Dietary fatty acids in human thrombosis and hemostasis\textsuperscript{1–3}

Howard R Knapp

ABSTRACT The effects of fatty acids on hemostasis are controversial. It has been difficult to show convincing effects of saturated or monounsaturated fatty acids that are clearly related to hemostatic variables in humans. Unsaturated fatty acids alter platelet aggregation and processes related to coagulation and fibrinolysis. Indirect evidence exists that n–6 polyunsaturated fatty acids may exert favorable effects on thrombotic processes in vivo, but large clinical trials have failed to show benefits of 5–6 g linoleic acid (18:2n–6) or linolenic acid (18:3n–3)/d. Only long-chain n–3 fatty acids prolong the template bleeding time, and they may exert some beneficial effect on erythrocyte flexibility. It appears unlikely that n–3 fatty acids lower fibrinogen or interact with the fibrinolytic system directly. One prospective secondary prevention trial showed benefits that may have resulted from either an improved hemostatic profile or an antiarrhythmic effect. A similar time course of clinical improvement was noted with reduced rates of cardiac mortality and postoperative thrombosis in Norway during World War II, and this was associated with a drastic dietary alteration involving increased consumption of n–3 fatty acids and reduced consumption of saturated fatty acids. Further work is needed to develop better tools to examine in vivo hemostasis so that the mechanisms and eventual clinical utility of n–3 fatty acids can be elucidated in well-designed clinical trials. Am J Clin Nutr 1997;65(suppl):1687S–98S.

KEY WORDS Dietary fat, hemostasis, fatty acids, platelets, thrombosis, coagulation, n–3 fatty acids

INTRODUCTION

Only a few studies have been done on the role of individual fatty acids in human hemostasis; these were prompted first by epidemiologic observations and then by studies involving modification or supplementation of the diet with fats enriched in particular groups of fatty acids. A description of some of the epidemiologic studies will be given as an introduction, but the main body of this review presents sections comprising studies on particular types of fatty acids, first discussing studies involving dietary fat modification and then the few studies available on individual fatty acids. In many dietary studies only different food items were provided to the subjects; these are discussed if the composition of the foods was known and reasonably controlled. However, the unknown effects of various types of antioxidants, isoprenoids, steroids, and even inorganic constituents (e.g., selenium content of fish) on hemostatic variables makes the inclusion of such works problematic and they generally were excluded.

Some attempt has also been made to limit this summary to human studies with either clinical endpoints or hemostatic indexes that are generally agreed to reflect in vivo processes. There appears to be little agreement, for instance, on the predictive value of ex vivo platelet aggregation or thromboxane production for eventual vascular events. Although many studies examine oxidative damage to lipoproteins in parallel with malondialdehyde formation in plasma, such measurements are confounded by an unknown degree of lipid peroxidation occurring ex vivo and during sample processing and are related only hypothetically to hemostasis or atherogenesis in humans. More widely accepted indexes include platelet granule constituents in plasma, metabolites of platelet and vascular eicosanoids, template bleeding time, and thrombosis in vivo, and this article will largely focus on clinical investigations involving such measurements. Finally, the topic of this workshop section is hemostasis rather than atherogenesis, so studies involving only the latter area will be excluded.

Before a discussion of intervention studies, mention of the observational data that led to them seems appropriate. The conclusion of the Seven Countries Study (1) that dietary saturated fat and plasma cholesterol correlated with cardiovascular disease led to several trials of dietary modification to increase polyunsaturated fat at the expense of saturated fat. These included the study in Helsinki (2), in which these dietary changes were associated with both lower plasma cholesterol and reduced cardiovascular endpoints in men but not women. Interestingly, population (3) and intervention (4) studies in which this occurred in the setting of certain dietary modifications revealed a benefit within 1 y. It is widely believed, however, that only indirect evidence has been presented thus far linking dietary saturated fatty acids with enhanced thrombogenesis in humans (5, 6), aside from platelet aggregation studies on individuals in different geographic areas (7, 8). These latter studies are difficult to interpret, as there were other differences in addition to the types of dietary fats between the regions, such as alcohol intake.

Although many cross-sectional, migratory, and generational studies on atherosclerosis have been published, the lack of reproducible laboratory measurements of hemostasis before the

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1960s makes comparison with older reports difficult. The clinical observations in Norway during World War II, however, are of interest in that a discrete, drastic change in the diet of that country, but not in that of its close neighbor Sweden, was associated with a sharp decline in cardiovascular death within 6 mo (3). In addition, there was a similar rapid decline in the incidence of postoperative thrombosis (9). Rates of both of these clinical events returned to their prewar levels within a few months of the war’s end, when the usual diet of the population was restored. Although only an approximate analysis of the dietary change was possible, there was the near elimination of dairy products and the substitution of cod meat and cod liver oil (10). The percentage of energy derived from fat was believed to have declined from ~34% to 23% during the Nazi occupation and amounts of n−3 polyunsaturated fat substantially increased. Interestingly, the reduction in postoperative thrombosis was not accompanied by increased intraoperative hemorrhage (9).

