Activation of Factor VII During Alimentary Lipemia Occurs in Healthy Adults and Patients With Congenital Factor XII or Factor XI Deficiency, But Not in Patients With Factor IX Deficiency

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Factor VII activity (FVIIc), a risk marker for coronary heart disease, is increased during postprandial lipemia. Factor VII activation accompanies lipolysis of triglyceride-rich lipoproteins, but the nature of this association and whether it is causal remain uncertain. To explore this issue, four patients with homozygous factor XII deficiency, four with complete factor XI deficiency, six with factor IX deficiency, and their respective age- and sex-matched controls were given two isocaloric dietary regimens, one providing on average 136 g fat and the other 19 g fat. Blood was taken before breakfast, immediately before lunch at 195 minutes, and at completion of the study at 390 minutes. All samples for each subject and matched control were assayed as one batch for FVIIc, activated factor VII, and factor VII antigen (FVIIag). Activation of factor VII was observed with the high-fat regimen but not with the low-fat regimen in all controls, factor XII-deficient patients, and factor XI-deficient patients. No factor VII activation was observed during either regimen in factor IX-deficient patients, but a normal postprandial responsiveness of factor VII to dietary fat was restored in one patient who replicated the study after factor IX therapy. Plasma FVIIag was not altered postprandially in either regimen in any group of patients or controls. Factor IX apparently plays an obligatory role in the postprandial activation of factor VII, although the mechanism remains to be determined.

THE COAGULANT ENZYME, factor VIIa (FVIIa), circulates in human plasma at a concentration of about 4 ng/mL[1,2] with a half-life of approximately 2.5 hours.[3] Its precursor, the single-chain zymogen factor VII (FVII), measured as factor VII antigen (VIIag), has a circulating concentration of about 450 ng/mL (10 nmol/L)[4] and a half-life of 4 to 5 hours.[5] The conversion of FVII to FVIIa in vitro is accomplished by factors Xa,[6] IXa,[7] XIIa,[8] and thrombin,[9] but the relative roles of these clotting factors in the rate of generation of FVIIa in vivo are unknown. Plasma factor VII coagulant activity (FVIIc), as routinely measured by a one-stage clotting assay, is a function of the concentrations of both FVII and FVIIa in recalcified test plasma.[10]

Clinical interest in factor VII has been raised by the discovery of an increased risk of fatal coronary heart disease (CHD) in middle-aged men with a high FVIIc.[11,12] This association is strong and independent of blood cholesterol, plasma fibrinogen concentration, and blood pressure.[13] In the Northwick Park Heart Study, a difference of 1 SD in FVIIc (ie, 25% of standard) was associated with a difference of almost 50% in the risk of fatal CHD during the following 16 years.[11] An understanding of the basis for a high FVIIc is therefore of importance for cardiovascular medicine.

Epidemiologic and clinical studies have established that FVIIc is associated positively and independently with plasma triglyceride concentration[14-18] and dietary fat intake.[19,20] Furthermore, the relation between FVIIc and dietary fat is independent of serum cholesterol concentration, body mass index (BMI), alcohol intake and smoking habit.[20] In adults on a typically Western diet (30% to 40% energy supplied as fat), the associations of FVIIc with dietary fat intake[21,22] and plasma triglyceride concentration[17,18] clearly operate through FVIIag in the fasting state. However, when nonfasting blood samples are analysed with a bioassay that possesses sufficient sensitivity to FVIIa, the association between FVIIc and triglyceride concentration also appears to operate through FVIIa.[23] This latter relation is probably a consequence of the activation of factor VII that occurs during postprandial lipemia.[24-27] A high-fat diet may therefore increase the risk of fatal CHD not only through its long-term effects on plasma cholesterol concentration and atherogenesis, but also through associated increases in FVII and FVIIa, which raise the risk of a fatal coronary thrombosis in the event of rupture of an atheromatous plaque.

The mechanism of activation of factor VII after a high-fat meal is unknown. However, in vitro incubation of triglyceride-rich plasma with lipoprotein lipase to release free fatty acids from very–low-density lipoproteins and chylomicrons induces a prompt activation of factor XII.[28] Furthermore, factor XII is activated when citrated plasma is incubated with micellar stearic acid at a low temperature[29] (to reduce the inhibitory effects of C1 inhibitor on factor XIIa and kallikrein[30]). The generation of factor XIIa during incubation of plasma at a low temperature is accompanied by the conversion of FVII to FVIIa,[31] partly by direct catalysis and partly by way of intermediate sequential activation of factor XI and factor IX.[32,33] These findings raise the possibility of similar effects in vivo after a fat-rich meal. Studies have therefore been conducted in patients with homozygous factor XII deficiency, homozygous factor XI deficiency and factor IX deficiency to determine whether the contact phase of coagulation (factor XII and factor XI) mediates in the activation of the extrinsic pathway (factor VII) after fat consump-

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tion, and if so, whether this action is direct or indirect through factor IX.

