Postprandial coagulation factor VII activity: the effect of monounsaturated fatty acids

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(*Received 30 April 1996 – Revised 24 July 1996 – Accepted 31 July 1996*)

The present study investigated the effect of monounsaturated fatty acids (MUFA) on postprandial coagulation factor VII activity. Fifteen healthy male volunteers consumed three meals containing equal amounts (40 g) of fat, but providing different proportions of MUFA (12, 17 and 24 % energy) in random order. Fasting and postprandial blood samples were drawn every hour for 9 h. The magnitude of the postprandial triacylglycerolaemic response and the postprandial plasma non-esterified fatty acid (NEFA) concentrations were not significantly different following the three meals. Coagulation factor VII was activated during postprandial triacylglycerolaemia but the area under the curve of postprandial coagulation factor VII activity was not significantly different following the three meals. Regression analysis showed that fasting factor VII activity was the single most important factor affecting postprandial factor VII activity, irrespective of plasma lipid concentrations and meal fat composition. Peak postprandial factor VII activity was attained significantly earlier following the high-MUFA meal compared with the low-MUFA meal (6·33 (SD 2·16) h, 3·60 (SD 1·81) h respectively; $P = 0{\cdot}016$). Regression analysis showed that meal MUFA content was the primary determinant of time to peak postprandial factor VII activity. Although the magnitude of postprandial coagulation factor VII activity was not affected by meal MUFA content, peak postprandial factor VII activity occurred earlier and fasting activity levels were quickly restored following the high-MUFA meal. A short-lived increase in factor VII activity may be more beneficial than a prolonged thrombotic response.

Dietary fatty acids: Factor VII activity: Postprandial triacylglycerolaemia

Postprandial triacylglycerolaemia has been identified as an important factor in relation to CHD. It has been demonstrated that the magnitude and duration of postprandial triacylglycerolaemia is positively related to the pathogenesis and progression of atherosclerosis (Patsch *et al.* 1993; Karpe *et al.* 1994; Roche & Gibney, 1995*a*). Plasma triacylglycerol (TAG) concentrations also affect the process of thrombosis. Coagulation factor VII is a vitamin K-dependent plasma protein, the activity of which is positively related to plasma TAG concentration. The Northwick Park Heart Study, a prospective cardiovascular survey, demonstrated a significant and positive relationship between coagulation factor VII activity and CHD mortality (Meade *et al.* 1986, 1993; Ruddock & Meade, 1994). It has also been demonstrated that patients with CHD have high levels of factor VII activity (Orlando *et al.* 1987; Carvalho de Sousa *et al.* 1988).

Coagulation factor VII activity is positively related to plasma TAG concentration (Miller *et al.* 1991) and therefore, following a fat-rich meal, plasma TAG concentration and coagulation factor VII activity both increase. The exact nature of the association between plasma TAG concentration and factor VII activity has not been elucidated but there are two hypotheses which attempt to explain this relationship. First, plasma TAG

concentration could exert an effect on factor VII in terms of mass concentration, whereby the fractional catabolic rate of factor VII may be reduced by its physical binding to TAG-rich lipoproteins (TRL) formed during postprandial lipaemia (Carvalho de Sousa *et al.* 1989). Second, it has been proposed that TRL are capable of converting the inactive single-chain factor VII zymogen to its activated double-chain conformation. Miller *et al.* (1991) demonstrated increased postprandial factor VII activity, which could be related to plasma TAG concentration of the previous postprandial blood sample (160 min earlier). Therefore it was proposed that the catabolism of TRL activated factor VII and it has since been shown that TRL-induced activation of factor VII is associated with reduced concentrations of the single-chain zymogen. This hypothesis is supported by work which demonstrated that lipoprotein lipase (*EC* 3.1.1.34; LPL)-deficient hypertriacylglycerolaemic subjects exhibited normal factor VII activity despite massive hypertriacylglycelolaemia (Mitropoulos *et al.* 1992), while non-LPL-deficient hypertriacylglycerolaemic subjects had elevated factor VII activity (Mitropoulos *et al.* 1989). Treatment of hypertriacylglycerolaemic plasma of LPL-deficient patients with the enzyme LPL led to the stimulation of the contact phase of coagulation. The authors attributed enhanced coagulation to the high concentration of fatty acids which accumulate on the surface of the TRL and endothelial cells when LPL hydrolyses the core of TRL.

