FISH OILS IN HEALTH AND DISEASE:

LESSONS FROM THE ESKIMOS

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I INTRODUCTION

Cod-liver oil, the old traditional household remedy, that for decades has been used as a dietary source for vitamins A and D, may be enjoying a resurgance of importance due to the discovery of new health properties. There is reasonably good evidence to suggest that consumption of fish may provide some protective effects against the development of coronary heart disease. Epidemiological studies have indicated that atherosclerotic vascular disease is remarkably uncommon amongst Greenland Eskimos. This apparent cardiovascular protection may be related to their salutary lipid and hemostatic patterns that probably reflect their high fish oil intake.

This Grand Rounds presentation will attempt to explore the whole spectrum of actions of the marine omega-3 polyunsaturated fatty acids upon lipid and lipoprotein levels as well as prostanoid and leukotriene metabolism. Particular attention will be paid to their theoretical antinflamatory or immunosuppresive actions and also to their effects on platelet function and thrombosis. The perspective of dietary fish oil supplements as an aid in the prevention of atherosclerosis is very tempting and provocative. The therapeutic potential of these supplements in hyperlipidemic states, hypertension, diabetes mellitus and autoimmune diseases will also be discussed.

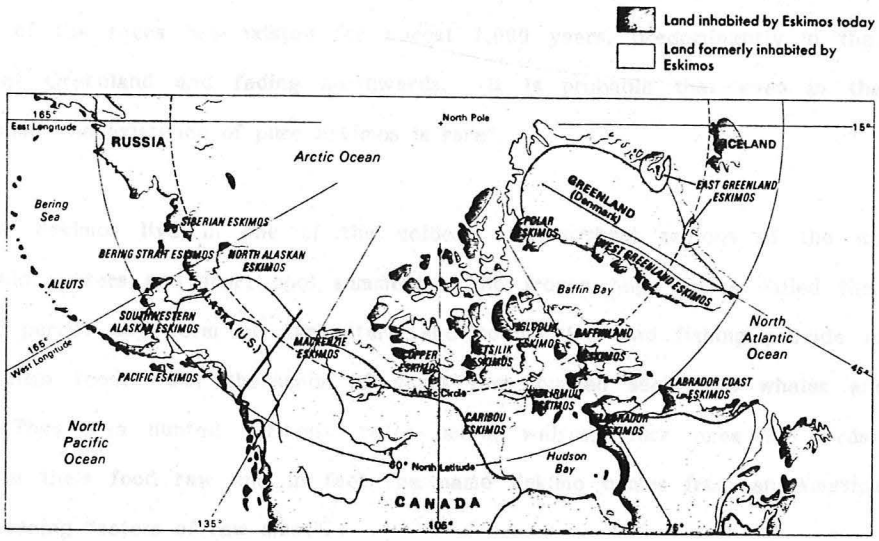
II THE MESSAGE FROM THE ARCTIC

The prevalence and severity of atherosclerotic vascular disease vary considerably among different geographic and ethnic populations (1). Since the Eskimo people may be considered an "experiment of nature" that could provide some insights in the pathogenesis of atherosclerosis, it would be appropriate to consider some background information about their special way of life.

1. ETHNIC AND DEMOGRAPHIC CHARACTERISTICS

The Eskimo land stretches from the northeastern tip of Siberia across Alaska and northern coast of Canada to Greenland (Fig. 1). Approximately 120,000 Eskimos live today in Russia, Alaska, Canada and Greenland. Most of them live in towns or small settlements that are progressively becoming more westernized (2).

Fig. 1



Eskimos have a characteristic appearance with short stature, light brown skin, straight black hair, narrow dark eyes, high checkbones and broad noses. They resemble in appearance the Siberian people of Northern Asia and to a lesser extent, the American Indians. The prehistoric ancestors of both the Eskimos and the American Indians lived in Siberia and probably crossed the land bridge that connected it to what is now Alaska, more than 10,000 years ago. From Alaska, the Eskimo later spread eastward in several migration waves across the Arctic North America to Greenland. The European ethnic influence on Greenland Eskimos is best summarized by Bang and Dyerberg(3): "During some 40 centuries Eskimos have repeatedly invaded Greenland, at

first exclusively from East Asia. The Eskimos spread southwards on the west and east coasts of Greenland. During the 10th and the following centuries Scandinavians — predominantly Norwegians and Icelanders—invaded Greenland and settled on the southern part of the west coast, but later on spread northwards. Remnants of their buildings and tools are found as far to the north as the UmanaK district. These Scandinavians disappeared in the 15th century, but in the 18th century a new invasion of Scandinavians started and is still going on. Consequently, the possibility of a mixing of the races has existed for almost 1,000 years, predominantly in the southern parts of Greenland and fading northwards. It is probable that even in the district of UmanaK the existence of pure Eskimos is rare".

