

**Editorial**

**Cardiovascular Effects of Fish Oils**

**Beyond the Platelet**

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There has been considerable interest recently in the potential of fish oils to prevent coronary artery disease. The article by Braden et al, "Dietary Fish Oil Accelerates the Response of Coronary Thrombolysis With Tissue Plasminogen Activator: Evidence for a Modest Platelet Inhibitory Effect In Vivo" in a recent issue of *Circulation* is another in a series of rigorous studies on the effects of fish oils on experimental thrombosis performed by these Vanderbilt University investigators. They have nicely demonstrated that feeding high levels of the two important polyunsaturated fatty acids of fish oils, namely eicosapentaenoic acid (EPA) (C20:5ω-3) and docosahexaenoic acid (DHA) (C22:6ω-3), significantly shortens the time for recombiant tissue-type plasminogen activator (rt-PA) to cause reperfusion in thrombosed coronary arteries in dogs. However, the high intake of fish oils failed to delay the development of an occluding thrombus when the endothelial surface of a cannulated coronary artery was injured electrically and failed to reduce the incidence of reocclusion as compared with control animals fed the usual dog chow.

The study was designed, as the investigators state, "to determine whether fish oils inhibited platelet-dependent events in vivo." It was nicely designed for that purpose. However, that is not to say that it was ideally designed to isolate the effects of only thromboxane and prostacyclin on the parameters measured. In any in vivo manipulation that results in a blood clot and its lysis, one must consider the potent effects of thrombin on platelet aggregation, as well as those of thromboxane. Thrombin is a more aggressive aggregator of platelets than is thromboxane. Aspirin will inhibit platelet aggregation caused by thromboxane, but thrombin will aggregate platelets even in the presence of aspirin except at very low concentrations.

In a recent study, Fitzgerald, Wright, and FitzGerald used this same canine model to examine the effect of a dose of aspirin that was selected to achieve a more than 95% inhibition of serum thromboxane B$_2$ (TXB$_2$) in the dog. Compared with the effects of the dose of fish oil chosen in this study, which inhibited serum TXB$_2$ by 88%, they found the effects of fish oil at this dose were comparable to those of the dose of aspirin selected for the previous study. The modest effects of both agents in this model are probably due to the effects of thrombin and other platelet aggregators, for example, ADP and collagen. Clinical experience with the high rate of restenosis after coronary angioplasty despite large or small doses of aspirin confirms the limited role of thromboxane in such circumstances.

By focusing their research on comparing effects of fish oils with those of aspirin on cyclooxygenase activity, these Vanderbilt University investigators have clarified the relative merits of these two very different chemical and pharmacological agents on thromboxane and prostacyclin biosynthesis under a variety of clinical and experimental conditions. Their studies, like the one recently reported, are carefully designed to exclude measurements of actions of fish oils other than those on platelet activity and on thromboxane and prostacyclin biosynthesis. They have maintained a conservative tone in their cautious evaluation of the possible merits of fish oils in the treatment of cardiovascular disease. Their conservatism is a needed reminder that there is much we don't yet know about the effects of fish oils in humans and that much remains to be carefully evaluated.

It is the many effects of fish oils not produced by aspirin, however, that add greatly to the interest in fish oils as potential preventive or therapeutic agents for atherosclerotic diseases. Unlike the action of aspirin, fish oils can increase prostacyclin activity by adding the active trienoic prostaglandin I$_3$ (PGL$_3$) to PGL$_2$, which is only slightly, if at all, reduced by fish oils. When one looks beyond the effects of fish oils on cyclooxygenase, there are increasing numbers of documented actions of EPA and DHA, which should be antiatherogenic. When ingested, these fatty acids from fish oils are incorporated into the sn-2 position in cell membrane phospholipids, displacing arachidonic acid (AA) (C20:4ω-6), which is derived from vegetable oils. The major demonstrated effects of the fish oil fatty acids are briefly reviewed.

