Dietary fat, thrombosis links reviewed

Both atherosclerosis (the build-up of lipid-containing deposits on the inner walls of coronary arteries) and thrombosis (the formation of clots which can block blood vessels in the heart and elsewhere) in humans can lead to adverse clinical outcomes such as heart attacks and strokes. Dietary lipids have been associated with both processes, although more is known about the association with atherosclerosis than with thrombosis. Some reports have suggested that saturated fatty acids are prothrombogenic compared to polyunsaturated fatty acids and that C6–3 polyunsaturated fatty acids are antithrombogenic compared to C6–6 polyunsaturated fatty acids. Some of the limited research on dietary lipids and thrombosis has been criticized for poor experimental design, control, statistical analysis, data interpretation, and methodology of questionable validity.

The objectives of the workshop were twofold: to evaluate critically the current knowledge base concerning the relationship between dietary fatty acids and thrombosis, emphasizing study design, methodology, and interpretation, and to identify important unanswered questions and directions for future research.

The workshop began with a presentation by Dr. W.H. Glinsmann, Associate Director for Clinical Nutrition at the U.S. Food and Drug Administration (FDA), on why FDA is interested in dietary fatty acids and thrombosis. This was followed by presentations by recognized experts on diet and thrombosis in six sessions: (1) thrombosis, (2) fatty acid metabolism and thrombosis, (3) platelet-macrophage-vessel wall interactions, (4) lipoprotein (a) and thrombosis, (5) thrombogenic potential of dietary long-chain fatty acids, and (6) clinical correlates. This report summarizes the presentations.

FDA's interest

According to Glinsmann, FDA ultimately is concerned with the safety of the U.S. food supply and therefore needs a clear understanding of health effects of dietary fatty acids, including not only their impact on blood cholesterol levels, but also their effects on thrombosis. In regard to thrombosis, FDA is concerned about what type of information is required and the validity of methodology for predicting thrombogenic risk of diets with major changes in fatty acid composition. FDA will be taking the state of this science into account in developing final regulations (by November 1992) covering such issues as nutrition labeling and health messages for foods. Questions dealing with dietary fatty acids which FDA will be considering in coming months include: Should there be a recommended dietary allowance (RDA) or a reference daily intake (RDI) for C6–3 fatty acids? If so, what should these values be? Should the definition of saturated fatty acids (which now includes the sum of C12, C14, C16 and C18 saturates) be modified to exclude C18 or be extended to include long-chain saturates such as C20 and C22? Are these longer-chain saturates atherogenic or thrombogenic? FDA will solicit comments from the food industry and elsewhere in addressing such issues.

With respect to development of fat-modified products, Glinsmann emphasized the need to identify the appropriate target populations as well as populations at potential risk. He used the term “nutriceutical” in referring to products (such as those that might be enriched with C3 fatty acids) which could provide both essential nutrients and therapeutic value. When considering products for general food use, Glinsmann noted that issues of safety are paramount. When the product is intended for therapeutic use, a risk-benefit analysis is appropriate.

Thrombosis

By way of background, the basic scheme of blood coagulation involves conversion of the soluble protein fibrinogen into insoluble fibrin, the principal component of a clot. This transformation is catalyzed by the enzyme thrombin, which in turn is generated from its active precursor, prothrombin. The conversion of prothrombin to thrombin is controlled by a complex series of interactions among various factors (details not discussed here, but may be found in many biochemistry texts). Clotting factors are found in blood cells known as platelets.

The key issue addressed during this session was: What are the most useful tests of thrombotic risk now available? The widely used method of platelet aggregation was viewed as a good test of inhibition of platelet function (e.g., aspirin appears to promote bleeding by inhibiting platelet function) but not as a reliable indicator of platelet activation. Also, it has not been established whether bleeding time, a commonly used measurement, is an indicator of bleeding risk. S. Fredd of the FDA's Division of Gastrointestinal and Coagulation Drug Products, emphasized the importance of establishing valid “surrogate endpoints” as indicators of clotting ten-

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**Fatty acid metabolism, thrombosis**

As noted by A.A. Spector of the University of Iowa, the only dietary fatty acid change that has been shown to affect thrombosis in humans is increased intake of marine oils rich in ω-3 fatty acids. When this occurs, platelet membranes accumulate ω-3 fatty acids, particularly eicosapentaenoic acid (EPA), which replaces some of the arachidonic acid in the membranes. The result is reduced thromboxane \( A_2 \) production in response to platelet activation and reduced tendency for platelets to aggregate. Spector indicated that the dietary fat modifications needed to alter platelet functionally probably are much larger than those usually occurring in the human diet, making it unlikely that such changes would be significant under ordinary conditions.

