Dietary fat and platelet function

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Platelets are needed for haemostasis. Platelets adhere to damaged tissue, activation being mediated by exposed collagen, thrombin and ADP. The activated platelets then release compounds that cause vasoconstriction, such as thromboxane A₂, and activate the clotting cascade culminating in the formation of a stable fibrin plug. Overactive platelets, however, may play an important role in causing coronary vasospasm and acute myocardial infarction. For almost two decades it has been widely held that the substitution of vegetable oils for animal fats in the diet decreases the reactivity of platelets. A reappraisal of this view seems long overdue in the light of new knowledge concerning the control of platelet function.

Total and saturated fat intakes

The typical western diet provides between 120 and 140 g of fat daily, or about 40% of the energy intake. About half of this is saturated fat derived mainly from dairy foods and meat. In contrast the typical diet in most developing countries only supplies about 50 g of fat, which contains proportionately less saturated fat.

High intakes of saturated fat increase arterial thrombosis tendency, as measured with the aorta loop technique and platelet aggregation induced by ADP in vivo in the rat [1]. However, measurements of platelet aggregation in vitro show an increased response to thrombin, but not to ADP or collagen [2]; the addition of stearic acid to the diet increases this response [3].

Measurements of platelet aggregation in vitro have shown that farmers with high intakes of dairy fats have a high response to thrombin compared with farmers who use mainly vegetable oils [2].

The response to thrombin can be reduced by decreasing the total fat intake and partially replacing the dairy fats with vegetable oils and margarine, rich in linoleic acid [2, 4].

Polyunsaturated fats

Human diets contain two series of polyunsaturated fatty acids, the ω6 and ω3, derived from linoleic acid (18:2ω6) and α-linolenic acid (18:3ω3) respectively. Linoleic acid and α-linolenic acid are made by plants but not by animals. A human requirement for dietary linoleic acid has been established and is in the order of 1% of the energy intake, an amount greatly exceeded by most human diets [5]. Both linoleic acid and α-linolenic acid can be further desaturated and chain-elongated in animal but not plant tissues to form long-chain derivatives. Thus food of animal origin can provide both linoleic acid and α-linolenic acid as well as their long-chain derivatives. Linoleic acid is usually the predominant dietary polyunsaturated fatty acid and most diets supply only very small amounts of the long-chain derivatives, with the notable exception of those containing large amounts of seafood. The fat from fish and marine mammals contains high proportions of eicosapentaenoic acid (20:5ω3, EPA) and docosahexaenoic acid (22:6ω3) derived from the marine food chain.

Arachidonic acid metabolism and platelet function

Prostaglandin (PG) metabolites of arachidonic acid (20:4ω6) with opposing effects, thromboxane (TXA₂) and prostacyclin (PGI₂), produced in different locations, are believed to regulate platelet-vessel wall interactions [6]. Platelets contain an exceedingly high proportion of 20:4ω6, which is converted via the prostaglandin endoperoxide PGH₂ mainly into thromboxane A₂ (Fig. 1). This substance is a powerful vasoconstrictor and stimulator of platelet aggregation. It has a short half-life
and is rapidly degraded to thromboxane B2. Low doses of collagen or ADP generate small amounts of thromboxane B2 and result in second phase platelet aggregation, which is readily inhibited by a wide variety of drugs, particularly non-steroidal anti-inflammatory drugs that inhibit prostaglandin production [7]. However, far greater amounts of thromboxane are produced when platelet aggregation is stimulated by thrombin or high doses of collagen even though aggregation induced by these agents operates independently of the prostaglandin pathway. Prostacyclin antagonizes the effects of thromboxane. It is produced by the vessel wall and monocytes from endogenous 20:4w6 or from PGH2 derived from platelets. Changes in the balance between thromboxane and prostacyclin production are believed to be reflected in the template bleeding time. For example, if thromboxane production is diminished and prostacyclin production maintained then the bleeding time will be prolonged.