These findings support the hypothesis that postprandial TRL enhances postprandial factor VII activity. That being the case, factors which influence the pattern of postprandial triacylglycerolaemia, namely dietary fat composition, could influence postprandial factor VII activity; however, the results of such studies conflict. *In vitro* investigations have shown that micellar long-chain saturated fatty acids (SFA) are potent stimulants of coagulation factor VII activity, while oleic acid and polyunsaturated fatty acids (PUFA) fail to activate factor VII (Mitropoulos *et al.* 1994; Mitropoulos, 1994). Mitropoulos *et al.* (1994) demonstrated that postprandial factor VII activity was significantly greater following a high-SFA diet compared to a high-PUFA diet. Conversely, Tholstrup *et al.* (1994) demonstrated no significant increase in fasting factor VII activity following a diet rich in SFA, which was derived from shea butter, compared with a diet rich in oleic acid derived from high-oleic acid sunflowerseed oil. Furthermore Salomaa *et al.* (1993) showed that fat-rich test meals composed of either *n*-6 PUFA or SFA caused equivalent activation of factor VII.

The present study was designed to extend information relating to dietary fats and coagulation factor VII activity. The acute effect of meals with varying monounsaturated fatty acid (MUFA) and SFA compositions on postprandial factor VII activation has not been studied. The purported effect of dietary fat composition on factor VII activity could be mediated by either altered triacylglycerolaemia or by the different fatty acid composition of plasma lipids following the different test meals. This study investigated the effect of three test meals varying in SFA and MUFA on postprandial factor VII activation and whether this effect was mediated through altered postprandial TAG concentrations or different postprandial fatty acid composition.

METHODS

Study design

This study was approved by the Ethics Committee of the Federated Dublin Voluntary Hospitals. The trial was conducted on an out-patient basis and subjects gave written consent before participation in the trial. Fifteen healthy normolipaemic male volunteers

aged between 18 and 30 years (mean 22·6 (SD 2·85) years), weighing 75·8 (SD 7·5) kg and of normal BMI (23·0 (SD 1·1) kg/m^2) participated in the trial. All subjects were screened to ensure that their habitual dietary fat intake was between 35 and 45 % of energy, with a composition which reflected usual Irish dietary intake (approximately 17 % SFA, 12 % MUFA and 5 % PUFA as energy) and that they did not consume *n*-3 PUFA supplements. All participants were non-smokers and were not being prescribed any medications.

Postprandial investigations

The study was conducted in the Nutrition Laboratory at Trinity College Medical School, St James's Hospital. Each postprandial study began between 07.30 and 08.00 hours following a 12 h overnight fast. All subjects abstained from alcohol and refrained from strenuous exercise for 24 h before the postprandial investigation. During postprandial investigations subjects abstained from food (including chewing gum) and drinks, with the exception of caffeine-free, low-energy (< 4 kJ/ml) drinks, e.g. decaffeinated black coffee, sugar-free drinks and water.

The test meals were freshly prepared on the morning of each postprandial investigation and were well tolerated. Each subject received 135 g white bread, 36 g strawberry jam and a milk shake consisting of 40 g of the test oil, 40 g dried skimmed milk powder (Tesco, Cheshunt, Herts.) and 40 g Nesquik strawberry flavoured milk shake mix (Nestlé, Vevey, Switzerland), mixed with 200 ml water (Évian, France). Subjects were randomly allocated to receive the high-, medium- and low-MUFA test meals once monthly for three consecutive months. The fatty acid compositions of the test meals consumed are presented in Table 1.

Collection of blood samples

A 21-gauge, 32 mm venous catheter (Abbott, Ireland Ltd, Dublin, Ireland) was inserted into the antecubital vein of the non-dominant forearm with the subject in the sitting position. To ensure that the cannula remained patent throughout the 9 h study period it was flushed with sodium citrate (3·8 g/l) in saline solution (9 g NaCl/l) (Phoenix Pharmaceuticals Ltd, Gloucester, Glos). Two fasting blood samples (18 ml taken at −10 and 0 min) were drawn before the test meal was consumed. The test meal was consumed under supervision to ensure that all food was eaten within a 20 min period. Further blood samples were drawn every hour for 9 h, blood was collected in heparinized vacutainers for lipid analysis and in monovettes, containing 0·106 M-sodium citrate, for factor VII analysis. All samples were immediately centrifuged (2500 rev./min; 15 min) at room temperature. The plasma was harvested, vortex-mixed and portioned. Plasma for lipid analysis was immediately frozen

Table 1. *Fatty acid composition of the test meals (including the fat in the bread)*

	g/40 g			% Energy		
Test meal	SFA	MUFA	PUFA	SFA	MUFA	PUFA
Low-MUFA	20·0	14·0	5·7	17·2	12·0	5·0
Medium-MUFA	14·6	20·0	5·4	12·3	17·1	4·6
High-MUFA	6·3	28·3	5·4	5·4	24·1	4·6

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

(−20°) for subsequent analysis. Plasma harvested from the sodium citrate tubes was portioned (0·5 ml), immediately snap frozen in liquid N_2 and stored (−70°) until factor VII activity was investigated.