The Eskimos live in one of the coldest and harshest regions of the world with long cold winters and short cool summers. The frozen huge plains called the "tundra" do not permit any form of agriculture and so hunting and fishing provide almost all the Eskimo food. For thousands of years they hunted seals and whales and caught fish. They also hunted caribous, polar bears, walrus, musk oxes and birds. Often they ate their food raw and in fact the name Eskimo comes from an American Indian word meaning "eaters of raw meat".

The Eskimos' way of life began to change in the 19th century with the arrival of European whalers and fur traders. Since the 1950's, the western civilization has overwhelmed the traditional way of life of the Eskimos living in Alaska, Canada and Greenland with programs designed to improve education, health care, housing, commerce and industry. Eskimos have become commercial fishermen; the harpoons, kayak and umiak giving way to modern fishing equipment and motorboats: The snowmobile has

replaced the dog team. Most Greenland Eskimos work in small towns mainly in the fishing industry. Even today, the Eskimos in northern Greenland live mainly by hunting seals and continue to follow many of their old customs and traditional lifestyle.

Although genetic differences in the susceptibility to atherosclerosis among ethnic groups cannot be excluded, environmental factors probably play a major role. This environmental concept can be further strengthen if the "westernized" Eskimos show in the years ahead, an increase in cardiovascular morbidity and mortality similar to the phenomenon that occurred with the Japanese living in Hawaii (4).

2. ESKIMOS VITAL STATISTICS

The epidemiological evidence for the low frequency of cardiovascular diseases in this population was initially based on anecdotical observations made by different Arctic explorers. Alfred Bertelsen, member of the first Danish expedition to study Eskimos in 1903, did not even mention ischemic heart diseases when he reported causes of death in west Greenland between 1901 and 1930 (5). Kromann and Green (6) conducted an epidemiological survey in the Upernavik district, one of the still remaining whaling and sealing populations of northwest Greenland. Approximately 1800 Eskimos were studied over a 25 year period and all the diagnoses in the regional hospital, between 1950-74, were analyzed. The incidence of myocardial infarction was remarkably low. Compared with the Danish population, 40 diagnosed cases would have been expected, but only 3 possible myocardial infarctions were found. Table 1 also show the different distribution of cancer types and the extremely low prevalence of thyrotoxicosis, asthma and diabetes mellitus.

Table 1 (Ref. 6) Standardized numbers with the age structure for 1963–74 as the standard are given in parentheses

	1950–62		1963–74		1950–74	Expected no. 1950–74
	♂	♀	♂	♀	♂ + ♀	(♂ + ♀)
Cancer						
All forms	4 (5.0)	11 (11.1)	13	18	46	53
Upper respiratory tract, incl. salivary glands	0 (0.0)	4 (4.2)	4	1	9	1.3
Lungs	0 (0.0)	0 (0.0)	2	2	4	13.7
Digestive tract	3 (4.0)	0 (0.0)	2	4	9	5.1
Breasts	0 (0.0)	3 (3.1)	0	2	5	5.6
Urogenital system	0 (0.0)	3 (2.9)	1	4	7	8.1
Sarcoma	1 (1.0)	1 (0.8)	3	1	6	1.5
Others, unspecified	0 (0.0)	0 (0.0)	1	4	5	17.1
Apoplexy	6 (10.1)	5 (5.5)	8	6	25	15.0
Epilepsy (grand mal)	5 (5.9)	5 (7.0)	2	4	16	8.0
Peptic ulcer	4 (4.5)	2 (2.1)	10	3	19	29.0
Rheumatic fever	5 (6.0)	1 (1.1)	2	3	11	
Chronic polyarthritis	3 (3.7)	5 (5.4)	0	1	9	
Psychosis	2 (1.8)	0 (0.0)	3	5	10	8.0
Chronic glomerulonephritis	0 (0.0)	2 (1.9)	2	0	4	
Chronic pyelonephritis	0 (0.0)	1 (1.3)	0	1	2	
Acute myocardial infarction	0 (0.0)	1 (1.0)	1	1	3	40.0
Psoriasis	0 (0.0)	1 (1.1)	1	0	2	40.0
Bronchial asthma	1 (1.0)	0 (0.0)	0	0	1	25.0
Diabetes mellitus	0 (0.0)	0 (0.0)	1	0	1	9.0
Thyrotoxicosis	0 (0.0)	0 (0.0)	0	0	0	7.0
Multiple sclerosis	0 (0.0)	0 (0.0)	0	0	0	2.0