**Influence of dietary ω6 fatty acids**

Most human diets contain only small amounts of arachidonic acid (20:4w6), about 0.1 g daily, obtained from the consumption of offal, meat and eggs, and so most of the 20:4w6 in the platelets and blood vessel wall must be derived indirectly from dietary linoleic acid. The desaturation and chain elongation of linoleic acid is carefully regulated so that increasing the dietary intake of linoleic acid above a threshold value of between 1 and 2% of the energy intake does not lead to any further increase in platelet 20:4w6. The consumption of preformed arachidonic acid, however, greatly enhances the proportion of 20:4w6 in plasma and platelet lipids. This change is accompanied by an increased sensitivity to second phase platelet aggregation induced by ADP [8, 9] and an increased urinary excretion of prostaglandin metabolites.

Human platelet and plasma lipids contain small amounts of dihomo-γ-linoleic acid (20:3w6),
the precursor of the monoenoic prostaglandins. This fatty acid is not converted into a thromboxane or prostacyclin in vitro [7] but is converted mainly into PGF\(_2\). Unlike arachidonic acid (20:4\(w_6\)), the addition of 20:3\(w_6\) to human platelet-rich plasma inhibits platelet aggregation. Naturally occurring fats and oils do not contain 20:3\(w_6\). Therefore 20:3\(w_6\) in platelets must be derived from dietary linoleic acid (18:2\(w_6\)) or dietary \(\gamma\)-linolenic acid (18:3\(w_6\)), which is found in the oil of the evening primrose. Second phase aggregation induced by ADP was inhibited about 4 h after feeding with 1 g of 20:3\(w_6\) to human volunteers [10]. The generation of PGE\(_1\), PGE\(_2\) and PGD\(_2\) was increased. PGE\(_1\) and PGD\(_2\) both have an anti-aggregatory effect on platelets. The increased production of PGE\(_2\) could have resulted from the inhibition of thromboxane synthetase, because there was little evidence of conversion of 20:3\(w_6\) into 20:4\(w_6\). Sim & McCraw [11] showed that dietary 18:3\(w_6\) was approximately half as potent as 20:3\(w_6\) in inhibiting second phase aggregation induced by ADP in man. However, these feeding studies were poorly controlled and involved a small number of subjects, treated for varying periods.

The effect linoleic acid has on platelet aggregation depends upon the amount consumed. A dietary deficiency of linoleic acid drastically reduces the amount of 20:4\(w_6\) in both platelet and arterial wall lipids. Subsequently the capacity to produce both thromboxane A\(_3\) and prostacyclin (PGI\(_2\)) is reduced [1,12]. Arterial thrombosis tendency is low. Second phase platelet aggregation induced by ADP or low doses of collagen does not occur but the response to thrombin is increased [13]. Dietary linoleic acid restores the level of 20:4\(w_6\) in platelet and arterial lipids, prostaglandin production and second phase platelet aggregation.

Intakes of linoleic acid far greater than those required to cure symptoms of dietary deficiency do lower arterial thrombosis tendency in rats and increase the capacity to produce prostacyclin PGI\(_2\) [1,12]. When vegetable oils and margarines, rich in linoleic acid, replace dairy and other hard fats in the diet the linoleic acid intakes can be increased from 4% to 12% of the energy intake, which is equivalent to an additional daily intake of 16–20 g of linoleic acid. Under these conditions platelets aggregate less readily, as measured in vivo by the filtragometer method [14,15]. Increased platelet life-span [16] and a reduction in heparin thrombin clotting time have also been reported [17,18]. In these studies it is impossible to discriminate between the effects of a decreased saturated fat intake and those of an increased linoleic acid intake. Moreover, as edible oils were used rather than pure fatty acids it is also possible that the effects could be attributed to other constituents in the oils.

Bleeding time is not prolonged by high intakes of linoleic acid [14,19], but this is not surprising since prostaglandin production is not inhibited [12]. Indeed, in rats the capacity to produce both thromboxane and prostacyclin may increase with the intake of linoleic acid [20].

The mechanism by which high intakes of linoleic acid reduce the sensitivity of platelets to activation is uncertain. This might be mediated by an increased production of PGE\(_1\) [21]. However, high intakes of linoleic acid do not elevate the proportion of 20:3\(w_6\) in either plasma or platelet lipids. An increased capacity to produce prostacyclin PGI\(_2\) might explain the reduced rate of platelet aggregation measured by the filtragometer technique. The intravenous infusion of an oil emulsion rich in linoleic acid leads to an increased production of 6-keto-PGF\(_{1\alpha}\) in man [22]. However, it is now known that prostacyclin is produced in response to mild endothelial injury such as the insertion of an intravenous cannula or perfusion with an irritative substance [23].