On the basis of unblinded observations that linseed oil rich in α-linolenic acid (18:3n−3) altered platelet function and prolonged the template bleeding time, a large trial was carried out in Norway for 12 mo in 1965–1966 (11). This was a randomized, blinded comparison of supplementing the diets of 50–59-y-old men with 6.3 g linoleate/d (6690 men) compared with 5.5 g linolenate plus 1.5 g linoleate/d (6716 men), with close monitoring of vascular endpoints. There was no significant difference in myocardial infarction (52 compared with 53) or death (27 in each group); there were fewer thrombotic episodes in the linoleate group (10 compared with 16) but this was not significant. Thus, this 1-y study did not suggest the type of antithrombotic change seen with the dietary change in occupied Norway (3) or in the more recent Diet and Reinforcement Trial (DART) (4).

DART, the only large secondary intervention trial of n−3 fatty acids, involved >2000 men in Wales who had survived a myocardial infarction (4). At least three helpings of fatty fish, or ~15 fish oil capsules per week led to a significant (29%) reduction in both cardiac and total mortality within the first 4 mo. The reduction in total infarctions in the DART group consuming oily fish was not significant but survival was, and a post hoc analysis found that there was a dose-response relation between survival and intake of n−3 fatty acids (12). The early improvement in survival in DART suggests an effect of n−3 polyunsaturated fatty acids on thrombosis rather than atherosclerosis; no detectable benefit was reported for groups in the DART study that reduced their fat intake to 30% of energy or doubled their intake of fiber.

Thus, large trials indicate a possible effect of dietary fatty acids on hemostatic variables. I now turn to the evidence available from smaller intervention studies of each type of dietary fat.

SATURATED FATTY ACIDS

The idea that dietary saturated fats contribute to high plasma cholesterol concentrations and atherogenesis was fostered by the Seven Countries Study (1), but skepticism remains about the size of such an effect (13, 14). Nonetheless, the hypothesis that thrombotic processes are involved in both atherogenesis and its vascular complications prompted a series of early studies on the effects of saturated fatty acids on coagulation and platelets. Direct addition of saturated fatty acids to human blood quickly led to clot formation (15) and it was shown that this property was not shared by unsaturated fatty acids. Eventually, it was realized that the sodium salts of the saturated fatty acids used were actually added in a microparticulate form whereas the unsaturated fatty acids were more soluble. The presence of albumin to bind the fatty acids prevented the thrombotic effect, and several researchers speculated on the effects of lipolytic release of saturated fatty acids, especially stearic acid (18:0), in amounts that would saturate the available albumin binding sites. Conceivably, in times of extreme stress, 18:0 could be mobilized from adipose tissue (16) or it could be released during postprandial processing of a stearate-rich meal. Several studies examining the effects of added free saturated fatty acids on platelet function concluded that saturated fatty acids stimulate aggregation of human platelets (17, 18).

The effects of dietary stearate in coagulation have been studied in a variety of ways since the reports of the early 1960s and a body of literature [especially from Renaud et al (7,8)] has suggested for some time that both coagulation and platelet function are accelerated by diets high in saturated fat (19). This idea has been criticized by several other investigators. In their comprehensive review in 1963, Merskey and Marcus (20) concluded that there was no evidence that the type of dietary fat had any influence on coagulation and this opinion has been echoed regularly since then, including in the recent conclusion by Hoak (6) that there is no evidence that dietary stearate exerts a thrombogenic effect. Still, new approaches to monitoring the coagulation system in vivo may provide the opportunity to clarify this 35-y-old point of contention (21).

The activation of factor VII by saturated fatty acids at the surface of lipoprotein particles in vitro was reported (22) and it was subsequently suggested that increased plasma 18:0 is responsible for an activation of factor VII in subjects consuming high-saturated-fat diets (23). It seems unlikely, however, that the concentration of unbound stearate in plasma would get high enough to accomplish this in the same way as described in vitro. Some workers also reported that 18:0 interacted more favorably with the coagulation system (factor VII activity) than did palmitic (16:0), myristic (14:0), or lauric (12:0) acids (24), whereas others found no effect of dietary saturated fat on platelet function or thromboxane generation (25) or on the excretion of thromboxane and 6-keto-prostaglandin F1α (26).