PATIENTS AND METHODS

Patients with homozygous factor XII deficiency, homozygous factor XI deficiency, and factor IX deficiency were recruited through the Haemophilia Centre and Thrombosis Unit at the Royal Free Hospital (Dr Christine Lee) and the Haemophilia Centre at Hammersmith Hospital (Dr Michael Laffan). Each patient was sent a letter outlining the purposes and design of the study and inviting participation. Those who volunteered stated their food preferences and any special dietary requirements by self-completed questionnaire. They were also asked to identify an age (±5 years) and sex-matched healthy volunteer who was then recruited as the control in the same manner. Ethical approval had been obtained beforehand from the relevant institutional committee, and all participants were offered an interview to discuss the study before giving informed consent in writing.

Test meals. The dietary information given by questionnaire was used in conjunction with published tables of food composition[34] to design two regimens of acceptable test meals, each comprising a breakfast and lunch of foods of known fatty acid content. Each breakfast supplied 5,230 kJ (15% as protein), and the lunch half this amount. One regimen was rich in fat, with a target intake of 90 g (3,389 kJ) at breakfast and 45 g (1,695 kJ) at lunch, while the other regimen had a low-fat content with respective target intakes of 12 g (452 kJ) and 6 g (226 kJ). Breakfast commenced between 9:00 and 9:30 AM, and lunch 195 minutes later. All meals were consumed within 15 minutes. The mean interval between the experimental regimens was 16 days (range, 6 to 35).

On the day before each test, subjects were requested to select what they ate from a list of low-fat foods and to avoid high-fat foods on a prohibited list. Patients with factor IX deficiency had their test deferred if they had received factor IX therapy within 5 days of their appointment. Subjects then fasted overnight and had no more than one cup of tea or coffee before arrival. The order of the low-fat and high-fat regimens was randomized in the first stages of the study involving the patients with factor XII deficiency and their controls, but when the high-fat regimen was taken first some found the bulk of the isocaloric low-fat regimen very difficult and could not complete the breakfast. Therefore, for the remainder of the study the low-fat test regimen was given first, the weights of any foods not eaten by the patients were recorded, and when necessary the meals in their high-fat regimen were adjusted to the energy and protein intake of the first regimen while maintaining their fat content as closely as possible. Subjects were required to consume the meals in the second regimen completely. Controls were always tested after the patients so that the total energy and nutrient intake in their test meals could be matched to those of their respective patients.

One patient with factor IX deficiency replicated his study after receiving an intravenous injection of 3,720 IU highly purified factor IX (Alpha Therapeutic, Thetford, UK) approximately 90 minutes before the baseline of each test regimen. He consumed exactly what had been eaten on the previous occasions, and aliquots of plasma collected during the initial tests had been held at −70°C for assay with those taken after administration of factor IX.

Blood sampling and analysis. Blood samples were taken by venepuncture into 'Vacutainer' tubes (Becton Dickinson) with minimal venous compression immediately before breakfast (baseline), immediately before lunch (195 minutes) and on completion of the test (390 minutes). Ten milliliters was drawn into a glass tube containing 15 mg Na_2 EDTA as powder (held on wet ice) and 9 mL into a silicone-coated tube containing 1 mL 0.105-mol/L buffered citrate. All tubes were centrifuged immediately at 1,100*g* for 10 minutes at ambient temperature, and aliquots of plasma transferred with a plastic Pasteur pipette into plastic vials (Nunc) for storage at −70°C pending analysis. All samples belonging to each patient and control pair were assayed in random order as a single batch.

Factor VII. Each assay for factor VII was performed in duplicate on citrated plasma and the results averaged. Factor VII antigen (FVIIag) concentration (a close approximation to single-chain FVII concentration since only about 1% of factor FVIIag exists as FVIIa) was determined by a two-site monoclonal immunoenzymometric assay (Novo Nordisk Biolabs, Bagsvaerd, Denmark). Plasma FVIIc was measured by a one-stage bioassay using a rabbit brain thromboplastin (Diagen, Thame, Oxon, UK) and a factor VII-deficient substrate plasma prepared by a modification[10] of a method described by Lechner and Deutsch.[35] The results of the FVIIag and FVIIc assays were expressed as a percentage of the performance of an in-house standard plasma calibrated in terms of an international primary standard plasma (National Institute of Biological Standards and Control, Potters Bar, England; code labeled 84/665). Plasma FVIIa levels were measured by a one-stage clotting assay using a soluble mutant recombinant tissue factor that possesses cofactor activity for FVIIa but fails to support activation of FVII.[1] The coagulation times were converted to FVIIa concentration (ng/mL) by comparison with a standard curve given by a freeze-dried preparation of purified recombinant FVIIa (National Institute of Biological Standards and Control; code labelled 89/688) serially diluted with a commercial factor VII-deficient human plasma (George King Biomedical, Overland Park, KS).

Other assays. Plasma factor IX (FIXc), factor XI (FXIc) and factor XII (FXIIc) coagulant activities were assayed with a modification of the activated partial thromboplastin time using factor IX-deficient plasma (Diagen), factor XI-deficient plasma (Immuno) and factor XII-deficient plasma (Immuno), respectively, and a common standard plasma (Immuno Reference Plasma). The limits of detection in these clotting assays were less than 1% of standard. Factor IX antigen (FIXag), factor X antigen (FXag) and prothrombin antigen concentrations were measured using a modification of the Laurell electroimmunoassay using rabbit antihuman sera (Dako). Factor XII antigen (FXIIag) was measured with the same system using a goat antihuman factor XII serum (Nordic Laboratories). The limits of detection of factor XII antigen and factor IX antigen with these assays were 5% and 3% of standard, respectively. Factor XIIa was assayed with an ELISA which used a monoclonal capture antibody (Shield Diagnostics, Dundee, UK) having an affinity for factor XIIa more than 50,000-fold greater than for factor XII.[36] Protein C activity was measured by a chromogenic method using a commercial protein C activator (Protac, American Diagnostics) and S-2366 as chromogenic substrate.