**Influence of \(\omega3\) fatty acids**

It has been known for some time that rats fed on fat-free diets supplemented with \(\alpha\)-linolenic acid (18:3\(w_3\)) have a bleeding tendency. Platelet aggregation and prostaglandin production are inhibited both in vitro and in vivo in rats fed with linseed oil, which is rich in \(\alpha\)-linolenic acid [12,24]. This can be attributed to the displacement of arachidonic acid (20:4\(w_6\)) from platelet lipids by 20:5\(w_3\) (EPA), which is synthesized from \(\alpha\)-linolenic acid. EPA is the precursor of the trienoic prostaglandins [25]: thromboxane A\(_3\) is only weakly active but PGD\(_3\) and prostacyclin PGI\(_3\) are potent inhibitors of platelet aggregation. EPA is a more potent inhibitor of human platelet aggregation in vitro than is 18:2\(w_6\), 18:3\(w_3\), 18:3\(w_6\) or 20:3\(w_6\) [26,27]. Bleeding time is prolonged in rabbits infused with the EPA but can be prevented by pretreating the animals with aspirin [28]. EPA, however, is a poor substrate for cyclooxygenase, compared with arachidonic acid [25,26,29,30]. Furthermore, trienoic prostaglandins do not appear to be produced in significant amounts by rats even if their tissue lipids contain a high proportion of 20:5\(w_3\) [12,31]. However, EPA is an effective competitive inhibitor for platelet cyclooxygenase [29,30].

Owren et al. [32] claimed that a single dose of 20 g of dietary \(\alpha\)-linolenic acid decreases platelet adhesiveness. However, Borchgrevinck et al. [33] were unable to show any influence on bleeding
time or platelet adhesiveness in patients with coronary heart disease given 10-30 ml of linseed oil daily for 3 months. Sanders & Younger [34], however, showed that 20 ml of linseed oil taken daily for 2 weeks by healthy volunteers led to only a very small increase in the proportion of 20:5ω3 but no change in that of 20:4ω6 in platelet lipids. In contrast, small amounts of preformed 20:5ω3 as fish oil concentrate (MaxEPA, Seven Seas Health Care, Hull, U.K.) led to a large increase in the proportion of 20:5ω3 and a decrease in that of 20:4ω6 in platelet lipids.

Platelet function is impaired in Greenland eskimos [35]. Bleeding time is prolonged and second phase aggregation induced by ADP or by low doses of collagen, but not by 20:4ω6, is inhibited. The proportion of 20:4ω6 in platelet lipids was greatly diminished and was replaced by eicosapentaenoic acid (20:5ω3) and docosahexaenoic acid (22:6ω3). The average daily intake of 20:5ω3 and 22:6ω3 by these eskimos was about 5 and 6 g respectively but that of linoleic acid was low (about 5 g). A lack of 20:4ω6 for the formation of pro-aggregatory prostaglandins could explain their mild haemostatic defect.

Smaller amounts of 20:5ω3 may also affect platelet function. A study of Japanese fishermen showed that higher concentrations of ADP were required to result in half-maximal aggregation than in a group of farmers [36]. The fishermen were consuming about 250 g of fish daily, which would provide about 2.5 g of 20:5ω3. Relatively small amounts of 20:5ω3 as a fish oil fraction lower whole blood viscosity, which is believed to be a measure of platelet stickiness [37]. Siess et al. [38] reported that platelet aggregation was inhibited by low doses of collagen after 1 week in subjects who had consumed 500-800 g of mackerel daily. These changes were accompanied by an increase in the proportion of 20:5ω3 in the platelets and a decrease in 20:4ω6. Depending upon the time of year the fish was caught, this would have provided 4-8 g of 20:5ω3 and 7-14 g of 22:6ω3 [39]. Siess et al. attributed the inhibition of platelet aggregation to changes in platelet composition. Other studies which have brought about comparable changes in platelet lipid composition by using smaller amounts of 20:5ω3 and 22:6ω3 provided as fish oils over a longer period have failed to show any consistent change in platelet aggregation measured in vitro [40-43]. A recent uncontrolled study in patients with ischaemic heart disease suggested that plasma concentrations of β-thromboglobulin and platelet factor 4, which are indicators of platelet aggregation in vivo, were lowered after consumption of 20 ml of MaxEPA daily for 5 weeks [44]. This study requires confirmation as the measurement of plasma β-thromboglobulin is subject to large experimental errors, because platelets can be activated by the venepuncture.

