Influence of variation in fat composition on haemostatic variables

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The effects of saturated fatty acids on platelet function remain uncertain although hypercholesterolaemia is associated with increased platelet aggregrability. The consumption of n-3 fatty acids as fish oils leads to a reduction in the concentration of arachidonic acid (20:4n-6) in platelets and endothelial cells and its replacement with eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA). This change is accompanied by a prolongation of template bleeding time. The effect of replacing arachidonic acid with EPA and DHA is to decrease the production of thromboxane A_2 and increase that of the antiaggregatory prostacyclins. Despite the association between plasma triglyceride concentrations and impaired fibrinolytic activity, there is little evidence to suggest that the type of fat influences PAI-1, tPA or global markers of fibrinolysis. Some studies have reported that plasma fibrinogen concentration may be decreased by n-3 fatty acids but a large number have found no effect. Plasma triglyceride concentrations are strongly associated with increased factor VII coagulant activity (FVIIc). Despite their well known hypotriglyceridaemic effects, n-3 fatty acids do not decrease FVIIc. Postprandial activation of FVII is now well recognised and oleic acid appears to be among the most potent activators. These effects are, however, dose related. In view of their potentially prothrombotic influence, it would be wise to caution against high intakes of fat in the middle-aged and elderly population who are most at risk.

Key words: Fat, saturated fat, monounsaturated fat, EDA, DHA, haemostasis factor VII, endothelial dysfunction

Introduction

Most research concerning the influence of dietary lipids in relation to cardiovascular disease has focused on their influence on plasma lipoprotein metabolism. However, it is apparent that different types of fatty acids influence several physiologically relevant mechanisms especially those concerned with haemostasis and inflammation. As the importance of factors influencing thrombosis and thrombolysis on risk of coronary heart disease and stroke have become more firmly established, our knowledge concerning the effects of different types of fats on these factors remains limited.

Haemostatic markers of coronary risk

Coronary thrombosis is a major cause of sudden cardiac death[1], acute myocardial infarction[2], unstable angina pectoris[3] and silent myocardial ischaemia[4]. Platelet activation plays a major role in precipitating coronary events and drugs such as aspirin, which inhibit platelet activation, have been shown to be effective in the secondary prevention of myocardial infarction[5]. However, prospective studies have failed to show that indices of platelet aggregation are associated with increased risk. A hypercoagulable state may not only predispose to coronary thrombosis but accelerate the atherogenic process. Prospective epidemiological studies have found that raised plasma fibrinogen concentrations and increased plasma FVIIc are powerful predictors of risk of fatal CHD in middle-aged men[6-8] even after adjustment for other known risk factors such as blood pressure and plasma cholesterol. Levels of plasminogen activator inhibitor type 1 (PAI-1) and tissue plasminogen activator (tPA) are also elevated in patients with CHD[8,9] and are thought to be markers of endothelial dysfunction.

Influence of dietary fat composition on platelet function

It is widely held that dietary fat composition influences platelet function. Animal studies suggest that diets rich in butter or coconut increase the sensitivity of platelets to aggregation[10]. However, palm oil appears to be an exception in some studies[11]. Hypercholesterolaemia is known to be associated with an increased sensitivity of platelets to aggregating agents. Some studies have reported decreased rates of platelet aggregation when the intake of saturated fatty acids in the diet have been reduced[12]. We have found that platelet counts and plasma β-thromboglobulin concentrations are higher in a diet rich in butter fat, which is rich in saturated fat, compared to diets low in saturated fat (Sanders TAB *et al*, in press). Polyunsaturated fatty acids have different and sometimes opposing effects on platelet function. Arachidonic acid (20:4n-6) is necessary for the formation of eicosanoids that stimulate platelet aggregation and the blockade of their formation by aspirin is believed to explain why aspirin prevents heart attacks. In common with low doses of aspirin, the consumption of fish oil or oily fish containing long-chain n-3 fatty acids prolongs template bleeding time[13], which is believed to reflect platelet vessel wall interactions. However, unlike aspirin platelet aggregation is only mildly inhibited but platelet adhesion is decreased[14]. The consumption of fish oil leads to the displacement of arachidonic acid by eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA) from the platelet and endothelial cell membranes. The capacity to synthesise thromboxane A_2 from arachidonic acid (20:4n-6) is slightly reduced. The prolonged bleeding time is more likely to be due to increased endothelial cell prostacyclin (both PGI_2 and PGI_3) generation rather than decreased platelet thromboxane synthesis[15].

