

## Postprandial lipid metabolism and thrombosis

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The atherosclerotic basis of CHD and the pathogenic roles in this condition played by disturbances of lipoprotein metabolism, high blood pressure and cigarette smoking are well known (Grundy *et al.* 1987). In addition, the major cause of sudden cardiac death (Davies & Thomas, 1984), acute myocardial infarction (De Wood *et al.* 1980), unstable angina pectoris (Fuster & Chesebro, 1986) and silent myocardial ischaemia (Gurfinkel *et al.* 1994) is recognized to be the formation of thrombus at sites where the endothelium overlying atheromatous plaques has sloughed or fissured (Davies, 1996). The resultant contact of circulating blood with atheromatous material permits the formation of complexes of the plasma protein factor VII with its co-factor, tissue factor, present in abundance in the core of the plaque (Wilcox *et al.* 1989). These complexes are powerfully pro-coagulant, and their rapid formation in high local concentration overwhelms the inhibitory mechanisms which normally maintain the coagulant pathway at a basal level of activity (Bauer & Rosenberg, 1987). An amplifying cascade of linked proteolytic reactions is triggered, culminating in the generation of thrombin in sufficient quantity to produce the haemostatic mass of fibrin and aggregated platelets which is the thrombus (Macfarlane, 1964).

Factor VII exists in plasma mainly as a single-chain zymogen (FVII) but also in trace quantity as the derivative two-chain enzyme, activated factor VII (FVIIa; Wildgoose *et al.* 1992; Morrissey *et al.* 1993). It is the FVIIa–tissue factor complex that is responsible for the initiation of coagulation and, a priori, the magnitude of the thrombotic response to plaque disruption is likely to depend in part on the concentration of circulating FVIIa at that instant. This is the rationale of warfarin anticoagulant therapy which is known to reduce the risk of thrombosis in CHD (International Anticoagulant Review Group, 1970; Chalmers *et al.* 1977). Warfarin acts by suppressing the activity level of factor VII and other vitamin K-dependent clotting factors. On this account, the discovery of a high factor VII coagulant activity (FVIIc) in men at high risk for fatal CHD is of particular relevance (Heinrich *et al.* 1994; Ruddock & Meade, 1994). Plasma FVIIc is a function of the concentrations of FVIIa and FVII (Miller *et al.* 1994), and the question immediately arises as to the causes of an elevated factor VII, months or even years before the onset of an acute coronary event.

### POSTPRANDIAL LIPAEMIA AND FACTOR VII

The consistent epidemiological and clinical associations between hypertriacylglycerolaemia and an increased FVIIc (Constantino *et al.* 1977; Fuller *et al.* 1979; Stirling *et al.* 1984; Miller *et al.* 1985; Bruckert *et al.* 1989; Mitropoulos *et al.* 1989), coupled with early evidence for a reduction in FVIIc with successful treatment of hypertriacylglycerolaemia (Elkeles *et al.* 1980; Simpson *et al.* 1983), led to an examination of the effects on factor VII of the manipulation of plasma triacylglycerol concentration by dietary means.

In an earlier study, six healthy adults consumed two experimental diets in random order, one containing about 13% of energy as fat and taken for 2 weeks, the other containing 62% of energy as fat and taken for 3 weeks (Miller *et al.* 1986). Fasting blood

Table 1. *Blood lipids and clotting factors during two dietary regimens* (From Miller *et al.* 1986)

	Low-fat	High-fat	Difference between regimens		Statistical significance of difference: <i>P</i>
			Mean	SE	
Energy (kJ/d)	9339	11959	2619	578	< 0.01
Fat (% energy)	12.8	62.1			
Serum cholesterol (mmol/l)	4.24	4.70	0.46	0.18	0.05
Serum fasting triacylglycerol (mmol/l)	1.21	0.83	-0.38	0.12	0.03
Factor VII coagulant activity (% standard)	81.7	97.5	15.8	4.8	0.02
Prothrombin (% standard)	96.4	96.2	-0.2	0.8	NS
Factor X (% standard)	88.7	86.7	-0.2	2.1	NS
Fibrinogen (g/l)	3.1	3.0	0.1	0.2	NS

samples were taken before breakfast at 2 or 3 d intervals, and assayed for FVIIc, prothrombin, factor X activity, fibrinogen concentration and lipid concentrations. The mean averages of these results are presented in Table 1, showing that whereas fasting triacylglycerol concentration was higher during the low-fat regimen than in the high-fat period, FVIIc was lower on the low-fat diet. These results indicated that the positive relationship between FVIIc and triacylglycerol concentration in cross-sectional community studies was not due to any simple binding of factor VII to VLDL. Instead, FVIIc was significantly related to the fat content of the diet. Dietary energy intake was also increased during the high-fat regimen, although the excess was completely accounted for by fat. However, a second study (Miller *et al.* 1986; Table 2) showed that a 50% increase in dietary energy had no effect on FVIIc when provided as carbohydrate, but did have a significant effect when in the form of fat.

