

## Postgraduate Symposium

# Dietary monounsaturated fatty acids and haemostasis

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Diets high in monounsaturated fatty acids (MUFA) are increasingly being recommended as a highly-effective cholesterol-lowering strategy in populations at risk of CHD. However, the need for a re-appraisal of the benefits of diets rich in MUFA became apparent as a result of recent studies showing that meals high in olive oil cause greater postprandial activation of blood coagulation factor VII than meals rich in saturated fatty acids. The present review evaluates the evidence for the effects of MUFA-rich diets on fasting and postprandial measurements of haemostasis, and describes data from a recently-completed long-term controlled dietary intervention study. The data show that a background diet high in MUFA has no adverse effect on fasting haemostatic variables and decreases the postprandial activation of factor VII in response to a standard fat-containing meal. Since the same study also showed a significant reduction in the *ex vivo* activation of platelets in subjects on the high-MUFA diet, the overall findings suggest that there is no reason for concern regarding adverse haemostatic consequences of high-MUFA diets.

### Monounsaturated fatty acids: Haemostasis: Factor VII: Platelet aggregation: Saturated fatty acids

The haemostatic system has received considerable attention in recent years, not least because the evidence suggests a role for thrombotic events in the pathogenesis of cardiovascular disease. The demonstration of fibrin and platelet components within atherosclerotic plaques (Pearson *et al.* 1997) supports the idea that subclinical coronary thrombi contribute to the growth of the atheromatous plaque. Associations between atherosclerosis and thrombosis in patients with acute vascular occlusive events, e.g. acute myocardial infarction and unstable angina, have also been established (Fitzgerald & Barry, 1997). Furthermore, elevated fibrinogen concentrations (Meade *et al.* 1986; Ernst & Resch, 1993; Heinrich *et al.* 1994; Thompson *et al.* 1995; Mann, 1997), factor VII (FVII) coagulant activity (FVIIc; Meade *et al.* 1986; Heinrich *et al.* 1994; Ruddock & Meade, 1994; Junker *et al.* 1997), platelet aggregability (Meade *et al.* 1985; Trip *et al.* 1990; Elwood *et al.* 1991) and plasminogen-activator inhibitor-1 (PAI-1) levels (Hamsten *et al.* 1987) have been shown to be positively associated with CHD, although some of these studies were retrospective in design. Consequently, many investigators have addressed whether these and other haemostatic factors

are modifiable by dietary means. The aim of the present review is to summarise the evidence for effects of monounsaturated fatty acids (MUFA) on platelet aggregation and fibrinogen, and also on fasting and postprandial levels of FVII. Their role in coagulation will be briefly described, before discussing the specific studies that have investigated the effects of MUFA on these variables.

### Haemostasis

The haemostatic system is designed to maintain the integrity of the vasculature. This system maintains blood in the fluid state by controlling bleeding and playing a role in tissue repair. Vascular patency depends on the balance of a number of complex interrelated systems, i.e. the endothelium and its subjacent structures, the platelets, the coagulation and fibrinolytic systems, their plasma inhibitors, the deformation and flow characteristics of blood within the circulation, and vascular tone (Wessler & Gitel, 1984). Although the following description of haemostasis considers these events as separate entities, and therefore they appear to occur sequentially, this is not the case. In fact,

**Abbreviations:** a, Enzyme product of the zymogen precursor protein; FVII, blood coagulation factor VII; FVIIag, factor VII antigen; FVIIc, factor VII coagulant activity; MUFA, monounsaturated fatty acids; PAI-1, plasminogen-activator inhibitor-1; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TAG, triacylglycerols; t-PA, tissue plasminogen activator.

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tissue damage activates both platelets and the coagulation cascade simultaneously.