Influence of dietary fat composition on fibrinolysis

High plasma triglyceride concentrations are associated with increased PAI-1 inhibitor activity[9]. However, postprandial lipaemia is not associated with an increase in PAI-1 activity. Moreover, some studies have found decreased fibrinolytic activity on low fat diets compared with high fat diets. Most studies have found no effect of fat composition on fibrinolytic activity[16-18]. There have been reports of both an increase[19-21] and fall[22] in PAI-1 activity following the consumption of diets containing fish or long-chain n-3 fatty acids. However, we have been unable to demonstrate any influence of n-3 fatty acids on tPA or PAI-1 activity. This finding is consistent with there being no influence of n-3 fatty acids on fibrinolytic activity[13].

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Influence of dietary fat composition on plasma fibrinogen

Plasma fibrinogen concentration is elevated by cigarette smoking[23] and this has confounded many studies that have examined the effect of dietary fat on this variable. The majority of studies suggest that plasma fibrinogen concentrations are unaffected by the intake of dietary fat[16-18,24]. A reduced plasma fibrinogen concentration has been reported in a few studies with diets containing long chain n-3 fatty acids[25,26] but at least as many studies have reported no effect[13,27,28] or even an increase[29]. In our own most recent study (Sanders TAB *et al*, in press), where we excluded smokers, plasma fibrinogen concentrations on an n-3 diet were almost identical to those on a saturated fat diet but 10% higher on the n-6 diet compared with n-3 and saturated fat diets. This potentially important observation requires confirmation. It is possible that this effect could be mediated by the stimulating effects of linoleic acid intake on interleukin IL-6 production, which is known to increase plasma fibrinogen.

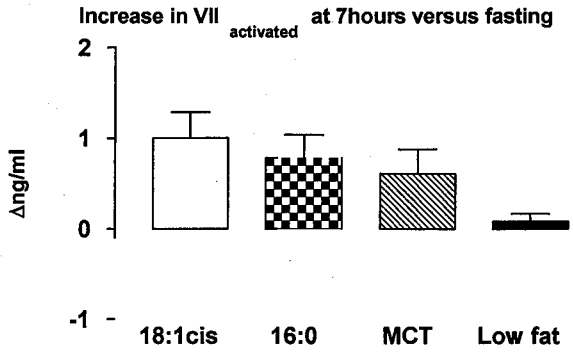
Influence of dietary fat composition on factor VII

An increase in $FVII_c$ occurs following the consumption of a high fat meal[30] and raised levels of $FVII_c$ are associated with habitual high fat intakes. High plasma triglyceride concentrations are also associated with increased $FVII_c$[31-33]. As the amount of fat provided in a meal is the primary determinant of the degree of post-prandial lipaemia, we postulated that the amount of fat consumed in a single meal may be more important than the average daily intake of fat in determining FVIIc levels. Indeed preliminary results from our laboratory support this finding[34].

Although a reduction in the intake of total fat is associated with a fall in FVIIc[17], the influence of dietary fat composition on this parameter is far from clear. The influence of different saturated fatty acids on FVIIc was studied by Tholstrop *et al*[18] who found that a particular type of stearic acid rich diet, where stearate was taken in the form of shea butter, led to lower levels of FVIIc compared with either a palmitate rich or laurate + myristate rich diets. On the other hand, Mitropoulos *et al*[35] argues that a raised plasma concentration of stearic acid is associated with increased FVIIc activity. Most previous studies have been unable to detect any significant effect of dietary fat unsaturation on factor VII[16,35,36]. We found a 7% increase in FVIIc on an n-3 diet compared with the saturated fat diet. This finding was unexpected as it was accompanied by decreased fasting and postprandial triglyceride concentration (Sanders *et al*, in press).