The above observation that diabetes mellitus was diagnosed in only one subject (1/1800=0.05%) with asymptomatic hyperglycemia, confirmed the previous report from Sagild et al (7) who found that diabetes was extremely uncommon in Greenland Eskimos. Between 1962-1964, these investigators screened 4,249 Eskimos from 3 different areas in Greenland, they performed postprandial urine tests and also reviewed all the records from 18 hospitals in Greenland searching for the diagnosis of diabetes mellitus. A medical history could not be obtained since the Eskimo language did not contain adequate words to describe the concept of diabetes. Table 2 outlines their main findings.

Table 2 (Ref. 7)

PREVALENCE OF DIABETES MELLITUS IN GREENLAND ESKIMOS (1962-1964)

Postprandial Urines	Hospital Records
• Population 4,249 Eskimos	• Population 32,000 Eskimos
• 24 cases positive glycosuria	• 10 cases of DM (No DKA)
1 definitive DM	3 Insulin Rx
2 probable DM	7 Oral Rx
• Prevalence 3/4, 249 (0.07%)	• Prevalence 10/32,000 (0.03%)

Autopsy studies (8,9) have indicated that the Alaskan Eskimos (Inupik and Yupik) despite greater western influence and more heterogenous ethnic background, comprising Eskimos, Indians and Aleuts, have also a low cardiovascular mortality (Table 3).

Table 3 (Ref. 9)

—Comparison of Primary Causes of Death

Cause	Gottmann (1956-1958)		Current Study (1959-1968)	
	No. of Cases	%	No. of Cases	%
All Causes	103	100.0	339	100.0
Infections	25	24.3	84	24.8
Malignancies	27	26.2	72	21.2
Congenital	8	7.8	37	10.9
Cardiovascular	6	5.8	35	10.3
Prematurity	14	13.6	25	7.4
Trauma	0	0.0	20	5.9
Tuberculosis	10	9.7	16	4.7
Others	13	12.6	50	14.8

Recent information from the Ministry of Greenland clearly indicates that death rate from ischemic heart disease in Greenland Eskimos is only 3.5% of all deaths (10,11), which is nearly ten-fold lower than in western countries (Table 4).

Table 4 (Ref. 10)

MORTALITY FROM ISCHEMIC HEART DISEASE
Males Age 45-64

	DEATH RATE (% of total death)
U.S.	40.4
Denmark	34.7
Greenland	3.5

3. THE QUEST FOR THE ESKIMO PROTECTIVE FACTORS

Rabinowitch was probably the first to report that total serum cholesterol levels in Eskimos were low despite their high marine fat diet (12,13). He served in the R.M.S. Nascopie with the Canadian Government Eastern Arctic Patrol of 1935 for the following reasons as he wrote: "Two different interests prompted this investigation. The purpose of the Canadian Government was to determine the general health of the Eskimos; whether contact with civilization is causing their deterioration; and, if so, the causes. Quite frankly, this was not the writer's interest. If there was a serious health problem amongst the Eskimos he was not aware of it. His interest was primarily in the alleged absence of diabetes, cancer, and arteriosclerosis, and the possible relationship between such absence and the peculiar dietary habits of these people".

Approximately 390 Eskimos were examined in the islands of Southampton, Baffin, Devon and Ellesmere. Rabinowitch made very interesting observation on the lifestyle and health conditions of the Eskimos in the Canadian Eastern Arctic. He noted that in the most northerly parts, where no contact with the white man had altered the diet, there was no evidence of arteriosclerosis.