### Platelet aggregation

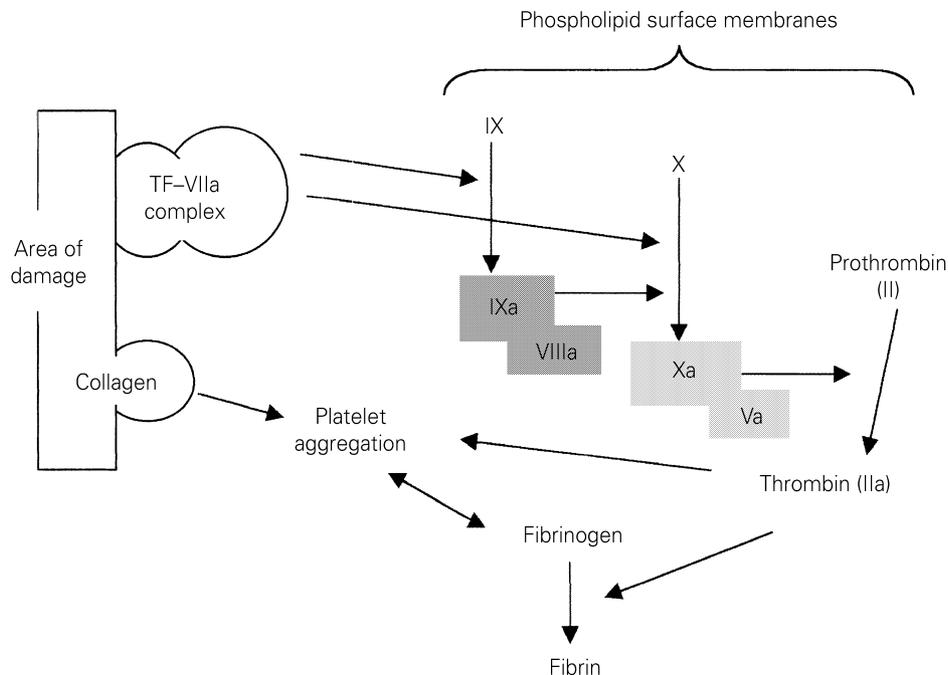
Platelets are small cell fragments that are produced by the disintegration of megakaryocytes, the large precursor cells found in bone marrow (Babior & Stossel, 1990a). The primary role of platelets is maintenance of haemostatic integrity of the blood vessels and cessation of bleeding after injury. Platelets circulate passively and do not adhere to intact endothelium because of the non-thrombogenic property of its surface coupled with its ability to produce prostacyclin and heparin, both of which are anti-thrombotic (Needleman *et al.* 1979). However, in response to vessel wall injury or exposure to foreign surfaces platelets rapidly undergo the process of adhesion, shape change, secretion and aggregation. After adsorption to the damaged site platelets release many of their cellular constituents into the ambient blood, making available the contents of their alpha and dense granules, as well as lipid mediators (e.g. thromboxane  $A_2$ ), and pro-coagulant receptors on the platelet surface. This process continues until the constricted lumen of the small vessel is completely occluded by a loose aggregate of platelets. This aggregate is transformed into a more definite plug by the activation of the blood clotting mechanism.

### Coagulation cascade

The coagulation of plasma is triggered when blood comes into contact with a pro-coagulant surface. In response, an ordered series of proteolytic transformations follows, in which the constituent zymogens of the coagulation pathway are converted to active enzymes in a simple waterfall sequence, as described by Davie & Ratnoff (1964) and Macfarlane (1964). This process culminates in the generation of thrombin, the enzyme that converts fibrinogen to fibrin, as well as activating platelets. By convention, each clotting factor or zymogen (inactive form of the protein digestive enzymes) of the coagulation pathway is given a roman numeral, and its activated form is denoted by 'a'.

There are two major pathways by which prothrombin (factor II) is converted to thrombin, the so-called intrinsic and the extrinsic. For simplicity only the extrinsic pathway will be discussed (Fig. 1), recognising that both pathways interact with each other and converge at the level of factor X activation. For an in-depth review of the coagulation process, see Miller (1993), Babior & Stossel (1990b), Zur & Nemerson (1987) and Prydz (1987).

The extrinsic pathway is activated when blood comes into contact with cell membranes that contain a specific protein termed tissue factor or thromboplastin. Tissue factor is found in many types of tissue, although some (e.g. brain, lung and placenta) display much greater specific activity than others (e.g. kidney, spleen; Zur & Nemerson, 1987).



**Fig. 1.** A simple schematic representation of the extrinsic pathway of coagulation, illustrating the involvement of platelets. The extrinsic pathway is activated when blood comes into contact with cell membranes with exposed tissue factor (TF). Factor VII is activated (VIIa) in the presence of factors XIIa, IXa, Xa and thrombin (where a denotes the enzyme product of the zymogen precursor protein). The sequential proteolytic transformations are dependent on phospholipids and take place on cell membrane surfaces provided by activated platelets, endothelial cells or leucocytes. Platelets are attracted to many proteins in the vessel wall, including collagen. Once platelets are activated they release many cellular constituents including fibrinogen. (■, □) Factor X-converting complex and prothrombin-converting complex respectively.