Plasma cholesterol and triglyceride concentrations were measured by enzymic assays with reagents from Sigma (Poole, UK) and Wako Chemicals (Alpha Laboratories, Eastleigh, UK) respectively.

Statistical analysis. Data for factor VII and plasma triglyceride at each of the three time-points during the tests were analysed separately, expressing postprandial values as a percentage of the baseline value. Repeated-measures analysis of variance (ANOVA) was used to test the significance of differences within each deficiency state between patients and controls, between dietary regimens, and for interaction between these terms. Differences were considered significant when the likelihood of occurrence by chance alone was less than 5% ($P < .05$).

RESULTS

Table 1 shows that patients and controls were well matched for age, and were of similar mean height. The ranges of weight and body mass index (BMI) in each group

Table 1. Sex, Age, and Body Size

	M/F	Age (yr)	Height (m)	Weight (kg)	Body Mass Index (kg/m²)
Factor XII deficiency					
Patients	2/2	39 (23-55)	1.74 (1.60-1.91)	79.8 (59.6-95.5)	26.7 (21.6-37.3)
Controls	2/2	38 (21-54)	1.74 (1.60-1.80)	72.3 (68.8-76.4)	24.1 (21.8-28.8)
Factor XI Deficiency					
Patients	3/1	45 (24-56)	1.72 (1.58-1.85)	73.6 (64.6-80.9)	25.1 (23.0-29.2)
Controls	3/1	46 (24-56)	1.74 (1.63-1.82)	76.9 (64.6-91.0)	25.4 (23.9-29.6)
Factor IX deficiency					
Patients	6/0	40 (27-49)	1.80 (1.73-1.88)	78.9 (70.0-89.1)	24.7 (22.3-28.2)
Controls	6/0	41 (22-49)	1.82 (1.74-1.91)	86.5 (68.0-103.2)	26.1 (22.5-30.9)

Results are the mean (range).

were wide, but only one female patient with factor XII deficiency and one control for a patient with factor IX deficiency were obese (BMI >30 kg/m²). Table 2 summarizes the hemostatic status of patients and controls. Only one of four patients with factor XII deficiency (a male aged 23 years) had any detectable factor XII. Among patients with factor XI deficiency FXIc was absent in three and estimated to be only at trace levels in the fourth (since this patient had a stop codon in exon 5 true factor XI activity must have been completely absent). In patients with factor IX deficiency FIXc ranged from nil to 8% of standard. FIXag was too low to be quantified in five patients, but was 81% of standard in the patient with an FIXc of 8% of standard. All patients with factor IX deficiency had normal levels of FXIIc, factor X antigen, prothrombin antigen and protein C activity. The controls had normal levels of the hemostatic factor deficient in their matched patients.

Baseline levels of FVIIc, FVIIa, FVIIag, plasma cholesterol or plasma triglyceride did not differ significantly between the two dietary regimens in any group of patients or controls. On this account each subject's paired results were averaged to produce Tables 3 and 4, which show no statistically significant differences for any of these indices between patients and their matched controls except for a reduction in FVIIa (Table 3) and plasma triglyceride concentration (Table 4) in factor IX-deficient patients.

The design of the study ensured that corresponding meals taken by patients and their matched controls were very similar in energy, protein, total fat, and fatty acid composition, and these data were therefore pooled. Table 5 gives the mean intakes at breakfast and for breakfast and lunch combined. The mean difference in energy intake of 686 kJ between the low-fat and high-fat breakfasts in the study of factor XII deficiency was explained earlier. Overall, the average fat intake during the high-fat phase was 91 g (3,416 kJ) at breakfast and 136 g (5,121 kJ) for the meals combined, the respective figures for the low-fat phase being 11 g (414 kJ) and 19 g (715 kJ). Stearic acid intake averaged 10 g (382 kJ) at breakfast and 15 g (570 kJ) for breakfast and lunch combined in the high-fat phase, and 0.75 g (28 kJ) and 1.38 g (52 kJ), respectively, in the low-fat phase. Corresponding linoleic acid intakes were respectively, 2.6 g (99 kJ) and 3.8

Table 2. Hemostatic Factor Levels in Patients and the Ranges in Respective Age- and Sex-Matched Controls

Deficiency	Patients	Respective Controls
Factor XII (n = 4)		
Factor XII coagulant activity (% standard)	Undetectable in 3 patients, 7% in one patient	102-122
Factor XII antigen (% standard)	Undetectable in all patients	85-128
Activated factor XII (ng/mL)	Undetectable in 3 patients, 0.6 in the patient with 7% XII coagulant activity	2.9-7.9
Factor XI (n = 4)*		
Factor XI coagulant activity (% standard)	Undetectable in 3 patients, 1% in one patient	73-135
Factor IX (n = 6)†		
Factor IX coagulant activity (% standard)	Undetectable in 2 patients, 5% in 3 patients, and 8% in 1 patient	103-124
Factor IX antigen (% standard)	Undetectable in 5 patients, 81% in the patient with 8% IX coagulant activity	91-123
Factor XII coagulant activity (% standard)	67-135	57-87
Factor X antigen (% standard)	75-103	92-106
Prothrombin antigen (% standard)	69-103	87-114
Protein C activity (% standard)	60-95	85-110

The result in each participant is the mean of 6 determinations. Samples for matched patients and controls were assayed simultaneously.