Because most humans are postprandial for a greater part of the day than they are in a fasting state, it was natural to examine the influence of newly arriving dietary fat on blood clotting variables. Two reports indicated that fibrinolysis is impaired after consumption of a meal rich in 18:0 (100 and 270 g of double cream) (27, 28). Other reports, however, found similar prothrombotic changes in platelet function (29–32) and hemostatic variables (33, 34) for comparisons of saturated and n−6 unsaturated fatty acid test meals or of the latter and saturated medium-chain triacylglycerols (35). Some workers reported that low-fat diets reduce and hyperlipidemia increases platelet aggregation on a chronic basis (36, 37), whereas other reports indicate less rather than more platelet aggregation during the lipemia after ingestion of, for example, 100 g whipping cream (38). Improved means of assessing the many interactive components of coagulation in vivo will go a long way to resolving the conflicting reports that regularly appear in this field.
MONOUNSATURATED FATTY ACIDS

Oleic acid (18:1) is usually considered to be the neutral reference point against which the antithrombotic effects of polyunsaturated fats and proaggregatory effects of saturated fats are compared (36). Not all studies agree that 18:1 (or dietary olive oil) is devoid of biological effects in humans, however, and there are reports of decreased platelet aggregation when 18:1 was replaced by linoleic acid (18:2n–6) in the diet (38). In diabetic patients with atherosclerosis and high plasma concentrations of von Willebrand factor, increased intake of 18:1 resulted in a reduction of von Willebrand factor but no change in fibrinogen or fibrinectin (39). This was hypothesized to be due to improved diabetic control with more monounsaturated and less polyunsaturated fat in the diet. Finally, a recent report from a group in Israel indicated a stimulation of plasmin activity by 18:1 in vitro (40). Saturated fatty acids were ineffective whereas 18:2n–6 had effects similar to those of 18:1; no studies focused on such variables in vivo. Likewise, because humans make large amounts of 18:1 and it comprises about half of the fatty acids in the US diet, 18:1 or olive oil has frequently been used as a presumably inert control in studies of polyunsaturated fats.

n–6 POLYUNSATURATED FATTY ACIDS

Linoleic acid

The hypocholesterolemic and antiatherosclerotic effects of dietary 18:2n–6 (2, 41), as well as suggestions that a low intake predisposes to myocardial infarction (42), prompted exploration of the potentially beneficial effects of this fatty acid in hemostasis as well. Most of the published studies claim reductions in ex vivo platelet aggregation measured either in the flow tube of Hornstra and ten Hoor or in platelet-rich plasma (Table 1). Hornstra et al (43) found a nonsignificant reduction in platelet count and reduced aggregation of platelets with 12% of energy as 18:2n–6. There was no change in bleeding time but there was a nonsignificant 13% decrease in platelet count. Fleischman et al (44, 45) reported similar findings with 5% and 8% of energy as 18:2n–6 in two studies. O'Brien et al (46) found no change in flow-tube clotting but decreased aggregation (Born method) in response to ADP and collagen but not thrombin; the platelet count fell 15% (NS). They also reported a decrease in plasma fibrinogen with 7% of energy as 18:2n–6 and a bleeding time that was prolonged compared with that of control subjects but not with the treated subjects' own baseline values.

Challen et al (47) provided 60 mL safflower oil/d to six subjects and found reduced aggregation to ADP but not collagen or thrombin in whole blood; no change in bleeding time was noted. Vericel et al (48) provided only 2 g 18:2n–6/d to elderly subjects and saw decreased aggregation in response to collagen and arachidonic acid (20:4n–6) but not thrombin. Bleeding time was not altered. In contrast with these variable findings, Nordøy et al (49) provided 18:2n–6 as 24% of energy and found no change in aggregation. Similar findings were presented by Boberg et al (50), except that some of their subjects appeared to have increased aggregation after eating 16.5% of energy as 18:2n–6. A study comparing a fish oil supplement with a supplement rich in 18:2n–6 (21%) and 18:1n–9 (Oleic acid; 66%) found similar reductions in collagen-induced aggregation with both supplements (51). Also, other studies reported no effect of 18:2n–6 on blood viscosity (52) and a lack of the ability to increase fibrinolytic activity that is seen with other long-chain polyunsaturated fatty acids (53).

The effects of large doses of safflower oil (54) or varied dietary 18:2n–6 content [3% compared with 8.3% of energy (55)] on the in vivo synthesis of the eicosanoids released from platelets and endothelium (thromboxane and prostacyclin, respectively) were investigated. This was done by gas chromatography–mass spectrometry measurement of urinary metabolites; both groups of researchers found small but significant increases in prostaglandin E metabolite excretion. The supplementation study providing 39 g 18:2n–6/d to eight men with mild hypertension found only a nonsignificant trend toward higher excretion of prostaglandin I,–M and no change in thromboxane A,–M (54), whereas the dietary study providing 8.3% of energy as 18:2n–6 to 10 healthy (8 postmenopausal) women found no change in prostaglandin I,–M and a reduction in thromboxane A,–M (55). It will be of interest to explore this point further to determine whether this is a real sex difference or merely a result of the small sample sizes studied. Although the synthesis of thromboxane by platelets ex vivo is not be-