When these cells are damaged their tissue factor is exposed to FVII in the blood, and once the tissue factor–FVIIa complex forms it can convert factor X to factor Xa with considerable efficiency. Activated factor X associates with factor V, plus Ca and phospholipid, to form the prothrombin-converting complex that generates thrombin from prothrombin. Like factor Xa, thrombin is a protease that converts factors V and VIII into forms that are much more potent than the native circulating factors. Thrombin has numerous substrates in the biological milieu, including fibrinogen, factor XIII and platelets, all of which are involved in clot formation. It also converts plasminogen to plasmin and activates protein C; components that are involved in clot lysis.

#### *Regulation of clotting and fibrinolysis*

The elimination of activated clotting factors (by the liver) and the destruction of the fibrin clot are two important processes for maintenance of haemostasis. Tissue factor pathway inhibitor, proposed to be the major regulator of the initiating events of the blood coagulation system, forms a quaternary complex with FVIIa, tissue factor and factor Xa (Rao & Rapaport, 1987). Antithrombin-III, a circulating protease inhibitor, most probably participates as a scavenger because of its abundance in plasma. Its most important targets are probably thrombin and factor Xa. In addition, thrombin combines with endothelial-derived thrombomodulin, resulting in protein C activation which ultimately limits thrombosis by destroying factors Va and VIIIa, and initiating fibrinolysis.

Fibrinolysis is accomplished by the action of plasmin on the fibrin clot. Plasminogen bound to the fibrin in the clot is activated to plasmin by the action of tissue plasminogen activator (t-PA) and urokinase. The activities of plasmin and t-PA, which is secreted as a single-chain protein from endothelial cells, are regulated by specific inhibitors, i.e.  $\alpha_2$ -antiplasmin and PAI-1 respectively. The digestion of a clot by plasmin results in fibrin-split products which are themselves potent inhibitors of clotting.

#### **Monounsaturated fatty acids**

The Seven Countries Study (Keys *et al.* 1986) sparked interest in the effects of diets rich in oleic acid-rich olive oil on cardiovascular disease. Consequently there is now an abundance of evidence that MUFA-rich diets are hypocholesterolaemic when substituted for saturated fatty acids (SFA; Mensink & Katan, 1987, 1989; McDonald *et al.* 1989; Berry *et al.* 1991; Foley *et al.* 1992; Mata *et al.* 1992; Sirtori *et al.* 1992; Wahrburg *et al.* 1992; Lichtenstein *et al.* 1993; Howard *et al.* 1995; Kris-Etherton *et al.* 1999; Williams *et al.* 1999). However, much less information is available concerning the effects of such dietary changes on haemostasis.

Since the first demonstration of an effect of diet on FVIIc (which reflects a combination of the plasma concentration of FVIIa and the protein concentration (FVII antigen; FVIIag)) in healthy individuals (Miller *et al.* 1986), numerous studies have investigated whether quantity as well as quality of dietary fat influences the activation of FVII.

In summary, the data from studies that investigated associations between dietary fat and concentrations of FVII (Miller *et al.* 1989; Pekkanen *et al.* 1995), and those that altered the habitual diets of volunteers (Miller *et al.* 1986; Marckmann *et al.* 1990, 1992, 1993, 1994), indicate that an increase in total fat results in a concomitant increase in FVIIc concentration. This increase is predominately due to an increase in the concentration of FVIIag. In comparison, although exceptions have been found (Tholstrup *et al.* 1994), it is fair to remark that dietary fat composition (quality) has little influence on fasting FVII concentrations (for review, see Marckmann *et al.* 1998).

The observed relationship between total dietary fat and FVIIc is apparent whether chronic (habitual) or acute (meal) effects of diet are being investigated. However, the mechanisms underlying acute and longer-term dietary effects on FVIIc may differ. The current opinion therefore is that when the habitual diet is high in fat the accompanying increase in FVIIc is due, at least in part, to a rise in FVIIag. In contrast, the transient increase seen in FVIIc after a fat-rich meal is due entirely to a rise in the concentration of FVIIa. This rise in FVII postprandially is positively associated with plasma triacylglycerol (TAG) concentration (Miller *et al.* 1989; Green *et al.* 1994; Humphries *et al.* 1994). This finding led to investigations into whether dietary fat composition, which is known to influence postprandial TAG concentration, could influence the postprandial rise in FVII activity.