Biochemical variables investigated

Analysis of plasma TAG (TAG PAP Uni-kit, Roche Diagnostics Ltd, Basel, Switzerland), cholesterol (Cholesterol oxidase Uni-kit, Roche Diagnostics Ltd) and non-esterified fatty acid (NEFA) (NEFA c Kit (acyl-CoA synthetase—acyl-CoA oxidase) method, Wako Chemicals GmbH, Neuss, Germany) concentrations were determined using enzymic colorimetric assays on a Cobas-Bio centrifugal analyser (Cobas Mira, Roche Diagnostics Ltd). The TAG inter-assay CV for 2·55 mmol/l was 5·30 %, the cholesterol inter-assay CV for 7·60 mmol/l was 4·07 % and the NEFA inter-assay CV for 0·769 mmol/l was 6·91 %.

Determination of coagulation factor VII activity

Factor VII activity was determined using two methods. First, factor VII activity was measured in response to rabbit brain thromboplastin (Simplastin Excel S, Organon Technika, North Carolina, USA), factor VIIc(rab). Second, the factor VII activity due to activated proportion of the protein was measured in response to bovine thromboplastin (Stago Diagnostica, Paris, France), factor VIIc(bov). In contrast to the factor VIIc(rab) assay, the factor VIIc(bov) is primarily sensitive to factor VII in its two-chain activated form (Kitchen *et al.* 1992; Marckmann *et al.* 1993). The one-stage chronometric assay was used to determine coagulation factor VII activity in response to both reagents. The Coag-A-Mate Coagulometer (Organon Technika Corp.) was used to measure clotting time. Each plasma sample was diluted (1 : 10, v/v) in imidazole buffer (pH 7·35). A 100 μl portion of diluted subject sample and 100 μl factor VII-deficient plasma were placed in a pre-warmed (37°) well of the coagulometer and incubated at 37° for 2 min. Clotting was then initiated by the addition of 200 μl calcified thromboplastin. All samples were analysed in duplicate. The bovine thromboplastin inter-assay CV for 90·70 (% reference plasma) was 4·84 % and the rabbit thromboplastin inter-assay CV for 101·52 (% reference plasma) was 3·64 %.

The concentration of factor VII antigen (factor VIIag) was determined by an ELISA method using specific rabbit anti-human factor VII antibody (Asserachrom VII : Ag, Stago Diagnostica).

Determination of plasma non-esterified fatty acid composition

The lipid component of 0·5 ml plasma was extracted using the Dole procedure and the NEFA fraction of plasma was isolated from the crude lipid extracts by TLC in a solvent system of petroleum ether (bp 40–80°)–diethyl ether–formic acid (80 : 20 : 2, by vol.), as previously described (Gibney & Daly, 1994). The NEFA fraction was visualized with a 10 g/l solution of 2,7 dichlorofluorescein (in 950 ml/l methanol) under u.v. light and identified by comparison with the NEFA standard, palmitic acid (Sigma P5917, Sigma, Poole, Dorset). NEFA were transesterified using BF_3 (140 ml/l in methanol; Sigma B1127) to allow identification of fatty acids by GLC, with a Shimadzu GC-14A Series Gas Chromatograph (Mason Technologies, Dublin, Ireland), as previously described (Roche & Gibney, 1994).

Statistical analysis

All statistical analyses were completed with the Apple Macintosh-compatible statistical package Data Desk 4.1 (Data Description Inc., New York, USA). Two fasting samples were analysed for all biochemical variables and the mean of these two samples was used for statistical analysis. Plasma TAG and NEFA concentrations and factor VII data were transformed to the natural log (ln) to give the data a normal Gaussian distribution. Repeated measures ANOVA, using meal as the independent variable, investigated changes in the postprandial variations of plasma TAG concentration, coagulation factor VII activity, plasma NEFA concentration and plasma NEFA composition.

The postprandial data were expressed in summary form, i.e. area under the postprandial response curve (AUC), the incremental area under the postprandial response curve (IAUC), maximum postprandial levels (C_{MAX}) and time to maximal postprandial levels (T_{MAX}) were used to investigate between-meal postprandial variations. The AUC were calculated using the trapezium rule, as recommended by Matthews *et al.* (1990). Two-way ANOVA, using subject and meal as the independent variables, was used to investigate significant differences of these summary variables. Post-hoc statistical analysis was completed using the least significance difference, which determines the criterion to identify a significant difference between group means (Snedecor & Cochran, 1989).