E.A. Emken of the U.S. Department of Agriculture's (USDA) National Center for Agricultural Utilization Research reviewed results from his recent studies on metabolism of deuterated long-chain fatty acids by humans. Emken's work has shown both stearic and palmitic acids to be fairly well absorbed by humans; however, there has been considerable variation among subjects on how rapidly and completely stearic acid is absorbed compared to palmitic acid. Conversion of stearic to oleic acid (and palmitic to palmitoleic acid) could partially reduce any possible thrombotic effect of both stearic and palmitic acids. Emken and coworkers have observed about 5–12% conversion of dietary linolenic acid to the antithrombotic EPA; however, elongation and desaturation of linoleic to arachidonic acid has been barely detectable in their studies.

Based on work on the distribution of \(^1^3\)C-enriched fatty acids among serum proteins (primarily albumin), lipoproteins and membranes, D.M. Small of the Boston University School of Medicine suggested that under normal conditions most plasma fatty acids are bound to albumin. If the level of plasma fatty acids increases (e.g., as a result of exercise, stress or fasting), the fatty acids redistribute to lipoproteins and membranes. Small added that fatty acids are transiently released during the activation of platelets; however, whether these fatty acids play a role physically or metabolically or only act as constituents of the platelet phospholipids is uncertain.

S.M. Prescott from the University of Utah noted that diets rich in marine fatty acids result in modest impairment of platelet function, including reduced platelet adhesion. However, the doses needed to see such effects are quite large (at least 6–10 grams/day of MaxEPA for about 30 days). In general, smaller doses (about 1–3 grams/day) appear to have little or no effect on platelets.

**Platelet-macrophage-vessel wall**

Some investigators have hypothesized that oxidized LDL may promote atherogenesis. J.A. Berliner and colleagues from the UCLA Medical School have found that minimally oxidized LDL can alter endothelial cells in several ways that might contribute to thrombogenesis. She suggested that minimally oxidized LDL may facilitate the formation of foam cells, which in turn may damage the endothelium to which platelets adhere or interact with platelets as they migrate across the endothelium. Either process could initiate thrombus formation. Berliner noted, however, that it is uncertain whether antioxidants suppress or promote atherosclerosis.

F.A. Fitzpatrick of the University of Colorado Health Science Center suggested that the mechanism whereby dietary EPA may inhibit platelet activity is by replacing arachidonic acid in the platelet membrane and acting as an alternative substrate for enzymes that form eicosanoids. Specifically, EPA is a poor substrate for the enzyme cyclooxygenase in platelets, so there is little formation of an important proaggregatory substance, prostaglandin (PG) endoperoxide \( H_2 \).
J.B. Lefkowith from the Washington University School of Medicine reported on cell culture studies indicating that EFA deficiency impairs the ability of macrophages to spread and adhere and thereby exert their antiinflammatory effect. Addition of arachidonic acid to the medium apparently restores the normal spreading and adhering ability of these cells.

R.E. DiCorleto of The Cleveland Clinic Foundation discussed results of cell culture studies involving the production of platelet-derived growth factor (PDGF) by bovine aortic endothelial cells in the presence of various fatty acids. PDGF promotes the growth of vascular smooth muscle cells and thus may stimulate the development of atherosclerosis. Addition of MaxEPA to the medium greatly inhibited the production of PDGF. Safflower oil also inhibited PDGF production but to a much lesser degree. DiCorleto suggested that the reported beneficial effects of fish oils on coronary artery disease may be due in part to decreased PDGF production (and subsequently decreased intimal thickening) rather than solely to changes in plasma lipids or coagulation parameters.