Meals rich in MUFA were shown not to differ from meals rich in polyunsaturated fatty acids (PUFA) or SFA with regard to acute effects on postprandial plasma FVII (Freese & Mutanen, 1995; Larsen *et al.* 1997; Roche & Gibney, 1997). Yet recent work describes more pronounced responses of FVIIc to test meals rich in MUFA compared with meals rich in SFA (Oakley *et al.* 1998). Similar observations were reported when fifty-two middle-aged men with moderately-raised non-fasting TAG concentrations were fed a high-palmitate meal or a high-oleate meal. Despite leading to the same degree of postprandial lipaemia, the high-palmitate meal did not lead to any postprandial change in FVIIc, whereas FVIIc was significantly increased 6 h from fasting after the high-oleate meal ( $P < 0.05$ ; Sanders *et al.* 1999). Notably, FVIIa concentrations did increase after the high-palmitate meal, although this increase was half that seen after the high-oleate meal. Based on these results, the benefits of diets rich in MUFA for those individuals at risk of CHD was questioned. However, the findings of Oakley *et al.* (1998) and Sanders *et al.* (1999) are in contrast with more recent reports which demonstrate lower levels of FVIIa when subjects consume background diets that are high in MUFA (Roche *et al.* 1998; Larsen *et al.* 1999).

The potential influence of diets habitually rich in MUFA on postprandial FVII was illustrated by the observation of different postprandial patterns of FVIIc in a cross-cultural study involving northern and southern European males. Again no significant effect of test meal composition was found (SFA v. MUFA), but FVIIc was significantly greater 8 h postprandially in northern Europeans than in southern Europeans ( $P < 0.03$ ; Zampelas *et al.* 1998). In turn, the study by Roche *et al.* (1998) cited earlier demonstrated

significantly lower postprandial FVIIa and FVIIag concentrations after approximately 40 % of the dietary SFA were replaced with MUFA in an 8-week cross-over dietary intervention study ( $P < 0.05$  in both cases; Roche *et al.* 1998). The beneficial influence of MUFA-rich diets was also evident when such diets were compared with diets rich in PUFA (Larsen *et al.* 1999). Diets rich in olive oil, rapeseed oil and sunflower oil were administered to eighteen healthy young individuals for 3-week periods. The olive oil diet was associated with lower non-fasting mean and peak concentrations of FVIIa than was the sunflower oil diet. Although not statistically significant, non-fasting mean and peak concentrations of FVIIa also tended to be lower after the olive oil diet than after the rapeseed oil diet. If these findings are indeed an effect of a chronic MUFA diet, then it could be suggested that the lower rate of IHD in Mediterranean countries may result in part from the anti-thrombotic effects of MUFA-rich diets.

#### *Effects of monounsaturated fatty acids on other haemostatic indices*

The data reporting changes in platelet aggregation in response to changes in fat composition are equivocal. Sirtori *et al.* (1986) reported significant decreases in aggregation in response to collagen and arachidonic acid when olive oil diets containing 17.5 % energy as MUFA were administered for 8 weeks ( $P < 0.05$  in both cases). In contrast, McDonald *et al.* (1989), Freese *et al.* (1994) and Vicario *et al.* (1998) found no significant change in aggregation when the MUFA content of the diets was increased. However, Mutanen *et al.* (1992) and Turpeinen *et al.* (1998) found that oleic acid- and linoleic acid-rich diets enhanced ADP-induced and collagen-induced aggregation respectively when compared with diets rich in SFA. Notably, the authors of studies investigating the effects of fatty acids on platelet function recognise that the predictive value of *ex vivo* platelet aggregation or thromboxane production is uncertain, and can partly explain discrepancy between laboratories.

The data illustrating effects of dietary fat content or composition on fibrinogen status are more consistent, and although the majority of studies have focused on fish oil supplementation it appears futile to reduce or change fat composition in an attempt to improve fibrinogen status (British Nutrition Foundation, 1992). PAI-1 activity levels were reported to be lower after MUFA-rich diets than after the comparative diet (Lopez-Segura *et al.* 1996; Avellone *et al.* 1998; Temme *et al.* 1999). In the study of Lopez-Segura *et al.* (1996) a significant decrease in PAI-1 antigen levels ( $P < 0.04$ ) was also demonstrated. However, recent work by Tholstrup *et al.* (1999) indicates no benefit from MUFA-rich diets on t-PA activity or PAI-1 antigen levels.

Clearly there is discrepancy in the data in terms of the influence of MUFA on FVII, platelet aggregation and fibrinolytic variables. In an attempt to clarify some of the confusion we investigated the effects of substituting dietary SFA with MUFA in student volunteers living in a University Hall of Residence. The effects of consumption of these diets for a period of 16 weeks were determined by measurement of a range of fasting and postprandial thrombogenic risk

indicators. Atherogenic risk indicators were also measured and have been reported previously (Smith *et al.* 2001).