Equally importantly, fasting FVIIc immediately before breakfast was found to be positively correlated with the amount of fat consumed during the previous 24 h ( $r$  0.52,  $P$  < 0.01), while the second study showed that this effect was discernible within 8 h of commencement of a fat-enriched diet (Table 2). Subsequent studies have demonstrated a postprandial elevation in FVIIc within 3 h of consumption of a fat-rich test meal (Miller *et al.* 1991).

Marckmann *et al.* (1993) gave four healthy male adults four isoenergetic diets of different fat (20% or 50% of energy) and fibre (2 or 4 g/MJ) levels, each for 2 d. The high-fat diets were associated with much higher postprandial peak FVIIc levels than the low-fat

Table 2. *The effects of dietary fat and carbohydrate enrichment on factor VII* (From Miller *et al.* 1986)

	Usual diet	Fat-enriched diet	Carbohydrate-enriched diet
Total energy (kJ)	7222	10854	10925
Fat (kJ)	2377	5904	2473
Carbohydrate (kJ)	3594	3636	6958
Factor VII activity (% standard):			
At 17.00 hours	101	111*	103
In fasting sample following morning	98	106*	99

Mean values were significantly different from those for usual diet (paired  $t$  test): \*  $P$  = 0.02.

diets, whereas fibre content had no effect on FVIIc whatsoever. These subjects, aged 21–30 years, cleared a fat load very efficiently, such that the postprandial and 24 h mean triacylglycerol concentrations were not significantly influenced by the dietary fat intake. The investigators, therefore, concluded that the magnitude of the postprandial increase in FVIIc was probably determined primarily by the mass of dietary triacylglycerol transported in chylomicrons and VLDL rather than the postprandial change in triacylglycerol concentration. Marckmann *et al.* (1993) also estimated the levels of FVIIa using a variant of the factor VII bioassay (employing bovine tissue factor rather than human tissue factor) and FVII as factor VII antigen (FVIIag) by ELISA. They demonstrated that the postprandial increase in FVIIc was due entirely to activation of factor VII with a rise in FVIIa. This result was subsequently confirmed in a larger study (Bladbjerg *et al.* 1994), and accorded with the findings of Miller *et al.* (1991).

Silveira *et al.* (1994) followed the postprandial responses to a standard test meal (containing olive oil, 50 g/m<sup>2</sup> body surface area) in thirty-three patients who had survived a myocardial infarction and ten healthy controls, the mean ages of both groups being 49 years. Substantial activation of factor VII was observed within 3 h of the oral fat load, but unlike in the younger subjects of Marckmann *et al.* (1993), peak factor VII activation was positively associated with rise in plasma triacylglycerol concentration. Hypertriacylglycerolaemic patients were found to have lower levels of fasting and postprandial FVIIa than the healthy controls, despite their higher triacylglycerol concentrations. Together, the findings of Marckmann *et al.* (1993) and Silveira *et al.* (1994) can be reconciled if factor VII activation is determined both by the rate of lipolysis of triacylglycerol and the rate at which the products of lipolysis are cleared from the circulation.