**Lipoprotein(a) and thrombosis**

Lipoprotein(a) [Lp(a)] is a low-density lipoprotein-like particle whose levels in plasma correlate both with atherosclerotic lesions and with increased risk of thrombotic events. As such, Lp(a) has been thought of by some as a “link” between atherosclerosis and thrombosis. S.V. Pizzo of the Duke University Medical Center suggested that Lp(a) may be involved in the regulation of fibrinolysis (the breakup of clots) and that the association between the increased incidence of thrombogenesis and elevated levels of Lp(a) may be due to suppression of normal fibrinolytic activity. He noted that Lp(a) competes with plasminogen, the precursor of the fibrinolytic enzyme plasmin, for vascular binding sites. Lp(a) displacement of plasminogen from these cellular binding sites may prevent plasminogen from interacting with plasminogen activators and thereby inhibit the rate of plasmin generation.

K.A. Hajjar of Cornell University Medical College added that this inhibition of plasminogen binding by Lp(a) may be the basis for regulating plasminogen generation at the endothelial cell surface. She also indicated that the tendency toward thrombosis, as indicated by elevated levels of Lp(a), may predispose an individual to atherosclerosis by the release of growth factors that can precede the formation of atherosclerotic lesions.

At this time, it is not known how blood levels of Lp(a) are regulated and, specifically, whether dietary factors can influence these levels.

**Thrombogenic potential of long-chain fatty acids**

J.M. Iacono of the USDA Western Human Nutrition Research Center expressed his view that the diet may influence platelet aggregation. He noted that in a study of farmers in Finland, Northern Italy and the United States, those with the highest intake of saturated fatty acids (the Finns) had the most hyperactive platelets to the agonist collagen but also the least active to epinephrine. There was no explanation for this apparent inconsistency in reactivity toward two different agonists, and such results (observed by others) have raised questions about the value of platelet aggregation as a measure of thrombotic risk. According to G. Hornstra from the University of Tromsø (Norway) noted that dietary fatty acids, particularly ω-6 and ω-3 polyunsaturated fatty acids, may have profound effects on the tendency to thrombosis in man. He described how some recent clinical work in which addition of ω-3 fatty acids to a low-fat diet resulted in decreased urinary excretion of proaggregatory thromboxane B2, thus suggesting that a low-fat, high ω-3 fatty acid diet may reduce clotting tendency. According to Nordøy, the optimal dietary concentrations of ω-6 and ω-3 fatty acids in relation to platelet function have not been defined. Some studies have indicated that an intake of ω-6 fatty acids above 10% of calories may abolish reported beneficial effects of ω-3 fatty acids on platelet function.

S. Renaud from the INSERM Unit in Bron, France, expressed his view that in previous coronary prevention trials, apparent benefits (such as increases in survival) occurring effectively within a year are most likely acting through changes in thrombosis rather than through changes in total cholesterol level. He presented results of epidemiological studies suggesting that intake of saturated fatty acids, and particularly palmitic and stearic acids, can be correlated with coronary heart disease and platelet reactivity. In the case of coronary heart disease, dietary calcium may reduce the association with palmitic and stearic acids, possibly by enhancing the excretion of these fatty acids. The fatty acids 20:3 (ω-9) and 22:3 (ω-9) present in plasma and platelet phospholipids and derived from dietary stearic acid by desaturation and elongation have been found to be positively related to platelet reactivity to the agonists thrombin and ADP. Furthermore, the
Clinical correlates
Vasospasm is an important clinical problem in patients with atherosclerosis. According to D.D. Heistad of the University of Iowa College of Medicine, one hypothesis to explain this effect is that platelets may adhere to atherosclerotic plaques and release serotonin and thromboxane A2 (which are vasoconstrictors) as well as ADP (which is a vasodilator). Recently he and his colleagues found that activation of platelets in vivo or infusion of serotonin produced vasodilation in normal monkeys and vasoconstriction in atherosclerotic monkeys. Regression of atherosclerosis restored the normal vascular responsiveness to serotonin. In addition, activation of leukocytes, which contain vasoactive substances, has resulted in vasoconstriction in atherosclerotic monkeys. Heistad added that dietary ω-3 fatty acids improve vessel relaxation in normal and diabetic animals, in patients with coronary artery disease, and in patients with heart transplants. On the other hand, there is no evidence that saturated fatty acids or ω-6 polyunsaturated fatty acids contribute to the abnormal responses of atherosclerotic arteries.