## **Methods**

### *Study design*

The study had a randomised single-blind parallel design. Fifty-one student volunteers living in a fully catered Hall of Residence at the University of Reading took part. The Ethics and Research Committee, University of Reading, approved the protocol and all students gave informed consent before taking part. All subjects consumed a reference (SFA) diet (% dietary energy; 37 total fat, 15.6 SFA, 11.8 *cis*-MUFA, 5.8 *cis*-PUFA, 0.7 *trans*-fatty acids) for 8 weeks followed by a MUFA-enriched diet for a further 16 weeks, the aim of which was to replace 24 % of dietary SFA with *cis*-MUFA, while keeping total fat, PUFA and *trans*-fatty acids consistent between the two diets.

The design and implementation of the intervention diets has been described in detail previously (Kelly *et al.* 2000; Nydahl *et al.* 2000). Differences in the composition of the intervention diets were achieved by altering the fats used in food preparation in the Hall kitchen, and by providing the volunteers with specially-manufactured spreads (manufactured by Van den Bergh Oils, Purfleet, Surrey, UK) and snack foods (manufactured by United Biscuits Ltd, High Wycombe, Bucks., UK and at the School of Food Biosciences, University of Reading). Students were required to eat the majority of their meals in the Hall dining room during the study and to substitute their habitual spreads and snacks for those provided by the investigators.

### *Dietary assessment and compliance*

Students were requested to complete a 4 d diet diary during week 6 of the reference diet (SFA) and week 12 of the MUFA diet. Typical portion sizes were determined by the investigators. These diaries were analysed using Foodbase nutrition database (Institute of Brain Chemistry and Human Nutrition, 1993) which had been modified previously to include manufactured foods and recipes used in the Hall kitchen and the composition of the fats and foods designed specifically for the study. Compliance to the diets was also assessed by measurement of plasma phospholipid-fatty acid composition (Fielding *et al.* 1996).

### *Anthropometric and biochemical measurements*

Subjects were weighed once every 14 d by the investigators. Subjects were instructed not to engage in any vigorous physical activity and to avoid alcohol for 24 h before blood sampling. They were provided with a standard low-fat meal on the evening before venepuncture and before postprandial investigations. Fasting (12 h) blood samples were collected from all subjects at the end of the reference diet, and at 8 weeks and the end of the MUFA diet.

Fasting venous blood samples were taken using an evacuated blood tube collection system with minimum venostasis, using a standardised protocol. Blood for lipid and glucose analyses were collected into lithium

heparin-containing tubes and plasma aliquots were stored at  $-20^{\circ}\text{C}$ . At the end of the study concentrations of plasma TAG, total cholesterol and glucose were analysed on a Monarch automated analyzer (Instrumentation Laboratories Ltd, Warrington, Cheshire, UK) using commercial test kits. Blood for haemostatic analysis was collected into two 4.5 ml sodium citrate (0.105 M)-, one 2 ml acid citrate- and one 4.5 ml  $\text{K}_3$  EDTA-containing Vacutainer tubes. All haemostatic assays were carried out on samples stored at  $-70^{\circ}\text{C}$  at the Wolfson Coagulation Laboratory (MRC Epidemiology and Medical Care Unit, London, UK). FVIIc was measured by a one-stage semi-automated bioassay using rabbit-brain thromboplastin (Diagen, Thame, Oxon, UK) and a FVII-deficient substrate plasma prepared as described elsewhere (Miller *et al.* 1994). The inter-assay CV was 3.2 %. t-PA activity was determined by a chromogenic assay (Chromogenix Coatest PAI; Quadrant Diagnostics Ltd, Epsom, Surrey, UK). FVIIag was determined by using an ELISA with specific rabbit anti-human factor VII antibody (Asserachrom VII:Ag; Diagnostica Stago, Boite Postal 226, 92602 Asnières Cedex, France). The inter-assay CV was 8.3 %. Fibrinogen was measured by a thrombin-clotting method, based on that of Clauss (1957), using a standard plasma (Immuno, Vienna, Austria). The inter-assay CV was 2.93 %. Fresh whole-blood samples, collected from twenty-nine volunteers at each time point, were used for platelet aggregation analyses. Using the impedance technique (Cardinal & Flower, 1980), a whole-blood Chrono-Log aggregometer (Labmedics Ltd, Stockport, Cheshire, UK) was used to measure aggregation in response to ADP (10  $\mu\text{mol/l}$ , final concentration), collagen (4  $\mu\text{g/ml}$ , final concentration) and arachidonic acid (1.0 mmol/l, final concentration). Reagents were supplied by Labmedics Ltd, except collagen which was obtained from Bio-Stat Ltd, Hazel Grove, Cheshire, UK. Haemostatic and aggregation analyses were carried out in duplicate.