<table>
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<tr>
<th>Dose</th>
<th>No. of subjects</th>
<th>Aggregation†</th>
<th>Platelet count</th>
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<tr>
<td>12% of energy</td>
<td>63</td>
<td>Flow tube, ↓</td>
<td>↓ 13% (NS)</td>
<td>No change</td>
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<td>5% of energy</td>
<td>66</td>
<td>Flow tube, ↓</td>
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<td>Fleischman, 1975 (44)</td>
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<td>8% of energy</td>
<td>28</td>
<td>Flow tube, ↓</td>
<td>—</td>
<td>—</td>
<td>Fleischman, 1974 (45)</td>
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<td>7% of energy</td>
<td>19</td>
<td>Flow tube, no change; ADP, Coll ↓; thrombin, no change</td>
<td>↓ 15% (NS)</td>
<td>↑ vs control, not vs pretreated</td>
<td>O'Brien, 1976 (46)</td>
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<td>60 mL safflower/d</td>
<td>6</td>
<td>WB; ADP ↓; Coll, thrombin, no change</td>
<td>No change</td>
<td>No change</td>
<td>Challen, 1983 (47)</td>
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<td>24% of energy</td>
<td>30</td>
<td>No change in ADP, Coll, thrombin</td>
<td>No change</td>
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<td>Nordøy, 1974 (49)</td>
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<td>2 g/d</td>
<td>16</td>
<td>Coll, AA ↓</td>
<td>—</td>
<td>No change</td>
<td>Vericel, 1986 (48)</td>
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<td>8% of energy</td>
<td>17</td>
<td>No change</td>
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<td>Boberg, 1986 (50)</td>
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† Aggregation measured either in a flow tube or in platelet-rich plasma. Coll, collagen; WB, whole blood; AA, arachidonic acid.
lieved to closely reflect the in vivo situation, several investigators examined the effects of dietary 18:2n−6 supplementation on this variable (25, 54, 56). In general, no alteration in ex vivo thromboxane generation has been observed.

Dihomo-γ-linolenic and arachidonic acids

Both 18:3n−3 and dihomo-γ-linolenic acid (20:3n−6) have been shown to exert antiaggregatory effects in vitro (57, 58), but the effects of the latter fatty acid ex vivo were different in different laboratories. Kernoff et al (59) fed 20:3n−6 to four subjects and found variably decreased ADP-stimulated aggregation with a more consistent decline in plasma heparin-neutralizing activity. A controlled trial of 1 g 20:3n−6/d in 33 subjects by Szczeklik et al (60) found no change in circulating platelet aggregates and a nonsignificant trend toward increased aggregation in response to ADP and collagen. There was a 20% decrease in the threshold dose of ADP needed for aggregation but no change in thromboxane B synthesis. Mikhailidis et al (61) found decreased platelet aggregation with 20:3n−6 administration to normal subjects but not diabetic subjects. Sim and McCraw (62) likewise detected a decrease in ADP-induced aggregation after single and daily doses of 1.5 mg 20:3n−6 methyl ester/kg to normal subjects.

Free 20:4n−6 causes platelet aggregation in vitro but in the presence of both platelets and endothelial cells actually inhibits aggregation (63). In two studies the pure ethyl ester was given to volunteers at doses of 6 g/d (64) and 10 g/d (65) without adverse events. In the latter study, supplementation was ended in two of four subjects when their platelets exhibited a 10-fold increase in ADP sensitivity. Unfortunately, bleeding times were not measured and this study was conducted before the discovery of prostacyclin, so no assessment of changes in the actual thrombotic-antithrombotic balance was possible. Studies in Australian aborigines, however, found a prolongation of bleeding time when the aborigines ate a low-fat diet enriched in 20:4n−6, which probably provided 2 g 20:4n−6/d, in the setting of their losing considerable amount of weight (66). Finally, Nowak et al (67) infused 120 μg 20:4n−6 over 5 min into the right atria of volunteers to study pulmonary 20:4n−6 metabolism. There were no untoward effects and the 20:4n−6 was converted to prostaglandin E compounds rather than to prostacyclin or thromboxane.

n−3 POLYUNSATURATED FATTY ACIDS

α-Linolenic acid

In 1964 Owren et al (68) reported that the addition of 18:3n−3 to men’s diets reduced ADP-stimulated platelet adhesiveness in a filtration system. Corn oil and safflower oil (both rich in 18:2n−6) and cod liver oil [rich in eicosapentaenoic acid (20:5n−3) and docosahexaenoic acid (22:6n−3)] had no effect. On the basis of this unblinded clinical study, a sizable trial was mounted comparing supplementation with either 18:2n−6 or 18:3n−3 in > 13 000 men aged 50–59 yr; the negative results were described in the introductory section (11). The notion that 18:3n−3 possessed special antiplatelet properties was supported by in vitro data indicating that this fatty acid and not 18:2n−6 inhibited the platelet aggregation stimulated by saturated fatty acids in vitro (69). A blinded clinical study, however, found that up to 30 mL linseed oil/d providing 15 g 18:3n−3/d had no effect on platelet adhesiveness or bleeding times in men with atherosclerosis (70). Platelet adhesiveness was also determined in a subgroup of men in the large, randomized, blinded clinical trial referred to earlier (11) and no difference in platelet adhesiveness was detected among those receiving 18:2n−6 or 18:3n−3 or control subjects receiving neither.