Discussing possible mechanisms for hemostasis (the arrest of bleeding) and thrombosis (the occlusion of a blood vessel), K.G. Mann of the University of Vermont suggested that the blood coagulation reaction system in man is continuously "on," with the products of the various reactions being neutralized by numerous inhibitors that regulate the clotting process. He believes that for most people, the blood coagulation response is normal and that the underlying cause of seemingly abnormal coagulation responses is "damage" (mechanical or cellular) to the vascular endothelial surface. This damage results in a series of cellular adhesive reactions involving platelets and potentially other inflammatory cells. The actual coagulation mechanism proceeds through the transformation of inactive to active proteolytic enzymes that give rise to the active blood clotting complex. Genetic and dietary factors may affect the underlying lesions, which can result in vasoocclusive events, such as a heart attack.

Conclusions, future directions
Among the general conclusions and areas of future research that could be drawn from the workshop are the following:

- More evidence in humans is needed to directly relate atherosclerosis (including elevated blood cholesterol levels) and thrombotic risk. An important research need is validation of suitable surrogate endpoints for measuring thrombotic risk.
- Current methods for assessing clotting risk may not lend themselves reliably to large-scale dietary trials. These methods must be applied with care. Measurement of platelet aggregation, for example, may be a good indicator of platelet inhibition, but not a reliable indicator of platelet activation. Research that contributes to the advancement of valid methodology is needed.
- The anti-thrombotic effects of ω-3 fatty acids have been demonstrated, and mechanisms related to eicosanoid metabolism and platelet-activating factor synthesis have been proposed. There appears to be no direct evidence that dietary long-chain saturated fatty acids are thrombogenic to humans. Although some epidemiological evidence suggests that saturated fatty acids may play a role in thrombotic events, more research is needed to establish whether there is a relationship to thrombotic risk and to elucidate a possible mechanism of action.

(The proceedings of the workshop will be published as a supplement to the American Journal of Clinical Nutrition.)

α-Linolenic focus of EFA workshop

The Department of Nutritional Sciences at the University of Toronto has hosted Essential Fatty Acid (EFA) workshops in May of 1987 and 1989. The Third Toronto EFA Workshop was held at the University of Toronto, May 17-18, 1991. The objective of the workshops has been to bring together a group of 50-80 researchers and students studying nutrition, metabolism, biochemistry or clinical aspects of EFA for two days to hear about and discuss important concepts and recent research in this field.

Eight-five participants from four continents participated, listening to invited speakers from Europe, the United States and Canada addressing this year's topic: α-Linolenic acid in Human Nutrition and Disease. This subject was chosen because of the importance of ω-3 fatty acids in human nutrition and health and because, despite being the precursor of the ω-3 family, the function of α-linolenic acid is one of the least understood of the EFA.

Stephen Cunnane opened the workshop with three questions: (a) Is α-linolenic acid metabolized to longer chain ω-3 fatty acids in humans? (b) Does α-linolenic acid need to be metabolized to longer-chain ω-3 fatty acids to be nutritionally or metabolically active? and (c) Does the high rate of α-linolenic acid oxidation in vivo pose a conceptual dilemma (destruction of an essential nutrient) or is its oxidation of biological importance? Harold Cook of Dalhousie University, Halifax, Nova Scotia, followed with an in-depth look at the significance of α-linolenic during brain development and noted that very early in post-natal development, Δ6 desaturase activity in the rat brain is actually more active than in the liver and may therefore contribute significantly to the brain's need for arachidonic and docosahexaenoic acids through synthesis in situ. However, prenatally and in later life, the liver undoubtedly contributes preformed

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docosahexaenoic and arachidonic acids for brain membrane development. Patricia Wainwright of Waterloo University, Waterloo, Ontario, illustrated the difficulties facing developmental biologists in establishing a definitive role for longer chain ω-3 fatty acids in behavioral and neurological development, this in spite of clear evidence in well-controlled studies of modification of brain fatty acid composition in response to changes in ω-3 intake.

Kristian Bjerve of Trondheim, Norway, reviewed data showing that, in elderly patients, long-term total parenteral nutrition devoid of ω-3 fatty acids has clinical consequences relating to both dermal lesions (folliculitis of the scalp) and an altered immune response, both of which can be normalized by small doses of pure α-linolenic acid. His recent studies in infants suggest that plasma docosahexaenoic acid levels are directly related to early behavioral development. Peter Singer of Lindenfels, Germany, reported a series of studies involving flaxseed oil supplementation in Defined classes of hyperlipidemia and in hypertension. Despite little evidence of ω-3 fatty acids in flaxseed oil (56 wt %) being converted to longer chain ω-3 fatty acids in plasma lipids, flaxseed oil was effective in lowering serum lipids and blood pressure, thus reiterating a clinically beneficial role for α-linolenic acid itself.