* Two patients, one with an estimated factor XI coagulant activity of 1%, were homozygous for Q 117 stop. A third patient was a double heterozygote (Q 117 stop; F283L). The fourth patient was not genotyped.

† Five patients had a stop codon (2 with R333 stop, 1 with R29 stop, 1 with W194 stop, and 1 with W407 stop). One patient with 8% factor IX coagulant activity and 81% factor IX antigen had a 3-basepair deletion resulting in loss of G183.

Table 3. Baseline Results for Levels of FVIIc, FVIIa, and FVIIag in Patients and Respective Controls (Mean ± SE)

Deficiency	FVIIc (% standard)	FVIIa (ng/mL)	FVIIag (% standard)
Factor XII (n = 4)			
Patients	106.4 ± 17.4	1.56 ± 0.33	124.8 ± 22.5
Controls	101.8 ± 15.1	1.61 ± 0.25	97.2 ± 15.4
All subjects	104.1 ± 11.0	1.59 ± 0.20	110.2 ± 13.5
Factor XI (n = 4)			
Patients	103.4 ± 4.7	1.13 ± 0.32	120.5 ± 4.8
Controls	103.5 ± 6.7	1.44 ± 0.21	108.6 ± 5.1
All subjects	103.5 ± 3.9	1.27 ± 0.20	114.4 ± 3.8
Factor IX (n = 6)			
Patients	65.2 ± 3.7	0.51 ± 0.04*	87.6 ± 6.2
Controls	80.3 ± 4.5	1.48 ± 0.10	87.9 ± 5.0
All subjects	72.4 ± 3.3	—	87.8 ± 3.9

Baseline values before the high-fat and low-fat regimens did not differ significantly in any group of patients or controls, and results were therefore averaged in each subject.

* $P < .0001$ *v* age- and sex-matched controls.

g (143 kJ) in the high-fat phase and 1.5 g (57 kJ) and 2.5 g (94 kJ) in the low-fat phase.

Factor XII deficiency. The postprandial responses to the two dietary regimens did not differ significantly between factor XII-deficient patients and their controls (Table 6). Plasma triglyceride increased during both dietary regimens, but significantly more so during the high-fat phase. Plasma FVIIc increased in both groups during the high-fat regimen whereas it declined during the low fat phase, this difference in response between dietary phases being statistically significant both at 195 and 390 minutes. The interaction term in the ANOVA was not statistically significant at either time point, indicating that the pattern of responsiveness of FVIIc to the two test regimens did not differ significantly between patients and controls (although the mean FVIIc for controls at 195 minutes was only 1% above their baseline value).

Table 4. Baseline Results for Plasma Cholesterol and Triglyceride Concentrations in Patients and Respective Controls (Mean ± SE)

Deficiency	Cholesterol (mmol/L)	Triglyceride (mmol/L)
Factor XII (n = 4)		
Patients	6.60 ± 1.17	1.24 ± 0.28
Controls	6.40 ± 0.95	1.21 ± 0.10
All subjects	6.50 ± 0.73	1.22 ± 0.14
Factor XI (n = 4)		
Patients	5.24 ± 0.09	1.22 ± 0.35
Controls	5.07 ± 0.30	1.22 ± 0.28
All subjects	5.16 ± 0.15	1.22 ± 0.21
Factor IX (n = 6)		
Patients	3.77 ± 0.27	0.79 ± 0.13*
Controls	4.73 ± 0.38	1.44 ± 0.24
All subjects	4.25 ± 0.25	—

Baseline values before the high-fat and low-fat regimens did not differ significantly in any group of patients or controls, and results were therefore averaged in each subject.

* $P = .04$ *v* age- and sex-matched controls.

The postprandial elevation in FVIIc on the high-fat regimen was due essentially to an increase in FVIIa. On the low-fat regimen, FVIIa had increased to 110% of the baseline value on average in patients by 390 minutes, whereas it had declined to 95% of baseline value in the controls, but this difference was not statistically significant. Similarly, FVIIa increased more in patients than controls during the high-fat regimen (to 151% and 133% of baseline value, respectively), although again the difference was not statistically significant. By contrast to FVIIa, FVIIag levels decreased slightly with time, the one exception being a nonsignificant average increase to 106% of baseline value by 390 minutes in controls on the high-fat regimen. Overall, FVIIag levels did not differ between patients and controls or between dietary regimens.

