5)	51–106
Serum cholesterol (mM)		5 4	(1 1)	3 0–9 0
Serum HDL-cholesterol (mM)		1 5	(0 4)	0 7–2 6
Smoking (package years)		3 1	(8 4)	0–65
Quetelet index (kg/m^2)		23 6	(2 9)	17 6–33 6
Fam history neg	100			
Fam history pos	21			

Table 2 Factor VII and fibrinogen polymorphisms and plasma levels

		n	Perc of subjects	Mean clotting factor level	(SD)
Factor VII	M1M1	99	(82%)	109 6%	(26 0)
	M1M2	19	(16%)	85 1%	(17 5)
	M2M2	3	(2%)	82 3%	(6 4)
Fibrinogen	H1H1	86	(71%)	2 88 g/l	(58)
	H1H2	33	(27%)	2 86 g/l	(55)
	H2H2	2	(2%)	3 60 g/l	(74)

Table 3 Univariate regression analysis of risk factors

Variable	Change in IMT (μm)	95%-confidence interval
Age (10 yrs)	37 7	25 4 to 50 2
Sex (m = 0, f = 1)	–28 6	–54 0 to –3 2
Systolic BP (10 mmHg)	17 9	9 5 to 26 3
Diastolic BP (10 mmHg)	21 6	9 8 to 33 8
Serum cholesterol (1 mM)	23 2	12 2 to 34 2
Serum HDL-cholesterol (1 mM)	–21 0	–51 7 to 9 7
Cholesterol/HDL-cholesterol ratio	17 5	9 0 to 26 0
Smoking (10 package years)	6 6	–8 8 to 22 0
Quetelet index (1 kg/m^2)	6 6	2 2 to 11 0
Fam history (neg = 0, pos = 1)	–13 8	–47 9 to 20 3
Plasma fibrinogen (1 g/l)	20 70	–1 6 to 43 0
Factor VII activity (10%)	2 0	–2 9 to 6 9
Fibrinogen polymorphism H1H2/H2H2[1]	–25 6	–53 7 to 2 6
Factor VII polymorphism M1M2/M2M2[2]	– 7 8	–41 4 to 25 7

[1]Increment compared to H1H1 genotype
[2]Increment compared to M1M1 genotype

of an M2-allele Those who carried this allele had about 20 percent less factor VII activity (Table 2)

The HaeIII polymorphism of fibrinogen had an allele-frequency for the H2-allele of 15 percent, 29 percent carried a H2-allele Contrary to the findings of Thomas et al (10), carriers of the H2-allele did not have higher plasma fibrinogen levels than those homozygous for the H1-allele (the level appeared higher in individuals who were homozygous for the H2-allele, but this included only two individuals)

Based on these data, we concluded that the MspI factor VII polymorphism was a valuable indicator of the "genetic factor VII level" The HaeIII fibrinogen polymorphism did not fulfill our expectations in this respect

Relation of Determinants and Vessel Wall Thickness

The intima-media thickness of the carotid artery (mean of 18 measurements at each side), was higher for men than for women, and increased with age, blood pressure (both systolic and diastolic), total serum cholesterol, cholesterol/HDL ratio and Quetelet-index, whereas it decreased with HDL-cholesterol (Table 3) No relation with package-years of smoking, nor with a positive family history of cardiovascular disease was found

The intima-media thickness increased with higher levels of fibrinogen, whereas the fibrinogen polymorphism showed a strong trend of association with intima-media thickness, albeit in the unexpected direction (lower IMT for carriers of the H2-allele) Intima-media thickness was not related to factor VII activity, nor to the factor VII polymorphism genotype (Table 3) Scatterplots of factor VII and fibrinogen versus intima-media thickness with the regression lines are depicted in figure 1 It may be noted that the association of IMT with fibrinogen levels (20 7 μm per 1 g/l increase in fibrinogen) was to a high extent determined by one outlying observation Leaving out this point (fibrinogen 4 26 g/l, IMT 752 μm) reduced the slope of the regression line to 13 7 μm per 1 g/l (CI95 –8 5 to 36 0 μm per g/l increase in fibrinogen)