**Leukotrienes**

Leukotrienes are potent proinflammatory and immunoactive factors. Leukotriene B$_4$ (LTB$_4$), pro-
duced from AA, is a chemoattractant for neutrophils. LTB₄ also causes neutrophils to become active and to aggregate. LTB₅, produced from EPA, on the other hand, is formed in only small amounts and has little physiological effect, so when EPA replaces AA in cell membrane phospholipids, the inflammatory component of atherosclerosis is diminished.

Platelet-Derived Growth Factor

Once platelets aggregate at a site of an injury to the endothelial cells lining the arteries, they release several factors, including platelet-derived growth factor (PDGF), a potent chemoattractant and mitogen responsible, in part, for the migration of smooth muscle cells from deeper layers of the arterial wall to the site of endothelial injury or dysfunction. PDGF, along with other factors, also causes circulating monocytes to migrate to the site of endothelial injury, to multiply, and to change into tissue macrophages, which are scavenger cells in the developing atherosclerotic process. Not only platelets but also the other cell types that participate in the development of atherosclerosis (i.e., endothelial cells, monocytes or macrophages, and smooth muscle cells) produce PDGF-like growth factors that stimulate cell growth in the arterial wall. When the low density lipoprotein (LDL) cholesterol level in the blood is elevated, it is primarily the macrophages that ingest the cholesterol to form the foam cells that contribute to the atherosclerotic plaques; these plaques gradually increase in size and narrow the blood vessels in the heart, brain, and periphery.

Fox and DiCorleto have shown that incorporating EPA into cultured aortic endothelial cells markedly reduces their production of PDGF-like protein.

Oxygen Free Radicals

An important means by which neutrophils and monocytes fight infection is to attack invading bacteria or foreign cells with lethal oxygen free radicals. These highly reactive oxygen species, however, can damage normal cells and in fact are thought to be responsible in part for the cellular debris component of the atherosclerotic plaque. Their action on the LDL particle is essential to convert that cholesterol-rich lipoprotein to a form recognizable by the receptors on the macrophages in the growing atheroma. Without exposure to oxygen free radicals and the changes they induce in the apoprotein, LDL is endocytosed only in small amounts by macrophages and foam cells do not form.

Fisher and associates have shown that feeding humans fish oils results in a large reduction in oxygen radical formation by their neutrophils and monocytes when these cells are activated in vitro. Whether oxygen free radical production is likewise depressed at its many other sources in our bodies, for example, cytochromes and oxidase reactions, is not yet known. This is an important issue because the more highly unsaturated fatty acids in fish oils are more vulnerable to damage from oxygen free radical attack than is AA.

Platelet Activating Factor

Platelet activating factor (PAF) is a phospholipid molecule now known to have many widespread physiological effects, largely adverse. At least one of these, the activation of platelets (as its name indicates), contributes to atherogenesis, as described above. PAF can be synthesized by several different cell types. It has been shown in humans that the production of PAF by monocytes is markedly inhibited when the ω-3 fatty acids are incorporated in their membrane phospholipids, replacing AA in alkyl phosphatidyl choline, the precursor molecule for PAF formation.

Interleukin-1 and Tumor Necrosis Factor

Interleukin-1 (IL-1) is a peptide molecule produced by several of the cell types discussed here that are incriminated in the development of atherosclerosis. It, together with another peptide factor, tumor necrosis factor (TNF), is responsible for several effects that are atherogenic: stimulate synthesis of adhesion molecules (proteins that cause monocytes to adhere to endothelial cells), stimulate production of cytotoxic oxygen free radicals by neutrophils and monocytes, and activate platelets, neutrophils, and monocytes. Again, it has been shown that feeding fish oil supplements to humans reduces the production by their cells of both IL-1 and TNF. So far, there has been no report suggesting that such damping-down of the immune system by fish oils creates an increased risk of infections or cancer.