RESULTS

Plasma triacylglycerol concentration

Postprandial plasma TAG concentration demonstrated a significant ($P \leq 0.0001$) postprandial repeat effect, concentrations increased significantly 1 h following meal ingestion and returned to fasting concentrations by 9 h. There was no significant difference in the postprandial plasma TAG responses following the three test meals. Table 2 presents the summary variables of the triacylglycerolaemic response; the area under the postprandial plasma TAG response curve (TAG AUC), the incremental area under the postprandial plasma TAG response curve (TAG IAUC), maximum postprandial plasma TAG concentration (TAG C_{MAX}) and time to maximum postprandial plasma TAG concentration (TAG T_{MAX}) were not significantly different between meals. Fasting plasma TAG concentrations were significantly correlated with TAG C_{MAX} (r 0·651; $P \leq 0.0001$) and TAG AUC (r 0·877; $P \leq 0.0001$).

Plasma non-esterified fatty acid concentration

Postprandial plasma NEFA concentrations are presented in Fig. 1. Plasma NEFA concentrations demonstrated a significant ($P \leq 0.0001$) postprandial repeat effect, concentrations were significantly reduced following meal ingestion and returned to near fasting values between 5 and 6 h postprandially. There was no significant difference in the postprandial profile following the three test meals. Figure 2 presents postprandial plasma NEFA total AUC and the IAUC. The NEFA AUC was significantly ($P = 0.0062$) lower following the high-MUFA meal compared with the low-MUFA meal. Fasting plasma NEFA concentration demonstrated a strong positive association with postprandial plasma NEFA AUC (r 0·731; $P \leq 0.0001$), therefore differences in fasting concentrations would have contributed to differences in NEFA AUC. The NEFA IAUC was significantly different between meals ($P = 0.0195$), and decreased with increasing meal MUFA content.

Table 2. *Postprandial plasma triacylglycerol (TAG) variables in subjects consuming low-, medium- and high-monounsaturated fatty acid (MUFA) meals*

(Mean values and standard deviations for fifteen subjects)

Test meal...	Low-MUFA		Medium-MUFA		High-MUFA	
	Mean	SD	Mean	SD	Mean	SD
Fasting TAG (mmol/l)	1·10	0·33	0·99	0·32	1·02	0·40
TAG AUC (mmol/l.9 h)	12·06	3·56	11·79	3·18	12·40	5·02
TAG IAUC (mmol/l.9 h)	2·15	1·84	2·86	1·44	3·24	2·20
TAG C_{MAX} (mmol/l)	1·80	0·54	1·98	0·73	1·91	0·45
TAG T_{MAX} (h)	5·20	2·27	3·93	2·25	5·27	2·37

TAG AUC, area under the postprandial TAG response curve; TAG IAUC, incremental area under the postprandial TAG response curve; TAG C_{max}, maximum postprandial TAG concentration; TAG T_{MAX}, time to maximum postprandial TAG concentration.

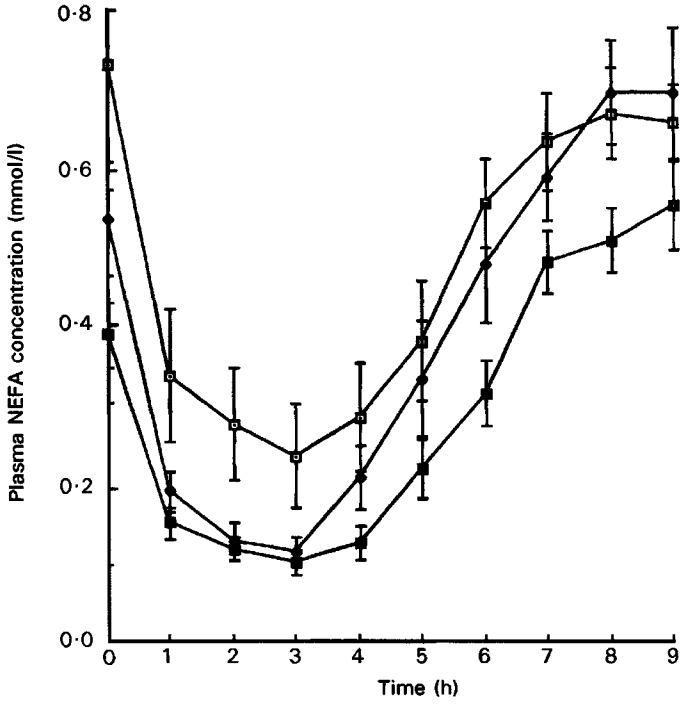

Fig. 1. Postprandial plasma non-esterified fatty acid (NEFA) concentrations in subjects consuming low- (⊡), medium- (◆) and high-monounsaturated fatty acid (■) meals. Values are means for fifteen subjects with their standard errors represented by vertical bars. For details of meals see p. 539 and Table 1.