Factor XI deficiency. The postprandial responses in patients with factor XI deficiency and their controls resembled those in the study of factor XII deficiency (Table 7). Thus the responses were very similar in patients and controls, and plasma triglyceride concentration increased significantly more during the high-fat regimen versus the low-fat regimen. At 195 minutes, FVIIc showed little change from baseline in either group during either dietary phase. However, by 390 minutes, FVIIc was significantly higher on the high-fat regimen in both groups, due to an increase above the baseline value in the high-fat phase and a decrease in the low-fat phase.

Table 7 also shows that on the low-fat regimen mean FVIIa increased on average to 113% of baseline at 390 minutes in patients, and to 105% of baseline in the controls. Although stronger, the patients' responses were not statistically significantly greater at either time point. During the high-fat phase the mean increase in patients was 59% above baseline at 390 minutes, as compared with 28% in their controls. This difference between patients and controls was not statistically significant, but in both groups the increase during the high-fat regimen was significantly greater than in the low-fat period. Plasma FVIIag tended to decline across the 390 minutes of observation in both groups and on both dietary regimens, the one exception being a nonsignificant average increase of 7% above baseline at 390 minutes in controls during the high-fat period. Overall, the decrease in FVIIag at 390 minutes was significantly greater in patients than in controls (for which we have no explanation) and on the low-fat regimen than on the high-fat regimen.

Factor IX deficiency. Table 8 shows that in contrast to patients with factor XII and factor XI deficiencies, patients with factor IX deficiency showed a clear difference from their controls in the responsiveness of factor VII to dietary fat. In both groups plasma triglyceride levels were significantly higher during the high-fat phase than the low-fat phase. Plasma FVIIc declined similarly in patients and controls in the low-fat period. However, unlike any other group, FVIIc also declined in the patients in the high-fat phase, whereas as expected it increased steadily in the controls to 117% of baseline value by 390 minutes ($P = .03$ for the subject x diet interaction term).

In the controls, FVIIa increased on average to 34% above the baseline value by 195 minutes and to 41% above baseline

Table 5. Energy and Nutrient Intake (kJ) at Breakfast and Over the Complete Test Period According to Dietary Regimen, Patients and Matched Controls Combined (Mean ± SE)

Period	Energy	Fat	Stearic Acid (18:0)	Linoleic Acid (18:2)
Breakfast				
Factor XII deficiency				
Low-fat	4,121 ± 276	322 ± 29	29 ± 3	27 ± 2
High-fat	4,807 ± 276	3,380 ± 218	379 ± 27	111 ± 14
Factor XI deficiency				
Low-fat	4,899 ± 192	448 ± 21	29 ± 3	69 ± 10
High-fat	4,736 ± 205	3,560 ± 167	379 ± 20	70 ± 8
Factor IX deficiency				
Low-fat	4,477 ± 126	452 ± 50	27 ± 5	68 ± 10
High-fat	4,502 ± 126	3,343 ± 88	387 ± 13	110 ± 15
Complete test period				
Factor XII deficiency				
Low-fat	6,673 ± 410	778 ± 96	71 ± 8	61 ± 8
High-fat	7,125 ± 402	5,033 ± 331	559 ± 41	157 ± 18
Factor XI deficiency				
Low-fat	7,146 ± 281	695 ± 42	49 ± 3	100 ± 14
High-fat	7,213 ± 285	5,422 ± 234	572 ± 29	105 ± 13
Factor IX deficiency				
Low-fat	6,757 ± 197	686 ± 46	41 ± 5	113 ± 10
High-fat	6,870 ± 184	4,979 ± 121	576 ± 21	158 ± 17

Control subjects were given meals of the same energy, fat, and protein content consumed by their matched patients.

by 390 minutes during the high-fat regimen, whereas it increased only by 1% and 3% above baseline at 195 minutes and 390 minutes, respectively, during the low-fat regimen. These differences between dietary phases were statistically significant (P = .01). By contrast, in the patients, mean FVIIa increased slightly during the low-fat regimen to a mean 14% above baseline at 390 minutes, whereas it decreased during the high-fat regimen to 7% below the baseline value at both 195 minutes and 390 minutes. These differences between dietary phases were not statistically significant. Plasma FVIIag decreased similarly in both patients and controls on both dietary regimens, the exception being a 3% average increase in FVIIag at 390 minutes in the controls on the high-fat meals (Table 8).

While the postprandial triglyceride responses did not differ significantly between patients and controls when concentrations were expressed as percentages of the baseline value (Table 8), the absolute values were lower in the patients. Thus, the geometric mean values during the high-fat regimen were 1.48 mmol/L in the patients and 2.92 mmol/L in the controls at 195 minutes (P = .07) and 1.36 mmol/L in the patients as compared with 2.64 mmol/L in controls at 390 minutes (P = .12). However, two patients with similar postprandial increases in triglyceride to the controls in the high-fat phase (1.76 mmol/L at baseline and 3.02 mmol/L at 390 minutes: 1.29 mmol at baseline and 3.37 mmol at 390 minutes), showed nevertheless decreases in FVIIa over the same period which accorded with the other four in the group (0.7 ng/mL at baseline in both, decreasing at 370 minutes to 0.5 ng/mL in one and 0.4 ng/mL in the other).