Despite these seemingly definitive results, a small series of papers have been published on the putative benefits of 18:3n−3, including one report that it lowered fibrinogen (71), but others found no effect of linseed oil on erythrocyte deformability or whole-blood viscosity (72). There has also been an interest in promoting a Mediterranean diet modestly enriched with 18:3n−3 in the secondary prevention of coronary artery disease (73). Instead of a dietary ratio of 18:2n−6 to 18:3n−3 of ≈20, the intervention group had a ratio of 4.5. This study randomly assigned 302 post-myocardial infarction patients to an experimental diet enriched with 18:3n−3 and 303 patients to a nonintervention control group. Subjects were followed for 5 y; there were 16 cardiac deaths in the control group and 3 in the experimental group. Nonfatal myocardial infarctions numbered 17 in the control group and 5 in the 18:3n−3-supplemented group; total mortality was 20 in the control group and 8 in the experimental group (P < 0.02). Possibly because nearly all of the patients in both groups also were given aspirin, no differences in platelet function were found. Although the results of this study are of interest, the many variables involved in significant dietary modification make it difficult to attribute the between-group differences to the intake of 18:3n−3 alone.

The interest in long-chain n−3 polyunsaturated fatty acids of marine origin has also stimulated studies on possible terrestrial sources of these fatty acids, and several papers have claimed effects of canola oil or flaxseed oil on hemostatic variables. Canola oil, however, usually has a much higher content of 18:2n−6 (21%) than of 18:3n−3 (8%), so supplementation studies would not be easy to interpret. One unblinded study found a prolongation of bleeding time when a group receiving canola oil was compared not with its own baseline but with the period when a mixed oil was ingested (74). The actual mean bleeding time during safflower oil ingestion was similar to that during canola ingestion (4.5±4.92 min) but was not statistically different from the 3.85 min during the mixed-oil period. Both oils lowered thromboxane and increased 6-keto-prostaglandin F1α in blood collected from the bleeding time incision, although this was significant only for the sunflower oil diet. No significant effect of either oil on collagen-induced aggregation was found. In another study, flaxseed oil with comparable amounts of 18:2n−6 (15%) and 18:3n−3 (21%) was administered in a carefully controlled manner (75); no effect of 6.3% of energy as 18:3n−3 for 56 d was detected on coagulation tests or bleeding times. In contrast, another study providing 4.6 g 18:3n−3/d to 15 hyperlipidemic subjects reported a decrease in platelet aggregation in response to thrombin but not collagen (76). Others reported that thromboxane production by platelets was not altered by dietary 18:3n−3 (or 18:2n−6) (77).

Eicosapentaenoic and docosahexaenoic acids

The great interest in the antithrombotic properties of marine oils has produced several thousand papers over the past 15 y, with at least 249 focused primarily on applications in human
HEMOSTASIS: HUMAN STUDIES

Most studies were conducted with oil preparations from different species of fish with a broad range of fatty acid compositions containing variable amounts of sterols (including vitamin D), tocophorols, retinoids, and other components. Studies involving increased consumption of fish include consumption of a large number of non–fatty acid components, both organic and inorganic. Thus, the interpretation of most human studies involving n-3 fatty acids was held by the Food and Drug Administration to be problematic, because not only could the effects not be attributed to a single fatty acid, they often could not even be reliably attributed to the fatty acids themselves or to the lipid fraction of the ingested material (78). Because of the potential health and medical applications of this fatty acid class, however, pure fatty acid ethyl esters and concentrated ethyl esters of carefully defined composition have been made available to investigators from both commercial and governmental (through the NMFS/DOC/NIH Fish Oils Test Materials Program) sources. Results from the smaller number of studies using these pure substances confirm many of the observations made using less refined or less well-characterized preparations.

This brief review will attempt to present the available data on the effects of long-chain n-3 fatty acids on hemostatic variables. Priority will be given to well-controlled studies carried out with pure fatty acid esters, but this small number of reports will be discussed in the context of the bulk of studies carried out with less ideal material. Conclusions will be drawn where possible, and suggestions made for studies needed to clarify important points.