Coagulation factor VII activity

Total coagulation factor VII activity (in response to rabbit brain thromboplastin; FVIIc (rab)) was not significantly increased during the postprandial state following either meal. The total factor VIIc(rab) AUC values were very similar following the low-, medium- and high-MUFA test meals (939·1 (SD 165·7), 898·41 (SD 191·1) and 930·3 (SD 129·3) respectively). Postprandial factor VII activity, measured in response to bovine

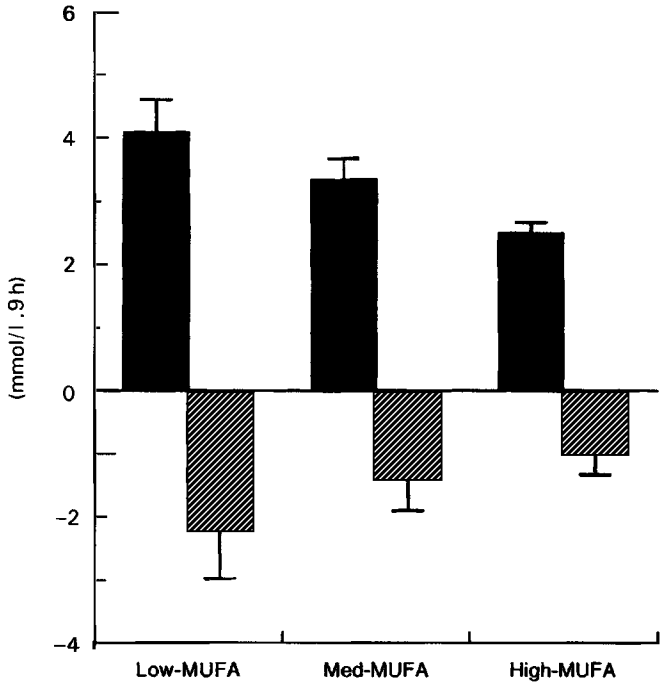

Fig. 2. Areas under the postprandial response curve (■) and incremental areas under the postprandial response curve (▨) for non-esterified fatty acids in subjects consuming low-, medium- and high-monounsaturated fatty acid (MUFA) meals. Values are means for fifteen subjects with their standard errors represented by vertical bars. For details of meals see p. 539 and Table 1.

thromboplastin (factor VIIc(bov)) is presented in Table 3. Activity increased significantly ($P = 0{\cdot}0028$) during postprandial triacylglycerolaemia, although there was no significant difference between meals. Summary variables of postprandial factor VIIc(bov) activity are presented in Table 4; the factor VIIc(bov) AUC and maximum postprandial factor VIIc (bov) activity (Factor VIIc(bov) Max) were not significantly different between meals. Factor VIIc(bov) T_{MAX} was achieved significantly earlier following the high-MUFA meal ($P = 0{\cdot}016$).

Plasma non-esterified fatty acid composition

The concentrations of individual plasma NEFA at 0, 3, 6 and 9 h following the ingestion of the high- and low-MUFA meals are presented in Table 5. The concentration of each fatty acid was significantly ($P \leq 0{\cdot}001$) reduced 3 h following meal ingestion, after which concentrations returned to or exceeded fasting fatty acid concentrations. Lauric (12 : 0) and myristic (14 : 0) acids demonstrated a significant ($P = 0{\cdot}001$) meal × repeat interaction, illustrating that concentrations were significantly increased following the low-MUFA meal but not after the high-MUFA meal. Fasting and postprandial concentrations of palmitic acid (16 : 0) and stearic acid (18 : 0) were significantly greater following the low-MUFA meal compared with the high-MUFA meal. Oleic acid (18 : 1) demonstrated a significant ($P = 0{\cdot}035$) meal × repeat interaction, postprandial oleic acid concentrations exceeded fasting concentrations following the high-MUFA meal.

Table 3. *Postprandial coagulation factor VII activity (% reference plasma), in response to bovine thromboplastin, following the ingestion of three test meals varing in monounsaturated fatty acid (MUFA) composition*

(Mean values and standard deviations for fifteen subjects)

Time (h)...	0	1	2	3	4	5	6	7	8	9
Low-MUFA										
Mean	72·95	73·95	76·59*	77·61*	78·04*	80·16*	78·25*	74·30	76·14	76·90*
SD	16·77	17·51	18·67	15·79	16·26	18·63	18·51	18·14	19·80	14·38
Medium-MUFA										
Mean	68·36	68·59	72·88*	75·64*	72·54*	71·03*	70·29	74·75*	69·31	69·32
SD	17·46	16·68	19·61	22·86	17·45	18·12	20·46	19·70	14·23	16·51
High-MUFA										
Mean	76·71	74·82	83·40*	81·44*	81·12*	79·46	78·40	76·81	78·07	73·34
SD	19·86	19·73	22·59	22·92	19·85	24·00	21·33	20·92	19·80	17·43

*Mean values were significantly different from fasting factor VII reactivity levels, $P < 0.05$.