Fibrinogen

Fibrinogen, another factor in the blood clotting cascade, has been identified as a cardiac risk factor when its blood level is increased and is also decreased by dietary fish oil.

Endothelium-Derived Relaxing Factor

Endothelial cell–derived relaxing factor (EDRF), as its name implies, promotes relaxation of arterial smooth muscle cells and diminishes constriction of arteries when they are exposed to several physiological and pharmacological vasoconstrictor agents. These actions of EDRF are enhanced by supplementing the diets of experimental animals with fish oils. Malis and associates have found that these potentially antiatherosclerotic actions persist even after exposure of arteries to anoxic conditions, as may occur with decreased blood flow during and after a heart attack. EDRF (nitric oxide) also acts with prostacyclin to maintain the antithrombogenic status of the endothelial surface of arteries.

Plasma Lipids

The causal role in atherogenesis of elevated LDL cholesterol levels in plasma is well established. The most consistent effects of fish oils on serum lipids and lipoproteins have been reductions in levels of serum triglycerides and very low density lipoprotein.
(VLDL). The effects, however, on LDL cholesterol have been variable. No significant effect of fish oil consumption (2–3 g/day EPA and DHA) on cholesterol levels of individuals with single LDL cholesterol elevations (phenotype 2A) has been shown, and increases in LDL cholesterol and apolipoprotein B with fish oils have been reported. Harris has reviewed the many reported studies that examined the effects of fish oil supplements on plasma lipid levels and concludes that there are only small and variable effects on total and LDL cholesterol levels reported. Small increases in high density lipoprotein (HDL) cholesterol were shown in the reports he reviewed. Only if the previous diet was high in saturated fat were significant reductions in LDL cholesterol seen when the saturated fats were replaced by fish oils. This is an action of polyunsaturated fatty acids that fish oils share with polyunsaturated fatty acids of vegetable origin and with monounsaturated fats as well, although the fish oils are quantitatively more effective in this action. From this experience in humans it would seem that fish oils exert little, if any, beneficial effect on plasma lipids. However, it has been shown in nonhuman primates that dietary fish oils will reduce the size of the LDL particle and lower its critical melting temperature, effects thought to be antiatherogenic. No comparable studies on the physical properties of lipoproteins in humans fed fish oils have been reported.

Other Effects

There are other physiological or pharmacological effects of fish oils that are potentially antiatherogenic for which the causative factor(s) have not yet been identified, including 1) decreasing the propensity of rat hearts to fibrillate when made ischemic—a potentially important action if reproducible in humans in light of the many acute arrhythmic deaths that occur with heart attacks; 2) decreasing blood viscosity by increasing deformability of red blood cells, which may improve blood flow through narrowed arteries; 3) decreasing blood pressure moderately in normal and mildly hypertensive subjects; 4) decreasing the rate of loss of serum albumin from the vascular compartment in diabetics (whether this endothelial leakiness to plasma proteins in diabetics contributes to the unusually high incidence of atherosclerotic diseases among diabetics is not clear, but such protein leakage does correlate clinically with the occurrence of the degenerative vascular complications of diabetes); 5) enhancing the thrombolytic activity of t-PA; 6) increasing vascular compliance; and 7) decreasing the vascular response to norepinephrine.

Animal Studies

It has been shown that fish oils added to the diet will inhibit intimal hyperplasia and atherosclerosis in animals made hypercholesterolemic by diets high in saturated fats and cholesterol. This has been clearly demonstrated in rabbits, dogs, swine, and monkeys. In rabbits and in rats, however, increased intimal foam cells and increased monocyte adhesion to endothelial surfaces, respectively, have also been reported. The protective action of the ω-3 fatty acids occurs with no or little improvement in blood lipid levels, indicating, as with aspirin, that the effects are at the level of the vessel wall, not the blood lipids. But whereas aspirin only reduces thromboxane production, the ω-3 fatty acids have multiple potentially beneficial effects.

