Fish Oil and the Prevention and Regression of Atherosclerosis

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Summary. Epidemiological studies in the seventies have put forward that dietary rather than genetic factors are responsible for the lower incidence of ischemic heart disease in Greenland Inuit and have generated a large body of both in vitro and in vivo experimental studies, exploring the putative favorable effects of fish (oil) on atherogenesis and its risk factors. The first part of this report reviews the in vivo animal studies, concentrating on the hypercholesterolemic models and the arterialized vein graft model. In the hypercholesterolemic animal studies, the results are inconclusive as the studies reporting a protective effect are matched by the number of studies showing no effect or an adverse effect. The diversity in species, dose of fish oil, duration of study, type of vessel studied and type of fish oil preparation (content of n-3 fatty acids, unesterified n-3 fatty acids, ethylesters or triglycerides) could all contribute. Furthermore, the definitions and criteria used in the literature to evaluate atherogenesis are diverse and it appears that while one parameter is affected, another is not necessarily modified in the same direction, stressing the importance of extending the analysis of the effects on atherogenesis to more than one parameter. We also believe that it is time to reach a consensus as to which animal model mimicks most closely a particular human situation. Only in appropriate models, investigating more than one atherosclerosis variable, can the effects of a putative anti-atherogenic drug or diet be verified. In the veno-arterial autograft model, mimicking the patient after coronary bypass grafting, dietary fish oil has been consistently effective in preventing accelerated graft intima proliferation. It could therefore be of interest to evaluate the effects of fish oil on graft patency in patients after coronary bypass surgery after a period of years.

The results from studies on restenosis after percutaneous transluminal angioplasty are also reviewed and it is concluded that the two large scale trials, that are currently underway, might reliably answer the question whether fish oil is effective as a non-pharmacological adjuvant in the prevention of restenosis.

Lastly, the studies on the effects of fish oil on the regression of experimental atherosclerosis are reviewed. In view of the small number of studies (i.e., four) investigating the effects of fish oil on the regression of atherosclerosis, it is premature to draw any conclusion, and therefore further experimental work is required.

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In the seventies it was shown that a cohort of Eskimos (Inuit), living in the Uummannaq district on the Westcoast of Greenland had low levels of plasma cholesterol, triglycerides and very low density lipoprotein (VLDL) and high levels of high density lipoprotein (HDL) [1]. Furthermore the official mortality statistics showed that the incidence of ischemic heart disease was low among Greenlanders. It must be kept in mind, however, that these mortality statistics are not very reliable as in Greenland most deaths occur at home or in small hospitals where diagnostic facilities are poor. The data do also not differentiate between native Greenlanders (80%) and Danes. The establishment of a computerized death register for Greenland has yielded a more specified judgment of the mortality data [2] and ischemic heart disease as a cause of mortality indeed seems to be lower among Eskimos living in Greenland (6.7% among males aged 45–64) than among Eskimos living in Denmark (32.8%) in the years 1979–1983. These findings have been attributed to the dietary habits of the Greenland Eskimos who traditionally consume about 400 g of seal and fish per day. Although only 130 Eskimos were included in those early studies [1] and no direct proof of the lower incidence of coronary artery disease was offered by means of coronary angiography or otherwise, the observation of low mortality from ischemic heart disease among Greenland Eskimos in combination with their low risk plasma lipid profile has initiated a considerable amount of studies investigating the effect of the long chain polyunsaturated n-3 fatty acid containing fish (oil) products on risk factors of atherogenesis. With respect to the other accepted risk factors for ischemic heart disease, it is important to note that the Eskimo's diet is low in saturated fat, the Eskimos are rarely obese, hypertension is uncommon,
and diabetes mellitus is unknown. On the other hand, most Eskimos are heavy smokers.