Table 4. *Postprandial plasma coagulation factor VII activity in response to bovine thromboplastin (factor VIIc(bov)) in subjects consuming low-, medium- and high-mono-unsaturated fatty acid (MUFA) meals*

(Mean values and standard deviations for fifteen subjects)

Test meal...	Low-MUFA		Medium-MUFA		High-MUFA	
	Mean	SD	Mean	SD	Mean	SD
Factor VIIc(bov) AUC (% reference plasma .8 h)	686·8	155·9	646·1	147·3	708·5	176·6
Factor VIIc(bov) Max (% reference plasma)	85·3	18·8	85·6	21·5	88·0	21·4
Factor VIIc T_{MAX} (h)	6·33	2·16	5·13	2·20	3·60**	1·81

AUC, area under the postprandial factor VIIc(bov) response curve; Factor VIIc(bov) Max, maximum postprandial factor VIIc(bov) activity; Factor VIIc(bov) T_{MAX}, time to maximum factor VIIc(bov) activity.
**Mean value was significantly different from those for low- and medium-MUFA meals, $P = 0.016$.

Correlation and regression analysis

The association of coagulation factor VII with age, body weight, BMI and plasma lipid concentrations was investigated; the significant correlations are shown in Table 6. Neither concentration of factor VII antigen nor factor VII activity was related to age, body weight, BMI or plasma NEFA concentration. The concentration of factor VII antigen was positively associated with fasting plasma cholesterol concentration and maximum plasma TAG concentration. Fasting and postprandial plasma TAG concentrations were positively correlated with both indices of factor VII activity in the fed and fasted states.

Stepwise multiple regression analysis was completed on factor VIIc(bov) AUC to determine the factors which affected the magnitude of the response. These analyses showed that fasting levels of factor VIIc(bov) activity (β 6·51; $P \leq 0.0001$) and fasting total factor VIIc(rab) activity (β 4·73; $P \leq 0.0001$) were most important factors in the regression analysis of postprandial factor VIIc(rab) AUC (r^2 0·912; $P \leq 0.0001$). Stepwise multiple regression analysis investigating the effect of plasma lipid concentrations on fasting total factor VIIc(rab) activity showed that fasting plasma TAG concentrations (β 24·17;

Table 5. *Postprandial plasma non-esterified fatty acid concentrations (μmol/l) following the ingestion of low- and high-MUFA meals*

(Mean values with their standard errors for fifteen subjects)

		Low-MUFA meal				High-MUFA meal				
Time (h)...		0	3	6	9	0	3	6	9	LSD
Fatty acid										
12:0	Mean	6·14	4·18*	12·65*	7·70	2·97†	1·23†	2·48†	3·15†	2·63
	SE	1·90	1·08	2·52	1·23	0·43	0·19	0·75	0·74	
14:0	Mean	20·24	7·66*	21·10	22·56	9·12†	2·48*†	7·38†	9·56†	4·90
	SE	5·01	2·21	1·90	4·02	1·13	0·44	1·35	1·07	
16:0	Mean	202·18	64·66*	153·42*	173·39	94·40†	24·71*†	76·55†	129·22†	35·51
	SE	43·72	18·29	16·14	13·73	8·31	4·45	12·44	13·79	
18:0	Mean	89·32	31·51*	68·05*	71·57	53·85†	15·41*	38·85†	52·96	19·86
	SE	23·06	10·12	8·92	7·76	10·19	3·98	6·94	6·60	
16:1	Mean	30·47	6·97*	13·08*	25·13	12·85†	2·78*	9·33	18·17	7·03
	SE	7·87	1·74	1·89	2·88	2·25	0·68	1·74	2·38	
18:1	Mean	263·86	65·74*	179·68*	241·80	133·16†	31·61*	122·74†	245·88*	56·54
	SE	56·34	18·39	21·90	18·59	34·38	6·79	18·80	26·99	
18:2*n*-6	Mean	95·43	27·84*	63·04	81·06	47·86†	14·46*	30·60†	65·21	20·28
	SE	24·64	8·14	16·28	7·00	6·15	3·73	4·37	8·13	

LSD, least significant difference.
*Mean values were significantly different from fasting values, $P \leq 0{\cdot}05$.
†Mean values were significantly different from those for the low-MUFA meal, $P \leq 0{\cdot}05$.