#### Postprandial investigations

Twenty-five of the fifty-one students participated in postprandial investigations which took place at the Human Investigation Unit at the School of Food Biosciences, University of Reading, at the end of the SFA (weeks 5–8) dietary period and at the end of the MUFA-enriched dietary period (weeks 13–15). After two baseline blood samples were drawn, subjects were requested to consume the test meal which provided 45 g fat, 93 g carbohydrate and 33 g protein, with total energy 3720 kJ. Blood was drawn at hourly intervals throughout the day and at 30, 90 and 150 min after consumption of the test meal.

Plasma samples collected into lithium heparin-containing vessels were subsequently analysed for plasma TAG, non-esterified fatty acids and glucose concentrations using a Monarch automated analyzer (Instrumentation Laboratories Ltd) using commercial test kits. Plasma samples collected into sodium citrate-containing vessels at 0 ( $\times 2$ ), 2, 4, 6 and 8 h postprandially were stored at  $-70^{\circ}\text{C}$  for analyses of FVIIc and FVIIag as described earlier. FVIIa was also measured at each time point according to the bioassay method described by Morrissey *et al.* (1993), which is a clotting assay employing a synthetic tissue factor reagent

which binds selectively to FVIIa. The inter-assay CV was 13.64 %.

#### Statistics

Results shown in the figures are expressed as means with their standard errors. Statistical analysis was performed using SPSS (version 9.0; SPSS, Chicago, IL, USA). Data were checked for normality, and log transformation was performed where necessary. Dietary data were compared using paired *t* tests. Repeated-measures ANOVA investigated changes in each fasting variable within the group and in the postprandial response for the analytes measured. Statistically significant changes were subsequently followed up by paired analysis of the different time points. The postprandial data were also expressed in summary form (i.e. area under the curve (AUC), incremental AUC, postprandial increase (difference between fasting and postprandial peak), peak and mean concentrations), and compared using paired *t* tests. A value of  $P < 0.05$  was accepted as statistically significant.

#### Results

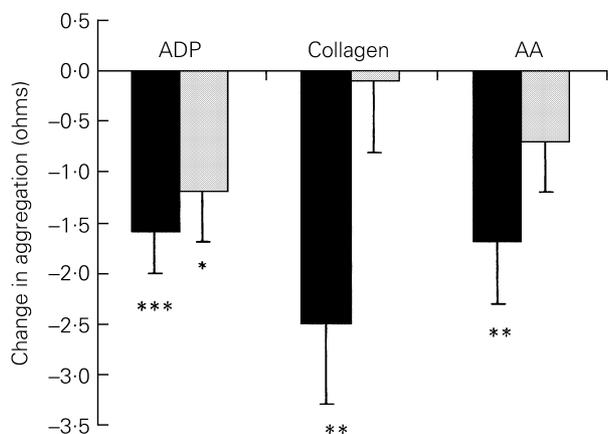
A brief synopsis of some of the pertinent findings relevant to the present review will be presented.

The dietary intervention largely achieved the target fatty acid composition of the reference diet, with the group as a whole reporting intakes (% dietary energy) of 14.9 (SD 2.1) SFA and 12.2 (SD 1.7) *cis*-MUFA compared with targets of 15.6 SFA and 11.8 *cis*-MUFA. The dietary intervention was successful in that it achieved a significant increase in the reported intake of *cis*-MUFA ( $P < 0.001$ ) with a corresponding reduction in SFA ( $P < 0.001$ ), without altering energy, total fat, *cis*-PUFA or *trans*-fatty acid intakes. During the MUFA dietary phase the subjects reported intakes (% dietary energy) of 11.0 (SD 2.1) SFA and 15.8 (SD 2.3) *cis*-MUFA compared with the target mean intakes of 11.8 SFA and 15.6 *cis*-MUFA.

Plasma phospholipid-SFA composition was significantly lower at the end of the MUFA-diet period compared with that at the end of the reference-diet period ( $P < 0.02$ ), while plasma phospholipid-MUFA content was significantly greater ( $P < 0.01$ ). There was no significant change in plasma phospholipid-PUFA composition as a result of the intervention.