The effect of factor IX therapy in factor IX deficiency. The results of the experiment in which one patient replicated his study after injection of factor IX are shown in Fig 1. Plasma FIXc in the basal state was 2% of standard and

Table 6. Responses (% of Baseline Value) of Plasma Triglyceride and Indices of Factor VII to Test Meals in Four Patients With Factor XII Deficiency and Four Age- and Sex-Matched Controls (Mean ± SE)

	Triglyceride		FVIIc		FVIIa		FVIIag	
Parameter	195 min	390 min	195 min	390 min	195 min	390 min	195 min	390 min
Low-fat regimen								
Patients	136 ± 26	179 ± 31	93 ± 2	93 ± 3	106 ± 12	110 ± 9	98 ± 5	94 ± 7
Controls	110 ± 12	136 ± 25	98 ± 1	94 ± 3	100 ± 5	95 ± 3	94 ± 6	95 ± 4
High-fat regimen								
Patients	169 ± 26	261 ± 123	113 ± 8	124 ± 9	121 ± 14	151 ± 11	92 ± 4	96 ± 9
Controls	217 ± 35	349 ± 98	101 ± 8	119 ± 6	112 ± 8	133 ± 14	102 ± 8	106 ± 7
ANOVA (P)								
Patients v controls	.73	.72	.60	.82	.61	.19	.48	.54
Low-fat diet v high-fat diet	.02	.05	.04	.0004	.01	.004	.89	.30
Subject × diet interaction	.14	.33	.12	.43	.75	.83	.39	.41

Table 7. Responses (% of Baseline Value) of Plasma Triglyceride and Indices of Factor VII to Test Meals in Four Patients With Factor XI Deficiency and Four Age- and Sex-Matched Controls (Mean ± SE)

	Triglyceride		FVIIc		FVIIa		FVIIag	
Parameter	195 min	390 min	195 min	390 min	195 min	390 min	195 min	390 min
Low-fat regimen								
Patients	139 ± 64	187 ± 73	100 ± 3	87 ± 5	106 ± 4	113 ± 5	86 ± 7	74 ± 5
Controls	108 ± 11	135 ± 44	96 ± 3	90 ± 3	98 ± 5	105 ± 10	101 ± 4	89 ± 3
High-fat regimen								
Patients	215 ± 37	273 ± 48	102 ± 2	114 ± 6	133 ± 11	159 ± 20	92 ± 6	88 ± 7
Controls	172 ± 18	211 ± 41	98 ± 3	105 ± 7	120 ± 9	128 ± 7	100 ± 4	107 ± 5
ANOVA (*P*)								
Patients *v* controls	.50	.38	.17	.68	.24	.11	.11	.03
Low-fat diet *v* high-fat diet	.01	.05	.48	.001	.01	.04	.53	.005
Subject × diet interaction	.75	.87	.97	.19	.79	.43	.37	.66

FIXag was too low to be measured accurately (ie, <3% of standard). These levels were essentially unchanged postprandially throughout both test periods. After injection of factor IX the baseline FIXc was 59% of standard before the low-fat regimen and 62% of standard before the high-fat regimen, decreasing postprandially to below 50% of standard by the end of each study period. The respective baseline FIXag levels after factor IX injection were 53% and 45% of standard, decreasing to 30% of standard or less at the end of each period.

Plasma triglyceride concentrations decreased similarly during the low-fat phase both before and after factor IX administration, whereas in the high-fat regimen they increased to peak values at 195 minutes (higher after factor IX injection) falling thereafter toward baseline values. Before factor IX administration FVIIc levels were 52% and 50% of standard at baseline in the low-fat and high-fat regimens respectively, decreasing slightly postprandially in both periods. After factor IX injection FVIIc levels had increased to 58% and 59% of standard, respectively, at baseline, remaining essentially unchanged postprandially during the low-fat phase but rising to 61% of standard at 195 minutes and 69% of standard at 370 minutes in the high-fat phase.

Figure 1 shows that the increase in baseline FVIIc after factor IX injection was due mainly to an increase in FVIIa. Further increases in FVIIa above baseline were observed at 195 minutes in both dietary phases, but more so during the high-fat regimen. In the low-fat phase FVIIa was the same (0.78 ng/mL) at 195 minutes and 390 minutes, an increase of 0.20 ng/mL above baseline. By contrast during the high-fat regimen FVIIa continued to rise beyond 195 minutes to peak at 1.21 ng/mL at 390 minutes, an increase of 0.64 ng/mL above baseline. Baseline and postprandial levels of FVIIag were slightly higher in both dietary phases after factor IX administration, the mean (SD) of the six individual average values being 58.4 (2.8)% standard in comparison with 54.8 (2.8)% standard before factor IX. No postprandial response in FVIIag was observed after administration of factor IX.