Table 6. *Pearson-product moment correlations of plasma lipid concentrations with indices of postprandial plasma coagulation factor VII activity in response to bovine thromboplastin (FVIIc(bov)) and rabbit thromboplastin (FVIIc(rab)) and the concentration of the antigen (FVIIag)*

	FVIIag	FVIIc(bov)			FVIIc(rab)		
	Fasting	Fasting	AUC	Peak	Fasting	AUC	Peak
TAG 0	0·35	0·53*	0·55*	0·58*	0·72**	0·71**	0·71**
TAG AUC	0·42	0·64*	0·69**	0·70**	0·77***	0·81***	0·80***
TAG IAUC	0·36	0·56*	0·65**	0·62**	0·51*	0·66**	0·60**
TAG C_{MAX}	0·53*	0·52*	0·59*	0·64**	0·70**	0·74***	0·74***
NEFA 0	0·05	−0·50*	−0·38	−0·21	−0·14	−0·15	−0·08
NEFA AUC	0·40	−0·30	−0·15	−0·04	0·07	0·07	0·16
NEFA IAUC	0·18	0·53*	0·44	0·29	0·25	0·25	0·18
TC 0	0·54*	0·47	0·51	0·54*	0·45	0·51*	0·50
TC AUC	0·41	0·39	0·48	0·56	0·38	0·49	0·47

TAG, triacylglycerol; NEFA; non-esterified fatty acids; TC, total cholesterol; AUC, area under the response curve; IAUC, incremental AUC; C_{MAX}, maximum postprandial concentration.
*$P \leq 0{\cdot}05$; **$P \leq 0{\cdot}01$; ***$P \leq 0{\cdot}001$.

$P = 0{\cdot}0003$) and postprandial NEFA AUC (β −3·19; $P = 0{\cdot}045$) were the significant factors in the regression model (r^2 0·454; $P \leq 0{\cdot}0046$).

Time to peak postprandial factor VIIc(bov) activity was the only variable which showed a significant difference between meals; maximum postprandial factor VIIc(bov) activity occurred significantly earlier after the high-MUFA meal than after the low-MUFA meal. The stepwise multiple regression model (r^2 0·596; $P \leq 0{\cdot}0001$) identified meal MUFA content as the most important factor (β −0·625; $P = 0{\cdot}006$), the higher the meal

MUFA content the earlier the increase in postprandial factor VIIc(bov) activity. This regression model also showed that the changes in postprandial plasma NEFA stearic acid ($\beta -0{\cdot}472$; $P=0{\cdot}002$) and oleic acid ($\beta 0{\cdot}172$; $P=0{\cdot}016$) concentrations were then the next most important variables which determined time to peak factor VIIc(bov) activity. The greater the reduction in postprandial plasma stearic acid concentration and the greater the increase in postprandial oleic acid concentration the earlier peak factor VIIc(bov) activity was achieved, thus explaining the earlier rise in factor VIIc(bov) activity following the high-MUFA meal.

DISCUSSION

The results of the present study confirm that coagulation factor VII is activated during postprandial triacylglycerolaemia. Furthermore the data show that altering the SFA : MUFA ratio of an acute test meal to reflect habitual dietary fat intakes of Northern and Southern Europe (Gregory *et al.* 1990; Kafatos & Mamalakis, 1993) does not influence the magnitude of the postprandial coagulation factor VII activity. Peak postprandial coagulation factor VIIc(bov) activity occurred significantly earlier after the high-MUFA meal, while a later and prolonged increase in coagulation factor VIIc(bov) activity followed the low-MUFA meal.

The literature relating to the effect of dietary fat composition on postprandial coagulation factor VII activity is confusing and the relevant studies need to be considered in terms of their study design. Mitropoulos *et al.* (1994) investigated the effect of isoenergetic high-SFA (62 % of energy), high-PUFA (62 % of energy) and low-fat diets (15 % of energy) on postprandial factor VII activity. Mean average coagulation factor VII activity was significantly higher following the high-SFA and the high-PUFA diets than following the low-fat diet (92·4, 85·8 and 79·3 % respectively). Lower factor VII activity following low-fat dietary treatment is in accordance with other studies (Miller *et al.* 1986; Marckmann *et al.* 1993; Roche & Gibney,1995*b*), which have also shown lower factor VII activity following low-fat dietary treatment. Mitropoulos *et al.* (1994) attributed the greater postprandial factor VII activity following the SFA-rich diet compared with the PUFA-rich diet to greater levels of plasma NEFA stearic acid derived from the high-SFA meal, compared with the high-PUFA meal (16·6 and 5·1 g/d respectively). This hypothesis is supported by *in vitro* investigations which showed that micelles rich in long-chain SFA were potent pro-coagulant agents. However, as in the present study, other investigators have shown no effect of test-meal fat composition on postprandial factor VII activity. Miller *et al.* (1991) demonstrated that postprandial factor VII activity was not significantly different when nine healthy adults consumed two 7 d diets of standardized energy and total fat content, but with high and low dietary PUFA : SFA ratios ($<0{\cdot}3$ and $>3{\cdot}0$ respectively). Salomaa *et al.* (1993) showed that while postprandial factor VII was reduced following a high-carbohydrate, fat-free meal, it was activated to a similar extent by an *n*-6 PUFA-rich meal and a SFA-rich meal.