Template bleeding time is prolonged by high intakes of fatty fish or fish oil supplements [40-43, 45], possibly because thromboxane A2 production is decreased. Arachidonic acid (20:4ω6) is partially displaced from the platelet lipids by 20:5ω3 and 22:6ω3. This reduces the amount of substrate for thromboxane formation. Furthermore, 20:5ω3 and 22:6ω3 present in the plasma lipids or released from the platelet lipids would also competitively inhibit the conversion of 20:4ω6 into thromboxane A2. The amount of thromboxane A2 generated during platelet aggregation is far greater than the amount needed to cause platelet aggregation. Indeed, it has been suggested that the main role of thromboxane A2 is to cause vasocstriction [46]. In rats dietary 20:5ω3 and 22:6ω3 inhibit the production of thromboxane A2 to a greater extent than that of prostacyclin (PGI2), with the consequence that bleeding times are prolonged and arterial thrombosis tendency is reduced [20, 31]. Dietary supplementation with menhaden oil, which is rich in 20:5ω3 and 22:6ω3, exerts a protective effect on experimental cerebral infarction in cats [47] and experimental myocardial infarction in dogs [48]. In both these studies infarct size was reduced and there was evidence of improved collateral circulation.

It is important to emphasize that different types of fatty fish or fish oils were used in these studies. It is difficult, therefore, to extrapolate the results from one study to another. Moreover, in studies where fatty fish replaced other items in the diet, it is impossible to separate the effects brought about by the addition of fish to the diet from those brought about by the exclusion of other foods. Studies using fish oil supplements are easier to interpret, provided that the subjects consume the oil. Compliance can be monitored by analysing the fatty acid composition of platelet and erythrocyte phosphoglycerides [40, 43]. However, fish oils are not all the same [49]. Some fish oils, especially those from salmon, mackerel and herring, contain a high proportion of eicosapentaenoic acid (22:ω3), which may cause a transient myocardial lipidosis similar to that caused by erucic acid (22:ω9) in several species of experimental animals [50]; Sinclair [51] when following an eskimo diet developed a thrombocytopenia, as did some of the subjects on a salmon oil diet studied by Goodnight et al. [41]. Similar changes occur in young men fed on high erucic acid rapeseed oil [52]. The requirement for vitamin E may be increased if large amounts of fatty fish or fish oils are consumed [53]. Furthermore, precautions should be taken...
to ensure that fish oil supplements do not contain peroxidized fatty acids, which are known to be toxic and can inhibit prostacyclin production [54]. The consumption of large amounts (15-50 ml/day) of certain fish-liver oils, especially shark-liver or halibut-liver oil, should not be encouraged because of their high vitamin A and D content. The earlier human studies tended to use cod-liver oil [19, 40] or salmon oil [41], but more recent studies [34, 43-45] have used an oil blend called MaxEPA, which has circumvented these objections.

A study by Dyerberg & Bang [55] on the influence of 10 ml of a 60% 20:5ω3 ethyl ester concentrate, which contained only a small proportion of 22:6ω3, in 20 subjects for 3 weeks gave disappointing results. It has been known for some time that fatty acid esters of fish oils, especially when prepared by urea fractionation, often yield different results from those expected [8, 49]. Firstly, the absorption of the esters may be poor compared with that of the triglyceride, and secondly, peroxidized fatty acids and their breakdown products, such as aldehydes, hydroxy acids and ketones, may be concentrated in the EPA rich fraction.