DISCUSSION

The significant increases in FVIIc and FVIIa after high-fat meals but not after isocaloric low-fat meals in the healthy controls in this study accorded with previous reports.[21,24-27] The postprandial response in FVIIc was due entirely to activation of factor VII, there being no effect of dietary fat consumption on postprandial levels of FVIIag (which is almost entirely FVII). The increase in FVIIa was demonstrable by 3 hours after ingestion of a fat-rich breakfast and the level continued to rise after a high-fat lunch. Because FVIIag

Table 8. Responses (% of Baseline Value) of Plasma Triglyceride and Indices of Factor VII to Test Meals in Six Patients With Factor IX Deficiency and Six Age- and Sex-Matched Controls (Mean ± SE)

	Triglyceride		FVIIc		FVIIa		FVIIag	
Parameter	195 min	390 min	195 min	390 min	195 min	390 min	195 min	390 min
Low-fat regimen								
Patients	103 ± 4	113 ± 10	98 ± 3	92 ± 4	104 ± 4	114 ± 23	91 ± 2	92 ± 4
Controls	123 ± 15	143 ± 12	101 ± 4	93 ± 5	101 ± 5	103 ± 7	98 ± 4	91 ± 3
High-fat regimen								
Patients	203 ± 29	188 ± 26	95 ± 3	93 ± 2	93 ± 5	93 ± 5	96 ± 3	96 ± 4
Controls	205 ± 15	189 ± 19	109 ± 8	117 ± 5	134 ± 13	141 ± 11	98 ± 3	103 ± 6
ANOVA (*P*)								
Patients *v* controls	.64	.47	.13	.006	.05	.22	.21	.36
Low-fat diet *v* high-fat diet	<.0001	.002	.59	.01	.09	.49	.31	.12
Subject × diet interaction	.51	.32	.28	.03	.003	.03*	.45	.48

* Significant increase on high-fat diet in controls only ($P = .01$).

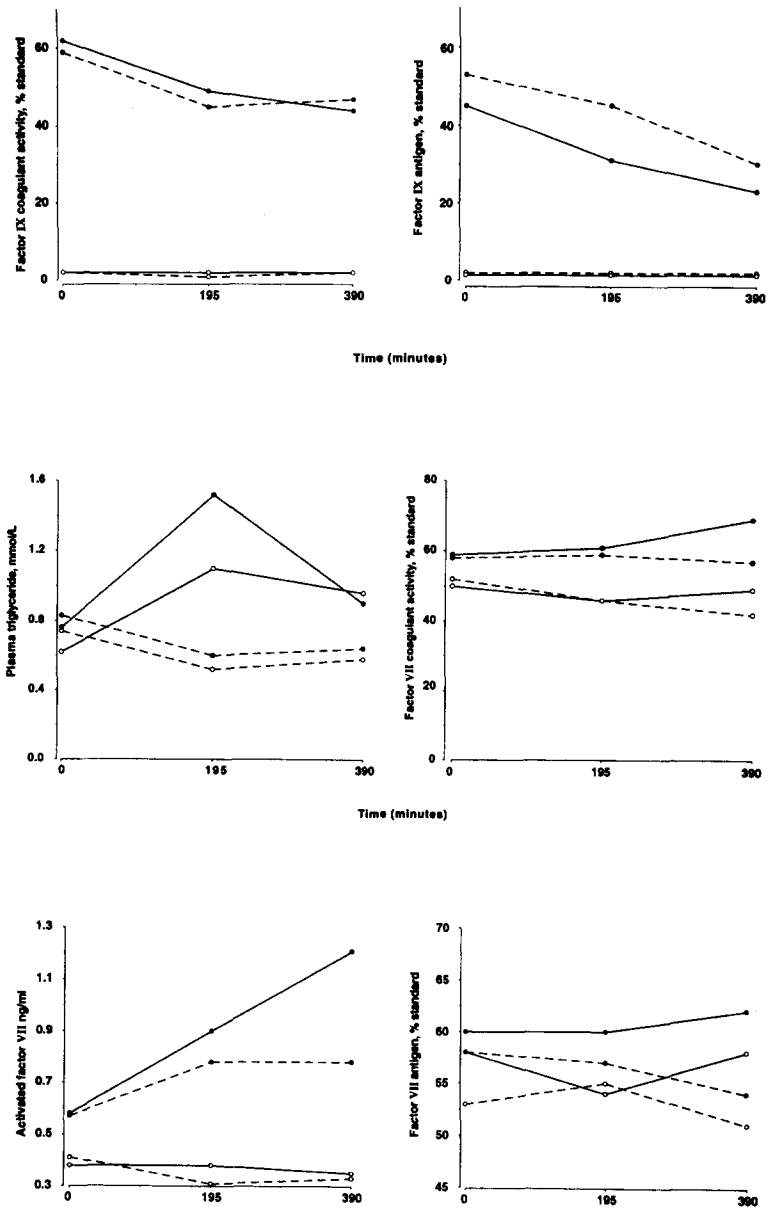

Fig 1. Factor IX coagulant activity, factor IX antigen concentration, plasma triglyceride concentration, factor VII coagulant activity, and activated factor VII and factor VII antigen levels during the low-fat regimen (-------) and high-fat regimen (———) in a patient with factor IX deficiency before (○) and after (●) intravenous injection of 3,720 IU highly purified factor IX.

often declined postprandially, irrespective of the type of meal, the response of FVIIc to a high-fat meal tended to be less marked than that in FVIIa (FVIIc by bioassay being a function of both FVIIa and FVII[10]).