The importance of lipoprotein lipase (EC 3.1.1.34; LPL) activity for factor VII is emphasized by a study of familial complete LPL deficiency (Mitropoulos *et al.* 1992). Despite their massive hypertriacylglycerolaemia, both FVIIc and FVIIag were normal in patients with this condition. How the magnitude of lipolysis and turnover of remnant particles might influence factor VII is not fully understood, but one possible explanation relates to the creation of a negatively-charged surface on triacylglycerol-rich lipoproteins which then activates the contact system of coagulation. Activation of this system depends on the interactions of factor XII, prekallikrein and high-molecular-mass kininogen with a suitable surface, on which factor XII is converted to the enzyme factor XIIa and prekallikrein is converted to kallikrein. Triacylglycerol-rich lipoproteins will provide such a surface when treated with LPL (Mitropoulos *et al.* 1992; McNally *et al.* 1996). The products of contact activation initiate a series of linked reactions involving factor XI, which lead to the activation of factor IX. Both activated factor XII and activated factor IX will convert FVII to FVIIa. This concept is supported by *in vitro* experiments which have demonstrated activation of factor VII following incubation of whole plasma with lipoprotein remnants produced by pre-incubation of VLDL with LPL (Mitropoulos *et al.* 1993). However, studies in patients with inherited deficiencies of clotting factors indicate that the previously described sequence of events cannot account entirely for the postprandial responses of FVIIc (Miller *et al.* 1996). Patients with complete deficiency of either factor XII or factor XI show a normal postprandial response of factor VII to fatty meals, thus excluding obligatory roles for these clotting factors in the process. However, patients with complete factor IX deficiency fail to show any change in FVIIc or FVIIa after a fat-rich meal, although a normal response is restored following factor IX-replacement therapy. Speculatively, perhaps a contact surface on triacylglycerol-rich particles and their remnants also leads to activation of factor IX and, hence, factor VII by pathways not involving factor XII and factor XI.

## POSTPRANDIAL FACTOR VII AND DIETARY FAT COMPOSITION

Activation of factor VII during alimentary lipaemia takes place whatever the fatty acid composition of the meal. In an early study (Miller *et al.* 1991), nine healthy adults consumed two diets of standardized energy and total fat content in random order, each for 7 d. In one diet polyunsaturated : saturated fatty acids (P : S) was less than 0.3, and in the other greater than 3.0. No difference in FVIIc or FVIIag was observed on the final days of the two regimens, either fasting or postprandially. Salomaa *et al.* (1993) gave ten middle-aged men two isoenergetic test meals each containing 1 g fat/kg body weight. In one meal the fat was supplied as cream and in the other as sunflower oil. Similar increases in FVIIc were observed after the two meals. T. De Grassi, T. A. B. Sanders and G. J. Miller (unpublished results) observed the effects of five different test meals of standardized energy, total fat, protein and fibre content on postprandial lipaemia and factor VII in sixteen adults. The meals were rich respectively in fatty acids 16:0, 18:0, 18:1n-9 *cis*, 18:1n-9 *trans*, and 8:0 + 10:0. There was a striking positive relationship between the magnitude of the postprandial lipaemia and the peak level of FVIIc after the meal. The high-18:1n-9 meal induced the greatest increases in plasma triacylglycerol concentration ( $P < 0.05$ , compared with 18:0) and FVIIc, whereas the 8:0 + 10:0 meal induced no significant change in either variable from its fasting level. In seemingly excluding any appreciable effect of fatty acid composition *per se* on factor VII, these results supported those of Silveira *et al.* (1994) in emphasizing a relationship between peak factor VII activation and the increase in plasma triacylglycerol concentration postprandially. A similar dose-response relationship between the fat content of the test meal and the postprandial rise in FVIIa has been described by Sanders *et al.* (1996). The relationship is not invariant, however. When Yahia & Sanders (1996) replaced 15 g olive oil with 15 g fish oil (MaxEPA; Seven Seas, Hull) in a 90 g olive oil test meal, a marked reduction in postprandial lipaemia occurred without any change in the postprandial increase in FVIIc.

## POSTPRANDIAL LIPAEMIA AND THROMBOSIS

A circadian rhythm to acute myocardial infarction and sudden cardiac death is well recognized, with peak rates of onset in the early morning and (to a lesser extent) in the late afternoon (Gnecchi-Ruscone *et al.* 1994; Muller *et al.* 1994). The explanation for this rhythm is uncertain, but a concordant diurnal variation in risk factors that have a role in plaque disruption or the strength of the thrombotic response to this disruption is one possibility. The most striking feature of this diurnal variation in the onset of an acute coronary episode is the rapid climb from a nadir at about 02.00 hours to a peak incidence about 6 h later at 08.00 hours. Morning increases in heart rate, blood pressure, platelet aggregability and suppression of fibrinolytic activity have been proposed as possible factors, but to this list could be added activation of factor VII following a heavy meal the previous evening. As mentioned previously, the magnitude of the thrombotic response to plaque disruption is likely to depend in part on the concentration of circulating FVIIa at that instant. This concentration will depend on the time and fat content of the previous meal. Current dietary advice for the prevention of CHD, which includes a reduction in the fat content of the diet, therefore, may benefit the patient not only by reducing plasma cholesterol levels but also by improving the coagulant status of the blood.

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