Bleeding time and bleeding episodes

The interest in medical applications of n-3 fatty acids started when Bang and Dyerberg (79) observed in 1980 that Greenland Eskimos had a prolonged bleeding time and an apparently low rate of atherosclerotic disease despite a high fat intake. Some reviewers have expressed doubts about the significance of this finding (80) but it has provoked concerns by regulatory agencies about the safety of fish oil supplements (78). Thus far, 24 reports (52, 81–103) showed significantly prolonged bleeding times whereas 15 studies (90, 104–117) showed no change or only a nonsignificant trend to prolongation. The latter studies tended to be those providing a lower dose of 20:5n-3 (six provided < 2 g/d) for a shorter time, but this is not true in all cases. The prolongation of bleeding time is usually modest and there have been no reports of serious bleeding.

Although detailed discussion of the many tests of hemostasis is beyond the scope of this review, it seems clear that whereas template bleeding time is not a good predictor of clinical bleeding episodes (80), prolongation of it likely reflects some alteration of platelet-vascular interactions. The balance between vitamin E status of the platelet and endothelium or the presence of lipid peroxides in the ingested preparation could also contribute to this effect. Template bleeding time does not simply reflect platelet function; reactivity of skin blood vessels to trauma, their production of pro- or anticoagulant substances, adherence of platelets to subendothelium, and blood viscosity could all contribute to alteration of the bleeding duration after a skin incision. Interestingly, one report describes n-3 polyunsaturated fatty acids blocking the shortening of bleeding time induced by exercise, although there was no prolongation of bleeding time at baseline, suggesting a vasoactive component (118). These many influences would be unlikely to all have the same dose- and time-response relation with the active components of marine oils, which could result in the wide variability in response found.

Bleeding-time prolongation has also been seen with ingestion of pure 20:5n-3 ethyl ester (99), so it appears likely that 20:5n-3 or some product derived from it is involved in this phenomenon. Thorngren and Gustafson (100) showed in 1981 that changes in ex vivo platelet aggregation and bleeding time were temporally unrelated and that neither appeared to be associated with the time course of altered platelet fatty acid composition or thromboxane synthesis. Certainly, from Thorngren and Gustafson’s work there appears to be little relation between thromboxane generation during serum formation and that synthesized in bleeding-time blood, indicating that the former has little relevance to the situation in vivo. Although 20:5n-3 decreases platelet thromboxane synthesis, the fact that aspirin and fish oil exerted additive effects on bleeding time showed that fish oil’s effects could not be due to simply thromboxane inhibition (100, 102). Thorngren and Gustafson’s observation has been confirmed by others (119) and was amplified in Leaf’s (120) excellent editorial in Circulation on this topic.

Perhaps of more clinical significance than the variable prolongation of bleeding time is the lack of significant bleeding in patients taking substantial doses of n-3 fatty acids and who undergo major surgical procedures or childbirth. Although Leaf et al’s (89) large trial of 8 g n-3 fatty acids/d in angioplasty restenosis was negative, none of the patients experienced significant bleeding during the 6 mo they took the preparation or during the angioplasty procedure itself (they were pretreated for 12–14 d). Several patients underwent emergency cardiac bypass surgery without abnormal blood loss; this was also noted in one report of a trial of n-3 fatty acids in coronary bypass patients (121) and in a preliminary communication about another trial (122). In a smaller angioplasty trial in Quebec, patients were pretreated for 28 d with n-3 fatty acids with no evidence of excessive bleeding at the puncture site (123). A trial in Denmark of the effects of 2.7 g n-3 fatty acids/d on pregnancy duration found no increase in blood loss at delivery (124). Finally, although dialysis patients are frequently found to have a bleeding diathesis, administration of n-3 fatty acids to either peritoneal dialysis patients (90) or hemodialysis (107) patients was not associated with any bleeding complications. The lack of excessive bleeding in these studies contrasts with the single report of increased epistaxis in hypercholesterolemic children given 5 g menhaden oil/d (125); in another paper, no bleeding problems were seen in children on dialysis receiving a similar dose of n-3 fatty acids for 8 wk (126).

Plasma viscosity (fibrinogen) and blood viscosity (erythrocytes)

A frequently mentioned effect of n-3 polyunsaturated fatty acids that could have relevance for vascular benefits is improved blood rheology. Reductions in fibrinogen and increased erythrocyte flexibility would be highly beneficial and could alone account for both the increase in bleeding time and decrease in blood pressure seen in many studies. Unfortunately, in neither area is there a consensus. Twelve studies reported a
reduction in fibrinogen (52, 83, 96, 108, 115, 127–133), but in two of these (115, 132) the same change was seen in the control oil (corn and olive oils) groups. In contrast, 28 reports showed no change in fibrinogen (86, 93, 97, 98, 110, 113, 114, 117, 134–152). One author has two papers reporting lower (96, 133), two reporting unchanged (145, 146), and one reporting higher (97) fibrinogen concentrations after the provision of n–3 supplements to different types of patients. Another group found lower fibrinogen concentrations with fish oil plus vitamin E and no change when extra vitamin E was omitted (108, 139). When these papers are not included, there are 4 describing lower fibrinogen with n–3 polyunsaturated fatty acids and 25 showing no change. Clearly, tocopherol status may be an important factor, but under usual conditions it seems likely that the benefits of n–3 fatty acids do not include a reduction of fibrinogen or plasma viscosity, to which fibrinogen is the largest contributor. Among authors not measuring fibrinogen, one describes lower and two describe unchanged plasma viscosity during fish-oil supplementation.