Human Studies

Epidemiological studies among the Greenland Eskimos and the Japanese show an association of low mortality from coronary heart disease with a high consumption of marine animals. Three retrospective studies of diet and heart disease mortality show an inverse relation between the quantity of fish ingested and the mortality from coronary heart disease. As few as two to three fish meals per week appeared to reduce mortality from heart attacks by as much as 50%. Two other retrospective dietary studies, however, failed to show a protective effect of fish consumption.

Recently, the first prospective, randomized clinical trial on the effects of fish on mortality from coronary artery disease was reported. Subjects who had a very recent heart attack were randomized either to advice to each fish two to three times a week or to no such advice. With slightly more than 1,000 subjects in each group, the results in 2 years showed a 29% reduction in both coronary heart disease mortality and all-cause mortality in the group advised to eat fish. There was no reduction in the occurrence of heart attacks in the fish-eating group, but fewer of the heart attacks were fatal. The beneficial effects of eating fish in this study may therefore reflect the demonstrated action of fish oil supplements to prevent ventricular fibrillation in experimental myocardial infarction. The effects described are generally ascribed to the fatty acid content of fish. Several of the cellular effects have been reproduced with the purified long chain polyunsaturated ω-3 fatty acids in fish oils, EPA and DHA. Nevertheless, benefits have been reported from eating fish that would have provided such small amounts of these fatty acids as to leave a lingering suspicion that other constituents in the fish may also be contributing to the effects.

The important study by Braden and associates can now be seen in context of the array of documented actions of fish oils. Their implied expectation that ingested fish oils should be able to prevent clot formation, to markedly shorten the time to reperfusion, or to prevent reocclusion in their experimental model to be of therapeutic merit raises interesting considerations. When we are evaluating an agent to cure a bacterial infection, we judge both its effectiveness in eradicating the bacteria and its toxicity to the host. But atherosclerosis and the degenerative diseases of aging are a different matter. They are due to misuse by the body’s own physiological mechanisms designed for its own protection. It is an appropriate
and protective response to endothelial injury that platelets, monocytes or macrophages, and smooth muscle cells aggregate at the site of injury; release thromboxane, PDGF, and other factors that cause vasoconstriction; and initiate an occlusive clot. This response, under the usual conditions in which our species developed, was designed to prevent exan- guinating hemorrhage from injury to a blood vessel. But atherosclerosis represents an apparent overreaction of the body to this injury. Perhaps an elevated LDL cholesterol available to be deposited into mac- rophages that have been marshalled to the site of endothelial injury to clean up cell debris and extravasated blood elements is the continued irritant that keeps the process going and prevents a self-limited healing process.

My point is that we should not be looking for therapeutic or preventive agents that would eradicate the clotting mechanism or the process of wound healing. The therapeutic results would then be worse and more immediate than the disease we are trying to cure or prevent. With atherosclerosis, as with the autoimmune diseases, we need to curb the body’s overly exuberant response to injury, not to abolish its responses. Thus, ideally, we should be seeking agents that will tactfully nudge the body back into healthful rather than self-destructive applications of its normal homeostatic responses. Not acting on a single aspect of the body’s response to endothelial injury, but modulating the entire cascade of factors involved in atherogenesis—curbing the excesses and correcting the deficiencies—would seem to be the appropriate approach. It is in this manner that fish oils seem to affect the atherosclerotic process. Perhaps the “mod- est platelet inhibitory effect in vivo” that these investiga- tors demonstrated is all the effect on platelets we should expect, or ask, of the fish oils.

Thus, there seems to be an increasing basis for optimism regarding the potential of fish oil ω-3 polyunsaturated fatty acids to prevent atherosclerotic disease and its main clinical manifestation, myocardial infarction. More clinical trials are needed, how- ever, before there can be certainty that all the pharmacological and physiological effects demonstrated for the ω-3 polyunsaturated fatty acids of marine animals do, in fact, add up to definitive clinical prevention of atherosclerotic diseases.

References

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