Multivariate Analysis

Since several of the determinants appeared to be interrelated, we performed a multivariate regression analysis The regression coefficients of this analysis are to be interpreted similarly to those of a simple univariate regression, but now the effects of other variables are adjusted for

When all classical determinants were entered into the model, age, blood pressure, total cholesterol and HDL-cholesterol emerged as independent determinants of intima-media thickness When diastolic blood pressure was entered in the analysis instead of systolic blood pressure, the diastolic regression coefficient became 11 0 μm/10 mmHg (95% CI –0 90 to 22 9) The effect of sex was attenuated as was that of fibrinogen, whereas the Quetelet index had no independent effect, nor had factor VII plasma levels In this analysis, as in univariate analysis, no effect of smoking or a positive family history could be observed (Table 4)

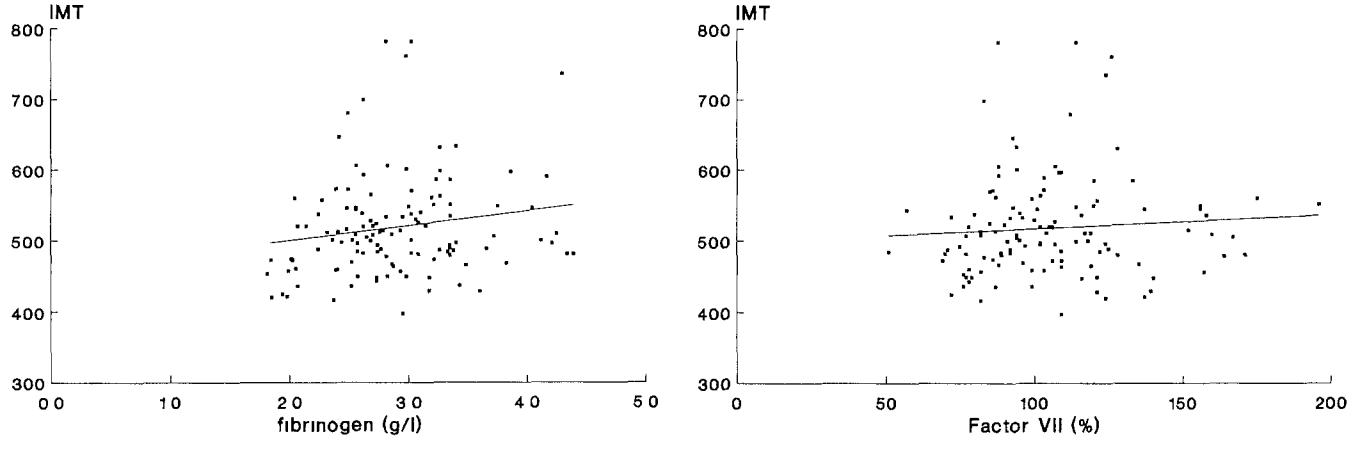

Fig 1 Scatterplot of fibrinogen (left) and factor VII (right) with intima-media thickness The regression line is indicated in the figures (fibrinogen 459 + 20 7 × fibrinogen level, factor VII 498 + 20 × factor VII level)

Discussion

We performed a cross sectional study to assess whether ultrasound measurement of carotid artery wall thickness could be of value in investigating the role of clotting factor levels in the development and progression of atherosclerosis

Intima-media thickness was clearly associated with several classical cardiovacular risk factors like age, sex, systolic and diastolic blood pressure, total and HDL serum cholesterol All these associations were positive or negative according to the expected (15) effect of the risk-factor on atherosclerosis Although the effects themselves are small, the associations are more than striking, since they were observed in a small study of a heterogeneous group of mostly young individuals We therefore conclude that ultrasonographic assessment of carotid intima-media thickness offers itself as a valuable research tool for non-invasive measurement of early atherosclerosis in healthy young and middle-aged subjects, as it is applied in studies with elderly or symptomatic subjects

It is notable that at the relative young age of our subjects, alterations were already visible in the arterial vessel wall, in a clear association with the presence or absence of cardiovascular risk factors