Erythrocyte flexibility provides the major component of whole-blood viscosity and a change in this variable would greatly affect a variety of pathologic processes. Although a favorable reduction in whole-blood viscosity has been claimed by the authors of at least 12 papers (127, 134, 136, 137, 148, 151, 153–158), a lack of any change has been presented in 9 (104, 109, 111, 117, 128, 150, 159–161). This area is complex, however, and simply measuring erythrocyte filtration is probably an inadequate method for detecting small but significant differences. Also, repeated measures are rarely carried out to establish the interindividual variability at baseline; further efforts to improve our technical capabilities in assessing the effects of diet on erythrocyte flexibility are needed to address this important variable, as well as the roles of tocopherol status, peroxide tone, and other influences on erythrocyte flexibility. One paper also suggested that erythrocyte osmotic fragility is lessened by n–3 fatty acids (162), but two others did not confirm this (163, 164).

Clotting factors and fibrinolysis

There is also little agreement on the effects of n–3 fatty acids on clotting factors and fibrinolysis. An epidemiologic study indicated an association between fish intake and lower factor VII and fibrinogen (165), but the differences between quartiles were extremely small and only significant because 45,000 subjects were involved. It is difficult to believe that the differences described would be biologically relevant. One intervention study described reduced factor VIII with n–3 supplements (113), but other authors carried out extensive investigations of the coagulation system without finding a significant change resulting from fish-oil ingestion (105, 141). Similarly, one group found that n–3 fatty acids reduce the expression of procoagulant tissue factor activity on monocytes ex vivo (166), but four other groups using slightly different systems failed to detect this type of effect (118, 145, 167, 168).

The idea that fish oils could have a favorable effect on fibrinolysis stems from, among other observations, the finding of improved myocardial infarction survival in the DART study described above (4). The currently available means of assessing fibrinolysis are constantly improving but the in vivo significance of examining just a portion of the complex overall system is always problematic. Three authors reported that fish oils reduce the activity of plasminogen activation inhibitor 1 (PAI-1) (98, 169, 170), and this would indicate a potential benefit in improved fibrinolysis. Seven other workers, however, found no change in PAI-1 during fish oil ingestion (114, 115, 144, 145, 171–173) and 11 other groups reported significant increases in this variable (132, 133, 140, 147, 174–182). Increased PAI-1 has been found in hypertriglyceridemia, but the increase seen with n–3 ingestion was unrelated to very-low-density lipoprotein or chylomicrons in most cases; if this were a true reflection of in vivo activity and were not accompanied by increased t-plasminogen activator, there could be a net change toward a procoagulant state. More work needs to be done to clarify this aspect of n–3 polyunsaturated fatty acid interactions with the hemostatic system.

Effects on platelets

There has been a great deal of discussion about the relation of ex vivo platelet aggregation to in vivo processes and there is some question as to whether ex vivo platelet hyperactivity indicates accelerated platelet-vascular interactions in vivo. Among the more controversial effects of n–3 polyunsaturated fatty acids are their effects on ex vivo platelet function. As mentioned previously, most polyunsaturated fatty acids have been reported to cause a variety of changes in platelet function that appear to have little relation to thromboxane release or even platelet lipid composition. Although 39 published reports present significant changes of one sort or another (81, 82, 86, 92, 94, 99–103, 105, 109, 110, 113, 148, 153, 155, 176, 182–202), 18 found no change (90, 96, 107, 114, 141, 146, 152, 158, 170, 173, 203–210) and several of these involved high-dose supplementation. One study using pure 20:5n–3 ethyl ester (150) and one reporting prolonged platelet half-life in vascular patients (207) found no effect on ex vivo aggregation in response to the usual agonists. Those (at least 38) that did report significant changes in aggregation presented many conflicting combinations of effects with different agonists. Aggregation induced by low-dose collagen is probably one the most widely reported activities to be influenced, followed by aggregation induced by ADP, epinephrine, and thrombin. Pure 20:5n–3 ethyl ester was found to have either no effect (150) or to influence all agonists (99, 192). Two studies using pure 22:6n–3 ethyl ester also found reduced aggregation (211, 212), although the chronic 22:6n–3 study was confounded by vitamin E deficiency (212).