There are three possible reasons why one study showed that the SFA-rich test meal had a more pro-coagulant effect than the PUFA test meal, while other studies (including the present study) have failed to demonstrate such an effect. First, in the present study, and in that conducted by Salomaa *et al.* (1993), only the acute effect of dietary fat composition was investigated, whereas Mitropolous *et al.* (1994) investigated postprandial coagulation factor VII activity when individuals had been adhering to background diets providing a very high level of dietary energy derived from fat (62 %). This chronic dietary intervention could have primed the coagulation system. The concentration of the factor VII antigen was

significantly greater following the SFA-rich diet than after the PUFA-rich diet. If more of the protein is present, as the result of dietary intervention, more activation can occur, therefore allowing increased activation of factor VII during the postprandial state.

Second, the quantity of fat used in the test meals by Mitropoulos *et al.* (1994) was much greater than that used in the present study (122 g *v.* 40 g). The quantity and composition of fats used in the present study were carefully chosen. The quantity represented the average amount of fat consumed by adult males in their main meal (40 g fat). The high-, medium-, and low-MUFA meals had fatty acid compositions similar to those consumed as part of the traditional Cretan diet, an Athenian diet and a typical UK or Irish diet (24, 17 and 12% of energy from MUFA respectively). It is possible that an excessive fat load stressed the thrombotic process and induced the differences between meals, which if usual amounts of fat had been consumed would not have been evident.

The final explanation of the absence of a difference between the high- and low-MUFA meals which provided low and high amounts of SFA relates to the hydrolysis of TRL. Zampelas *et al.* (1994) demonstrated that there was an inverse association between the degree of unsaturation of fatty acids and the magnitude of postprandial lipaemia. The postprandial triacylglycerolaemic response was greatest following a SFA meal, less following an *n*-6 PUFA meal and least following an *n*-3 PUFA meal. Zampelas *et al.* (1994) proposed that the more unsaturated the fatty acid the more the efficient is the hydrolysis of the postprandial plasma TAG. Therefore the SFA meal provided by Mitropoulos *et al.* (1994) would have led to greater concentrations of postprandial TRL which would cause greater activation of factor VII. A previous study demonstrated that total plasma TAG concentrations were not significantly different following an olive oil test meal compared with a soyabean oil test meal (De Bruin *et al.* 1993). Similarly, this study showed no effect of altered meal MUFA content on postprandial triacylglycerolaemia. TRL metabolism was not affected by varying proportions of MUFA, therefore TRL had a similar pro-coagulant effect on factor VII following the three meals and there was no difference in postprandial factor VII activity.

None of the previous studies investigated the extent to which postprandial factor VII activation is affected by baseline variables. Multiple regression analysis clearly showed that fasting levels of factor VII activity were the single most important determinant of the AUC of postprandial coagulation factor VII, and fasting total factor VII activity was in turn principally determined by fasting plasma TAG concentrations. These analyses highlight the need to control these variables when studying postprandial coagulation factor VII activity. Therefore the postprandial changes observed by Mitropoulos *et al.* (1994) may have been due to the chronic effect of a SFA diet which increased the concentration of the factor VII antigen, thereby promoting more factor VII activation.

The present study showed that the time course of factor VII activity was significantly affected by meal MUFA content; peak postprandial factor VII activity occurred significantly earlier following the high-MUFA meal compared with the low-MUFA meal. When specific fatty acids were examined it was shown that the greater the decrease in concentration of postprandial plasma NEFA stearic acid and the greater the increase in concentration of postprandial plasma NEFA oleic acid the earlier peak postprandial factor VII activity occurred. During postprandial lipaemia the NEFA fraction assumes the fatty acid composition of the test meal (Gibney & Daly, 1994), therefore it may be that the profile of postprandial factor VII activity is affected by meal fat composition. However, it is difficult to know the physiological significance of this effect; the factor VII activity AUC was not affected by the meal MUFA content but factor VII activity increased to a greater level during early postprandial lipaemia and rapidly returned to fasting levels following the

high-MUFA meal, compared with a prolonged increase in coagulation factor VII activity following the low-MUFA, high-SFA meal. The physiological significance of this effect must be determined as it is not clear whether a prolonged thrombotic response has a more adverse effect than a short, sharp increase in factor VII activity which quickly returns to fasting values.

This work was funded by AIR2.CT93.1430. We thank the School of Biological Sciences, University of Surrey, who completed analysis of plasma TAG and NEFA concentrations.

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