This study was conducted to test whether the contact system (factor XII, factor XI, prekallikrein and high molecular weight kininogen) and the intrinsic pathway (factor IX) had roles in the postprandial activation of factor VII. The contact phase is activated in vitro when blood is exposed to biological substances with a negatively charged surface,[29,37,38] although the relevance of this mechanism for physiological conditions in vivo remains uncertain. The initial in vitro reaction is conventionally thought to be the conversion of surface-bound factor XII to factor XIIa, principally by kallikrein[39] but also by autoactivation.[40] Surface-bound factor XIIa then activates prekallikrein.[41] The product kallikrein then activates factor XII in a reciprocal reaction[42] believed to be about 100-fold faster than factor XII autoactivation.[43] Factor XIIa also activates factor XI[44] which, like prekallikrein, binds to the activating surface as a complex with high molecular weight kininogen.[45] Factor XIa converts factor IX to factor IXa in the presence of calcium ions.[46] Factor IXa can then activate factor VII,[7] in pure systems in the presence of added procoagulant phospholipid and calcium ions,[47] but in plasma without the requirement of addition of either.[31,32] Other in vitro reactions of possible relevance are the direct activation of human factor VII by human factor XIIa,[7] the autoactivation of human prekallikrein[48] and factor XI[49] in the presence of a negatively charged surface, and the activation of human factor XI by thrombin.[50,51]

The clear postprandial activation of factor VII in patients with homozygous factor XII deficiency would appear to exclude an absolute requirement for factor XII in the reaction process. The genetic defects in these particular patients may have permitted the generation of trace quantities of a functional enzyme undetectable in plasma with the assays used in this study but nevertheless sufficient to support contact activation, but this possibility was thought to be very remote. An obligatory role of factor XI in postprandial factor VII activation was similarly excluded because two patients had normal postprandial responses despite possession of a stop codon in exon 5 of the factor XI gene which precluded synthesis of the protease domain of this clotting factor. Involvement of the contact system in the activation of factor VII postprandially in homozygous factor XII and XI deficiencies would therefore require an alternative pathway such as surface-dependent autoactivation of prekallikrein followed by direct activation of factor IX by kallikrein. Whether these reactions occur in vivo at significant rates is uncertain. Seligsohn et al[7] observed cleavage of ^{125}I-factor IX by kallikrein in a system containing partially purified factor VII and calcium, and Osterud et al[52] reported a slow rate of conversion of factor IX to FIXa by kallikrein. Mitropoulos et al[32] also have in vitro evidence for direct activation of factor IX by kallikrein. However, Enfield and Thompson[53] found no cleavage of ^{125}I-factor IX by kallikrein in a pure system.

The findings in factor IX deficiency indicated that factor IX plays an obligatory role in the mechanism linking alimentary lipemia to activation of factor VII, and excluded direct postprandial activation of factor VII by FXIIa[7] (factor XII levels were normal in factor IX deficiency), kallikrein,[7] or plasmin.[54] These enzymes may still, of course, activate factor VII in vivo: patients with factor IX deficiency had detectable FVIIa, albeit at subnormal concentration.

The analysis of the data for the factor IX-deficient patients was complicated by their low absolute triglyceride concentrations at baseline and postprandially. The possibility arose that the lesser alimentary lipemia in the patients (possibly reflecting increased lipoprotein-lipase activity at the endothelial surface[55]) may have contributed to their failure to activate factor VII during the high-fat regimen. However, this suggestion appeared most unlikely, partly because FVIIa decreased after the high-fat meals even in two patients with factor IX deficiency whose postprandial lipemic response was similar to that in the controls, and partly because administration of factor IX restored the postprandial response of FVIIa to normal in a third patient with low fasting and postprandial triglyceride levels.

Whether the response of factor VII to a fatty meal is due to the acceleration of factor VII activation by FIXa or stimulation of factor IX activation cannot be determined from these data. Bauer et al[56] have reported normal plasma concentrations of factor IX activation peptide in factor XI deficiency but very low levels in factor VII deficiency. They concluded that the basal rate of factor IXa generation in vivo is driven mainly by factor VIIa-tissue factor complexes. Yet the low levels of FVIIa in factor IX deficiency (reported also by others[2]) indicate that the basal rate of factor VII activation is determined mainly by the intrinsic (factor IX) pathway. These findings raise the possibility of reciprocal activation of factor IX by FVIIa and factor VII by factor IXa in vivo, the former pathway being dependent on tissue factor as cofactor for FVIIa, and the latter being accelerated by a cofactor function of lipolysed triglyceride-rich particles for factor IX. Mixed brain phospholipids support the in vitro activation of factor VII by factor IXa in the presence of calcium,[47,57] but no activation occurred when mixed brain phospholipids were replaced by plasma lipoproteins.[47] However, lipoproteins appear to lack activating properties until exposed to lipoprotein lipase.[28] Precisely how lipolysis could generate cofactor activity for the activation of factor VII by factor IXa is unknown. Another possibility might be that changes in the surface properties of lipoproteins or endothelial cells accompanying lipolysis augment the autoactivation of prekallikrein. Further understanding could perhaps be provided by using markers of factor XII, kallikrein and factor IX activation[36,56,58] and by examination of the responses of the coagulation pathway to fat-rich meals in factor VIII and prekallikrein-deficient patients.

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