In many of the studies mentioned above, changes in ex vivo aggregation did not correspond with bleeding-time prolongation or with changes in other hemostatic variables. Although such platelet-function studies provided much valuable information about platelet physiology, it is often difficult to extrapolate alterations in ex vivo aggregation or thromboxane synthesis to events occurring in vivo, where the platelets are interacting with endothelial cells, other blood elements, and atherosclerotic plaque (213). The few papers on the effects of n–3 fatty acids on human platelet adherence have the same lack of agreement as the larger number of papers on aggregation; two groups published that dietary fish oil has no effect on platelet adherence (150, 214) whereas Li and Steiner (189, 215) reported striking reductions, albeit with high doses of fish oil that could be exerting very different effects than those seen with lower doses. Likewise, a small decline in platelet count has been mentioned by several workers; this usually is not
significant and has only been clinically important in patients receiving large doses of salmon oil (86). Such a degree of thrombocytopenic response was not reported by any other workers, and Saynor and Gillott’s (130) interesting long-term follow-up study indicated that the reduction in platelet count usually seen is a transitory phenomenon, returning to baseline after \( \approx 3 \) mo of supplementation.

**Effects on platelet-vascular eicosanoids**

Thromboxane A\(_2\) is an extremely potent agonist for both platelet aggregation and vasospasm, whereas prostacyclin has the opposite effects. Alterations of their relative production were originally proposed as the basis for the potential vascular benefits of marine oils, with lowering of bioactive thromboxane and maintenance of prostacyclin synthesis (216). However, only a small percentage of maximal thromboxane synthesis is sufficient to stimulate complete platelet aggregation, and the amount made during blood clotting ex vivo bears little relation to in vivo processes (213). The fact that dietary fish oil leads to a reduction of maximal serum thromboxane generation ex vivo, therefore, may not be particularly relevant for in vivo thrombosis in human disease. This variable nonetheless appears to be one of the least controversial effects reported in fish-oil supplementation studies. Only a few workers failed to detect a decrease in serum thromboxane (106, 191, 204), whereas at least 23 papers describe a lowering of serum thromboxane generation as a significant effect of \( n-3 \) fatty acids (25, 54, 83, 92, 102, 103, 105, 114, 148, 170, 183, 185, 190, 194, 196, 199, 202, 203, 217–221).

In contrast with serum thromboxane, the noninvasive assessment of endogenous thromboxane synthesis via measurement of its urinary metabolites provides a clinically useful index of increased in vivo thromboxane production. There have been few studies on this important point, but two groups found that in either patients with atherosclerosis (220) or apparently healthy individuals (202) who excrete large amounts of dinor-thromboxane B\(_2\), there is a reduction toward the normal range with large doses of 20:5n–3 (10 g/d). Relatively small amounts of dinor-thromboxane B\(_3\), formed from the biologically less-active thromboxane A\(_3\) derived from 20:5n–3, were found during these fish-oil supplementation studies (220), and a reduction in total thromboxane was apparent. The situation is not the same in healthy individuals making normal amounts of thromboxane, however. Despite substantial reductions in serum thromboxane formation, these subjects have only a modest reduction in dinor-thromboxane B\(_2\) that is almost entirely replaced by the synthesis of dinor-thromboxane B\(_3\), without any reduction in total thromboxane synthesis occurring in vivo (54). A similar reduction in stimulated, but not basal, thromboxane synthesis was also seen in patients with coronary artery disease who were undergoing angioplasty (218). These findings again indicate the complexity of the effects of 20:5n–3 on in vivo processes related to hemostasis, in that the net effects of \( n-3 \) fatty acid supplementation may vary not only between different hemostatic variables, but also for the same variable at different levels of activity.

**SUMMARY**

The effects of fatty acids on hemostasis are controversial. It has been difficult to show convincing effects of saturated or monounsaturated fatty acids that are clearly related to hemostatic variables in humans. Unsaturated fatty acids alter platelet aggregation and processes related to coagulation and fibrinolysis. Indirect evidence exists that \( n-6 \) polyunsaturated fatty acids may exert favorable effects on thrombotic processes in vivo, but large clinical trials have failed to show benefits from 5–6 g/d of either 18:2n–3 or 18:3n–3. Only long-chain \( n-3 \) fatty acids prolong the template bleeding time and they may exert some beneficial effect on erythrocyte flexibility. It appears unlikely that long-chain \( n-3 \) fatty acids lower fibrinogen or interact with the fibrinolytic system directly. One prospective secondary prevention trial showed benefits that may have resulted from either an improved hemostatic profile or an antiarrhythmic effect. A similar time course of clinical improvement was noted with the reduced rates of cardiac mortality and postoperative thrombosis in Norway during World War II, and this was associated with a drastic dietary alteration involving increased consumption of \( n-3 \) fatty acids and reduced consumption of saturated fatty acids. Further work is needed to develop better tools to examine in vivo hemostasis so that the mechanisms and eventual clinical utility of \( n-3 \) fatty acids can be elucidated in well-designed clinical trials.

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