Manohar Garg of the University of Alberta, Edmonton, Alberta, opened the biochemistry session and demonstrated the importance of the ω-6/ω-3 ratio in modulating the effects of ω-3 fatty acids in arachidonic acid synthesis; when the ω-6/ω-3 ratio is high, the inhibition of ω-6 fatty acid metabolism by α-linolenic acid is much less effective. Also of potential clinical importance was the observation in rats that although both ω-3 fatty acid and linoleic acid lowered serum cholesterol in rats, linoleic acid, but not α-linolenic acid, was associated with increased liver cholesterol. Daniel Hwang of Louisiana State University, Baton Rouge, Louisiana, followed with an elegant review of the relation between α-linolenic acid and eicosanoid production in vivo. Again, the ω-6/ω-3 ratio as opposed to the total amount of these fatty acid classes seems to be the main determinant of eicosanoid modulation by α-linolenic acid. Using deuterated substrates and monitoring deuterated end-products in plasma lipids over 48 hours, Ed Emken, of the U.S. Department of Agriculture (USDA) in Peoria, Illinois, reported evidence that α-linolenic acid conversion to longer chain ω-3 fatty acids in healthy omnivorous human volunteers was greater than that of a similar amount of deuterated linoleic acid. Darshan Kelley of USDA in San Francisco, California, discussed the potential modulation of immune function by α-linolenic acid and reviewed recent evidence for specific immunomodulatory effects of flaxseed oil in humans.

The applied nutrition session was opened by Bruce Holub of University of Guelph, Guelph, Ontario, who reiterated regulatory/marketing issues originating from the presence of trans fatty acids in cooking oils several of which, prior to hydrogenation, may have appreciable amounts of α-linolenic acid. If α-linolenic acid is bred out of oilseeds so that trans fatty acid intake can be reduced, dietary intake of α-linolenic acid will drop further with undesirable clinical ramifications. Terry Dick of the University of Manitoba, Winnipeg, reported on the essentiality of α-linolenic acid to fish and on progress in development of fish feeds containing flaxseed as a source of α-linolenic acid for the aquaculture industry. Jeong Sim of University of Alberta, Edmonton, closed out this session with a lively review of studies on enrichment of ω-3 fatty acids in chicken meat and eggs using flaxseed as a source of α-linolenic acid. These latter two presentations suggested that dietary α-linolenic acid for human consumption need not be provided as an oil but can be incorporated into other premium quality foods contributing to a healthy diet with a higher-than-average ω-3 content.

The workshop concluded with a poster session in which new data concerning α-linolenic acid were reported: an alternative pathway of synthesis of longer chain ω-3 fatty acids involving retroconversion (Anne Coble-Voss and colleagues; Ohio State University, Columbus, Ohio); the anti-malarial effect of α-linolenic acid (Orville Levander and colleagues, USDA Beltsville, Maryland); the potential anti-tumor effects of flaxseed (Lilian Thompson and Maria Serraino, University of Toronto); effects of long-term low level fish intake (Andrew Sinclair and colleagues, Geelong, Australia); nutritional effects of perilla oil (Yang Lee-Kim and colleagues, Yonsei University, Seoul, Korea) and balance studies describing α-linolenic acid oxidation/depletion during short- or long-term energy deficit in pregnancy (Stephen Cunnane and colleagues, University of Toronto).

The Third Toronto EFA Workshop clearly emphasized that α-linolenic acid can be a source of longer chain ω-3 fatty acids in humans and can be active in lowering cardiovascular risk factors as well as in modulating immune function. Whether its rapid oxidation relates to these properties remains to be determined. (The proceedings of the workshop will be published in full in The American Journal of Clinical Nutrition during late 1991. Financial support for the workshop was received from the Ontario Ministry of Health, Health and Welfare Canada, Carnation Nutritional Products, T.J. Lipton Inc., Nutricia B.V., Flax Council of Canada, Wyeth Ltd., Canola Council of Canada and Arctic Fish Technologies.)