**Fish Oil and Factors Affecting Atherogenesis**

N-3 polyunsaturated fatty acids have their first double bond at the third carbon atom from the methyl end of the fatty acid. The very long chain fatty acids from the n-3 family, eicosapentaenoic (20:5n-3 or EPA), docosapentaenoic (22:5n-3 or DPA) and docosahexaenoic (22:6n-3 or DHA) are synthesized by algae and phytoplankton, organisms that are at the bottom of the marine food chain. All marine life may, therefore, ultimately be enriched with these fatty acids which may provide the required degree of unsaturation that allows cell membranes to remain fluid in cold water. Incorporation of dietary n-3 polyunsaturated fatty acids (20:5n-3 and 20:6n-3) in blood cell, vascular endothelial and vascular smooth muscle cell membrane of men leads to specific displacement of n-6 polyunsaturated arachidonic acid (20:4n-6). N-3 fatty acids are poor substrates for cyclooxygenase and compete with 20:4n-6 at the level of cyclo-oxygenase and lipoxygenase thereby generating a different family of prostanoids and leukotrienes which have weak or almost no activity as compared to their 20:4n-6-derived analogues. This interference of n-3 fatty acids with eicosanoid metabolism may alter vasomotor tone and thrombotic and inflammatory responses that are critical to plaque formation following endothelial injury. In addition to the effects on intracellular mediators such as prostaglandins and leukotrienes, more recent evidence shows that n-3 fatty acids also interfere with the phosphatidylinositol (PI) cycle [3-5]. This is an intracellular signal transduction pathway that links various stimuli such as mechanical stress, hormone- and growth factor-receptor interaction to their responses at the transcriptional level through the formation of the protein kinase activators Ca\(^{2+}\)-calmodulin and diacylglycerol [6-8]. Many processes generally believed to play a role in atherogenesis, such as expression of leucocyte adhesion molecules [8], mechanical stress responses of endothelium [7], secretion of "endothelial-derived factors" [9] and mitogenic responses of vascular smooth muscle cells [6] involve operation of the PI cycle, thereby providing n-3 fatty acids an additional mechanism to interfere in the atherosclerotic process.

In Table 1, by citing references 10-39, we have summarized the known effects of n-3 fatty acids on functions of various cell types and levels of certain blood constituents, all influencing the processes believed to be involved in atherogenesis. These processes, intima hyperplasia and lipid infiltration, thrombosis, vascular smooth muscle tone and inflammation are depicted horizontally, while the cells (endothelial cells, vascular smooth muscle cells, platelets, neutrophils and monocytes and blood constituents (lipoproteins and coagulation factors) that are involved in these processes are listed vertically (Table 1). At the sites where the columns and rows meet the known effects of n-3 fatty acids are mentioned. These effects, particularly the inhibition of platelet aggregation and the lengthening of bleeding time, have been reviewed several times [40-47] and we have therefore limited ourselves to discuss the effect of fish oil on the prevention and regression of atherosclerosis in different in vivo animal models and to review the effect of fish oil on restenosis following percutaneous transluminal coronary angioplasty.

**Animals used in the study on atherogenesis**

In studying atherosclerosis the choice of the species is important [48]. Rabbits have been used extensively in the study of atherosclerosis, but it is important to note that rabbits do not develop atherosclerosis naturally. However, when fed diets containing high levels of cholesterol massive amounts of cholesterol accumulate in the tissues and in the arterial wall [49]. In this species, the aortic arch and the intramural myocardial arterioles are affected [49] whereas in humans the abdominal aorta and the proximal main coronary arteries are involved. Importantly, the lesions in rabbits are chiefly fatty and complicated features such as fibrosis, ulceration and thrombosis seldom occur. When rabbits are fed casein in addition to saturated fat they do develop advanced lesions located in the abdominal aorta [50]. Swine do develop atherosclerosis spontaneously from 6 months of age onward (although serious lesions are only seen after 6-7 years) with the early occurring lesions closely resembling human lesions [51,52], and the location (abdominal aorta and proximal coronary arteries) being similar to that in man. Severe and accelerated atherosclerosis can be achieved in pigs by combining hypercholesterolemia with endothelial injury [53,54]. Dogs, on the other hand, hardly develop atherosclerosis, even after changing to a high cholesterol diet. Only after additional interventions, such as removal or destruction of the thyroid gland, lesions become severe and their severity are highly correlated with the degree of hypercholesterolemia. The location of the lesions in the arterial tree is similar to that in humans, but there is much more medial involvement than in other species and man [55]. Diet-induced atherosclerosis in rhesus and cynomolgus monkeys most closely resemble human atherosclerosis both anatomically and morphologically [56,57]. Nevertheless, it must be noted that also in this species there are hyper- and hyporesponders to a high cholesterol diet while the extent of atherosclerosis also varies considerably. These findings, however, appear to be characteristic for all species including man.