Plasma total cholesterol, LDL-cholesterol and HDL-cholesterol concentrations were significantly lower at the end of the MUFA-diet period than at the end of the reference-diet period ( $P < 0.001$ ,  $P < 0.01$  and  $P < 0.001$  respectively). No significant changes in plasma TAG were observed (RD Smith, CNM Kelly, BA Fielding, D Hauton, KDRR Silva, MC Nydahl, GJ Miller and CM Williams, unpublished results).

At week 8 of the MUFA diet there were significant reductions in whole-blood aggregation in response to ADP, collagen and arachidonic acid ( $P < 0.001$ ,  $P < 0.01$  and  $P < 0.01$  respectively), but the reductions were maintained by week 16 in response to ADP only ( $P < 0.05$ ; see Fig. 2). Details of these findings have been reported previously (Kelly *et al.* 2001). Fasting levels of FVIIag,



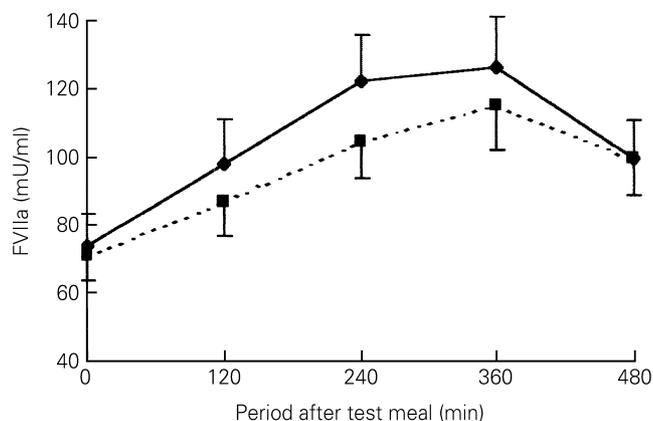
**Fig. 2.** Changes in whole-blood platelet aggregation in response to ADP (10  $\mu$ M), collagen (4  $\mu$ g/ml) and arachidonic acid (AA; 1 mmol/l) after 8 weeks (■) and 16 weeks (▒) of dietary monounsaturated fatty acids intervention. Values are means with their standard errors represented by vertical bars for twenty-nine subjects. For details of subjects and procedures, see p. 165. Mean values were significantly reduced by dietary monounsaturated fatty acid intervention: \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

FVIIc, fibrinogen and t-PA activity remained unchanged after 8 and 16 weeks of the MUFA intervention (data not shown).

The lipaemic responses of the volunteers were evaluated using TAG measurements in plasma. No significant differences were observed between the reference- and MUFA-diet periods (meal  $\times$  time), with no difference observed in peak TAG concentration or in the time to reach peak concentration between dietary treatments (KDRR Silva, CNM Kelly, AE Jones, RD Smith, SA Wootton, GJ Miller and CM Williams, unpublished results).

Plasma FVIIc increased significantly during postprandial lipaemia after the reference diet ( $P < 0.05$ ) but not after the MUFA-diet intervention. There was no difference in fasting FVIIc between the two diets, but the mean postprandial FVIIc after the MUFA intervention was significantly less than that after the reference (SFA) diet ( $P = 0.033$ ). The MUFA diet was associated with a lower postprandial peak FVIIc than the reference diet; differences between values approached significance ( $P = 0.072$ ). Differences in FVIIc AUC values on the MUFA diet (48542 (SD 11966) %·min) compared with the reference diet (46064 (SD 11462) %·min) were shown to be statistically significant ( $P = 0.05$ ). The difference in incremental AUC values approached significance ( $P = 0.09$ ).

Fig. 3 illustrates that FVIIa was significantly increased in response to the 45 g fat loads administered at the end of the reference ( $P < 0.001$ ) and MUFA ( $P < 0.001$ ) dietary intervention periods. There was no influence of background diet on the pattern of FVII activation and no significant difference in AUC or incremental AUC values after the reference diet compared with after the MUFA diet. However, although there was no difference in fasting FVIIa concentrations on either postprandial study day, the post-



**Fig. 3.** Postprandial responses in activated blood coagulation factor VII (FVIIa) following a standard test meal after an 8-week reference (saturated fatty acid) diet period (◆—◆) and after a 16-week monounsaturated fatty acid-rich diet period (■---■). Values are means with their standard errors represented by vertical bars for twenty-five subjects. For details of subjects and procedures, see pp. 164–165.

prandial peak concentration achieved after the MUFA diet (122 (SD 13) mU/ml) was less than that after the reference diet (142 (SD 16) mU/ml;  $P = 0.065$ ). Moreover, the postprandial increase (difference between fasting and peak values) in FVIIa after the MUFA intervention (52 (SD 10) mU/ml) was significantly less marked than that after the reference diet (68 (SD 13) mU/ml;  $P = 0.048$ ).