Most investigators have ignored the possible influence of 22:6ω3 on platelet function. However, it shows a similar inhibitory effect on platelet aggregation in vitro to that of 20:5ω3 (T. A. B. Sanders & N. R. Bolster, unpublished work). It would appear that a daily intake of 3-5 g of 20:5ω3 and 22:6ω3 taken for at least 3 weeks prolongs bleeding time by about 40%. This is considerably less than the amount consumed by Japanese fishermen. Whether smaller amounts consumed over a longer term would have any significant effect is unknown.

Plasma lipoproteins

The plasma lipoprotein environment seems to influence platelet reactivity. Platelet malondialdehyde formation, which is primarily an indicator of prostaglandin synthesis, is higher in hyperlipidaemic patients (Fredrickson types IIa, IIb, IV) than in healthy controls [56]. However, platelet thromboxane B2 production is enhanced by type II hyperlipidaemia but not type IV hyperlipidaemia [57]. Although individual lipoprotein fractions have been blamed, it is important to remember that several other plasma factors known to increase platelet reactivity are higher in patients with hyperlipidaemia, for example fibrinogen and clotting factors VII and VIII [58]. However, platelets from patients with type IIa hypercholesterolaemia contain an increased amount of free cholesterol relative to its principal solubilizer, phospholipid, and are more sensitive to aggregating agents in vitro [59]. Moreover, the addition of plasma from patients with familial hypercholesterolaemia to washed platelets from healthy volunteers increases the sensitivity of the platelets to aggregating stimuli compared with autologous plasma [60]. Furthermore, the incubation of cholesterol rich liposomes with washed platelets increases platelet sensitivity to aggregating agents and enhances the synthesis of thromboxane B2, by a partial redirection of arachidonic acid metabolism from the lipo-oxygenase pathway towards the cyclo-oxygenase pathway [61]. It was proposed that platelet membrane cholesterol is increased in the presence of high concentrations of low-density lipoprotein (LDL) cholesterol and that this makes the membrane more rigid and, therefore, more sensitive to aggregating stimuli [61]. However, in liver disease where the membrane cholesterol:phospholipid ratio is markedly altered, platelet sensitivity to aggregating agents is not increased [62].

Elevated concentrations of high-density lipoprotein (HDL) cholesterol are associated with a reduced risk of atherosclerotic disease but do not appear to lower the reactivity of platelets [63]. Platelets from patients with either type IV or type IIa or IIb hyperlipidaemia are less sensitive to the inhibitory effects of prostacyclin [61]. It is not possible to study the influence of high concentrations of triglycerides on platelet aggregation in vitro by the turbidimetric method. Consequently, the influence of plasma triglyceride and very-low-density lipoprotein concentrations have on platelet function is a moot point.

It is well known that dietary fat intake can modify the concentrations of plasma lipoproteins. Saturated fatty acids have a strong hypercholesterolaemic effect whereas polyunsaturated fatty acids have a weaker but opposite hypercholesterolaemic effect [64]. These effects are almost exclusively confined to changes in the proportion of LDL cholesterol. The C20-C22 polyunsaturated fatty acids of the ω3 series in addition show a powerful hypotriglyceridaemic effect [40, 43, 64]. It is therefore possible that some of the effects of dietary fat, especially saturated fat, on platelet aggregate are mediated by way of its influence on plasma lipid concentrations.

Conclusion

There is abundant evidence that a reduction in total fat intake and the partial substitution of vegetable oils rich in linoleic acid for dairy fats does reduce the rate at which platelets aggregate. This change, however, has no influence on haemo-
stasis measured by template bleeding time because thromboxane production is not inhibited. Ambiguous results were obtained from two prospective studies [65, 66] aimed at reducing the incidence of coronary heart disease by modifying the dietary fat intake in this way. Coronary vasospasm may result in acute myocardial infarction and angina pectoris [67]. This could be caused by thromboxane [68]. The consumption of modest amounts of fish oils leads to an increase in bleeding time and a reduction in thromboxane production. It is possible that the long-chain derivatives of α-linolenic acid play an important role in modulating the production of prostaglandins from arachidonic acid (20:4ω6). The low incidence of acute myocardial infarction among both eskimos and Japanese fishermen underlines the need for further studies to assess the potential long-term benefits of consuming large amounts of fish or smaller amounts of fish oil supplements.

References

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