The postprandial activation of FVIIc after a fatty meal, but not after a low-fat isoenergetic meal, is now well recognised and appears to be due to an increase in the fraction of factor VII circulating in the activated form[33]. The catalytic activity of lipoprotein lipase appears to be necessary to activate VII. Certain long chain free fatty acids such as stearic acid (18:0), elaidic acid (18:1trans) and behenic acid (22:0) have been found to activate FVII *in vitro*. It has been argued that factor VII is activated *in vivo* by these long chain free fatty acids because their structure enables them to stack on top of large triglyceride rich lipoprotein particles and act as a contact system to activate factor VII. Alternatively, chylomicron remnants could act as a contact surface for the activation of factor VII. We could find no increase in FVIIc following a low fat meal containing 15g fish oil but when the same amount was consumed with 75g of olive oil, there was a similar increase in FVIIc to that obtained with 90g of olive oil[38], despite decreasing postprandial lipaemia. These findings suggest that n-3 fatty acids only increase FVIIc in the presence of a high fat meal. This effect may be mediated by increased lipolytic activity due to an increased surface area of vascular endothelium exposed to blood in the postprandial period. Such an effect could be mediated via the synthesis of eicosanoids from the long chain n-3 fatty acids which have net vasodilator actions relative to those synthesised by n-6 fatty acids.

Figure 1. Influence of different fatty acids fed at 40% of the total fatty acids in a 90g test meal on activation of factor VII compared with a low fat (15g) isoenergetic test meal (results for 15 subjects).



We have consistently found that oils rich in oleic acid are potent at increasing FVIIc and that medium chain triglycerides have no influence (Figure 1). It is well known that MCT are transported via the hepatic portal vein in the post-absorptive state and do not lead to chylomicron formation. Our observation that MCT do not lead to postprandial lipaemia or an increase in FVIIc indicates that it is the postprandial lipaemia that acts on FVII and not the dietary fat per se. This suggests that the chain length of fatty acids influences FVIIc activity. Although the amounts of fat used in our test meal studies are high, it would be predicted that with increasing age and decreasing physical activity hyper-triglyceridaemia (and hence an increase in FVIIc activity) could be induced by lower intakes of fat. Further studies are needed to see if lower intakes of dietary fat can lead to an increase in FVIIc activity in an older population.

Conclusion

Our finding that olive oil and other oils rich in oleic acid increases FVIIc is important as there is a popular school of thought that believes that high intakes of monounsaturated fatty acids in the form of oleic acid are desirable for the prevention of CHD owing to their neutral effects on plasma low-density lipoprotein cholesterol concentrations. Our results show that high intakes of triglycerides containing long-chain fatty acids (both saturated and monounsaturated) induce activation of FVII and therefore oleic acid may not be neutral with regard to risk of fatal CHD especially in patients with atherosclerosis.

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脂肪組成的變動對血液凝集變量的影響

摘要

雖然高膽固醇血症與血小板凝集的增加有關，但至今爲止，飽和脂肪酸對血小板功能的影響仍未明確．攝食n-3脂肪酸（如魚油）可導致血小板和上皮細胞内的花生四烯酸（20: 4n-6）濃度減少，20碳五烯酸（EPA）和22碳六烯酸（DHA）濃度增加．這一轉變可使出血時延長．這種以EPA和DHA代替花生四烯酸的影響可使血栓烷A2的產生減少和抗凝集前列環素增加．某些研究報道n-3脂肪酸也許會降低血漿纖維蛋白原濃度，但大多數報導并非如此．血漿甘油三脂濃度與凝血因子VII（FVIIc）活性的增加有明顯關系．衆所周知的低甘油三脂血症，n-3脂肪酸不會降低FVIIc．餐后FVII的激活作用已廣爲人們所認識，其中油酸似乎是最有力的激活劑．這些也許與劑量有關，但回顧影響血液凝固的可能性，對處于危險狀態的中老年人群，應避免高脂肪的攝食．