There was no difference in the postprandial response of FVIIag after both dietary periods. Values decreased until 4 h after the test meal and returned to approximately baseline concentrations by 8 h (data not shown).

## Discussion

The call for re-appraisal of diets rich in MUFA was based on concern about the possible pro-thrombotic effects suggested by a number of acute test-meal studies using relatively large fat loads (Oakley *et al.* 1998; Sanders *et al.* 1999). In an attempt to clarify the long-term effects of diets rich in MUFA on haemostasis, a range of variables was included in the intervention described in the present report. The results from the present study show less postprandial activation of FVII as well as beneficial changes in whole-blood aggregation following a high-MUFA diet. These findings, as well as the recent work of Roche *et al.* (1998) and Larsen *et al.* (1999), demonstrate potentially-beneficial effects on haemostasis of diets habitually rich in MUFA. In particular, the data demonstrate that the tendency of fat-rich meals to be pro-thrombotic may be reduced if background diets rich in MUFA are consumed.

The present study appears to be the first to report platelet responses after a 16-week MUFA intervention period. Although the effects observed on whole-blood platelet aggregation are not large, they may be important when

translated to whole-population shifts in SFA and MUFA intakes. The mechanisms underlying the changes in platelet aggregation are not clear. It can only be speculated that the effects observed were due to changes in membrane microviscosity, as a result of which the activity, and/or efficiency of receptors and enzymes, were modulated. This effect may be in addition to, or independent from, any subsequent stimulation of the eicosanoid system.

The current study also adds to existing knowledge, based largely on short-term studies, that suggests that dietary changes involving the substitution of SFA with MUFA do not evoke an effect on fasting FVIIc, FVIIag, fibrinogen concentration or t-PA activity.

The mechanism underlying the postprandial activation of FVII is not completely understood, making it difficult to explain why the MUFA-rich diet resulted in less activation in comparison with the reference (SFA) diet. In the present study no differences between dietary treatments in the pattern of response in plasma TAG levels (which are considered to be a primary determinant of postprandial FVIIa concentrations) were evident. However, one possible explanation could be the difference in the size of the chylomicrons and chylomicron remnants produced, and the subsequent hydrolysis of these particles after each dietary treatment. The AUC, incremental AUC, peak and fasting concentrations of apolipoprotein B-48 were significantly lower ( $P < 0.001$ ,  $P < 0.0001$ ,  $P < 0.0001$  and  $P < 0.0001$  respectively) when subjects followed the MUFA diet compared with the reference diet (KDRR Silva, CNM Kelly, AE Jones, RD Smith, SA Wootton, GJ Miller and CM Williams, unpublished results), yet the retinyl ester responses were similar for both diets. Hence, the MUFA diet may have led to the production of smaller numbers of larger chylomicrons. The size of the chylomicrons may influence the ability of TAG-rich lipoproteins to promote the cleavage of the inactive single-chain zymogen of FVII to FVIIa. A simple hypothesis may be that larger particles provide less contact sites for activation of FVII. Studies suggest that lipaemia is necessary, but not sufficient, for activation of FVII; e.g. patients with familial complete lipoprotein lipase deficiency do not have increased FVIIc or FVIIag despite their massive hypertriglycerolaemia (Mitropoulos *et al.* 1992). Hence, in this present study the size of the chylomicrons may have affected the specificity of these particles for the lipolytic enzymes, lipoprotein lipase and hepatic lipase, and the generation of non-esterified fatty acids thought to provide the negative surface necessary to activate the contact system. The involvement of factor IX in FVII activation (Miller *et al.* 1996) emphasizes the need for studies of the contact system (factor XII, factor XI, prekallikrein and high-molecular-weight kininogen) in relation to diets differing in fat composition, because factor XIa activates factor IX.

### Conclusions

In conclusion, the present study confirmed that MUFA-rich diets attenuate postprandial activation of FVII. This finding is in contrast to some acute test-meal studies that have suggested that MUFA-rich diets could have adverse

consequences for haemostasis. Although studies investigating acute test-meal effects are important in understanding the mechanisms driving FVII activation, they should not be extrapolated to explain the putative effects of habitual diets that differ in fat composition. If coronary events are preceded for months or years by changes in haemostatic variables, an expedient means of ameliorating these changes in the long term is essential. The substitution of SFA for MUFA, without changing habitual total fat intake, may be considered an effective strategy in preventing age-associated increases in risk factors in younger age-groups.

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