Information from the literature about IMT in young healthy individuals is very scarce When we compare our results to those of the healthy control groups in two other studies (7, 16), the mean intima-media thicknesses are very similar (about 0 5 mm), as is the age relation compared to two studies that also included young subjects (6, 17) Interestingly, a much higher mean IMT of 1 mm was found in the Kuopio Ischaemic Heart Disease Risk Factor Study (8), which is conducted in an area with a very high incidence of coronary artery disease (eastern Finland) No comparison can be made on the effects of risk factors on intima-media thickness in young subjects, as no such studies could be found in the literature

We did not find associations of artery wall thickness with cardiovascular family history, nor with smoking, which is not in accordance with several other studies in which an effect of smoking on IMT had been found (7, 16) Although it has to been borne in mind that most studies on IMT, including ours, have been quite small which may easily have led to differences due to chance variation, it cannot be ruled out that these well-established risk factors for atherosclerosis may have a delayed effect, which will therefore not yet be apparent in young individuals

Our study showed no association of the level of factor VII, or its polymorphism with intima-media thickness Even in view of the limited number of subjects, an important effect of factor VII seems unlikely from these data This is less clear for fibrinogen Univariate analysis indicated a relation of fibrinogen with intima-media thickness that was (per 1 g/l) of about the same magnitude as for serum cholesterol (per 1 mM) This association largely disappeared in the multivariate analysis, which may be the result of correlations with other variables, especially age, on the other hand, it cannot be ruled out that part of the effect of age is mediated by fibrinogen Finally, the confidence intervals for the association of fibrinogen levels and artery wall thickness remained wide, and do not allow firm conclusions

The fibrinogen polymorphism had no relation with plasma fibrinogen levels in these 121 Dutch volunteers, whereas it was a clear predictor of fibrinogen levels in two studies among British and French individuals (9, 18) Recently, we also failed to find this association in an independent sample consisting of 199 patients with deep venous thrombosis and 199 healthy controls (19) Apparently, the association between this polymorphism and fibrinogen levels is not universal We did find a relation between the HaeIII polymorphism and IMT, which was in the unexpected direction (i e lower IMT in carriers of the H2-allele) Obviously, since the polymorphism and the fibrinogen level were not associated, this effect cannot be interpreted as mediated by the plasma level Still, this finding provokes thought, especially given our previous study on venous thrombosis (40 percent reduction of risk in H2-carriers) (19), and the results of the ECTIM study (10–20% reduction of myocardial infarction risk for those who carried the H2-allele) (18)

Table 4 Multivariate regression analysis

Variable	Change in IMT (μm)	95%-confidence interval
Age (10 yrs)	30 2	17 3 to 43 0
Sex (m = 0, f = 1)	–11 8	–38 8 to 15 2
Systolic BP (10 mmHg)	11 0	2 6 to 19 4
Cholesterol (1 mM)	14 0	3 0 to 25 0
HDL-cholesterol (1 mM)	–21 5	–53 2 to 10 3
Smoking (10 package-years)	–0 64	–13 8 to 12 5
Quetelet index (1 kg/m^2)	–1 3	–5 9 to 3 3
Fam history (neg = 0, pos = 1)	–15 4	–45 1 to 14 3
Plasma fibrinogen (1 g/l)	9 6	–11 3 to 30 5
Plasma factor VII act (10%)	–0 32	–5 3 to 4 7

These findings suggest that factor VII and fibrinogen have no detectable influence on early atherosclerosis Obviously, our study offers no information of the effect of clotting factor levels in advanced atherosclerosis with plaque formation We have studied healthy volunteers with clotting factor levels that generally were in the usual range We cannot exclude therefore, that extreme values of factor VII or fibrinogen (either at the lower or upper extreme of the spectrum) may have a noticeable and relevant effect on atherosclerosis

Our method of measuring atherosclerosis can be extended to other superficial arteries such as the femoral or popliteal, although it is likely that for research purposes measurements at the carotid arteries are sufficient for an adequate overall picture (20) We believe that automatic (off-line) detection of intima-media boundaries and contours could further improve the accuracy and precision of our measurements In addition, arterial vessel wall compliance may offer additional information in describing atherosclerosis

Intima-media measurements are valuable in young and middle-aged subjects, to study the determinants of atherosclerosis in its earliest stages Our study offers no support for the hypothesis that the clotting factor system is involved in the development and progress of early atherosclerosis

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