Between 1970 and 1978, Bang and Dyerberg conducted 4 Danish expeditions to the Umanak district in northwestern Greenland (3,11,14-23). They chose this region because life there was still rather primitive and most of the adults were seal hunters and occasionally involved in whaling and fishing. Table 5 show the magnitude of sealing and whaling in the Upernavik district in 1972, resulting in an average consumption of meat derived from arctic mammals of approximately 400 g/day.

Table 5 (Ref. 3)

Whales and Seals Reportedly Captured in the UmanaK District in 1970 and Estimation of the Meat Consumed from These Sources[a]

	Whales			Seales				
	Lesser Rorqual[b]	Narwhale[c]	Beluga[d]	Ringed seal[e]	Bearded seal[f]	Harp seal[g]	Hooded seal[h]	Total
Animals captured (*n*)	22	23	8	11,904	28	476	75	12,536
Average weight of meat and edible entrails (kg)	2,000	250	225	22	110	35	100	
Total weight of meat and edible entrails (kg)	44,000	5,750	1,800	261,888	7,080	16,660	7,500	340,678
Meat sold outside the UmanaK district (kg)	1,900							1,900
Meat and entrails consumed in the UmanaK district (kg)	42,100	5,750	1,800	261,888	3,080	16,660	7,500	338,778
Estimated whale and seal meat available per person (2400 inhabitants) per day (kg)								0.387

[a] Bang and Dyerberg (1972).
[b] *Balaenoptera acutorostrata* L.
[c] *Monodon moneceros* L.
[d] *Delphinapterus leucas* P.
[e] *Pusa hispida* S.
[f] *Eriguathus barbatus* E.
[g] *Pagophilus groenlandicus* E.
[h] *Cystophora cristata* E.

In August-September, 1970 the first expedition was carried out with the aim of determing lipid and lipoprotein levels in Eskimos (3,14,15). Table 6 shows a summary of the findings when 130 Greenlander Eskimos were compared with age and sex-matched Danish controls. In addition, 25 Eskimos living in Denmark had plasma lipid values significantly higher than those in Greenlanders and similar to those of the Danish controls, suggesting a role for an environmental rather than a genetic factor, to explain the findings.

Table 6

GREENLAND ESKIMOS vs DANES NORMAL SUBJECTS
The Eskimo Lipid Pattern

- Lower Cholesterol and Triglyceride Levels
- Lower LDL and VLDL Levels
- Higher HDL Levels in Males
- Lesser Age-dependent Rise in Lipids

The second (August-September, 1972) and third expeditions (April-May, 1976) were designed to explore the composition of the Eskimo food (16-18). The double portion technique was used to collect approximately 230 daily portions of food for 5-7 consecutive days. A sample population of Greenlanders were asked not to change their food habits during the study period and to collect and deliver in plastic bags quantitative duplicates of all their meals. Table 7 shows that the Eskimo food composition is characterized by a high polyunsaturated/saturated fatty acid ratio and by a shift from the n-6 fatty acid family (which is 50% lower than in Danish food) to a five-fold higher concentration of marine omega-3 polyunsaturated fatty acids. This shift in dietary fatty acid content was similar to the fatty acid pattern found in the Eskimos plasma (15). Eskimos daily intake of omega-3 polyunsaturated fatty acids originating from fish and arctic mammal is approximately 7 g corresponding to 13.1% of their total fatty acid intake which is considerably higher than the 0.8% found in the typical Danish diet.

Table 7 (Ref. 11)

Dietary fats in Eskimo and Danish food in per cent of total fatty acids.

	Eskimo	Danish
12:0	4.8	13.4
16:0	13.6	25.5
16:1	9.8	3.8
16:2-17:1	0.4	-
18:0	4.0	9.5
18:1	24.6	29.2
18:2	5.0	10.0
18:3	0.6	2.0
20:0	0.1	4.3
20:1	14.7	0.4
20:4	0.4	-
20:5	4.6	0.5
22:1	8.0	1.2
22:5-22:6	8.5	0.3
SATURATED	22.8	52.7
MONOENES	57.3	34.6
POLYENES	19.2	12.7
P/S RATIO	0.84	0.24
n-6 CLASS	5.4	10.0
n-3 CLASS	13.7	2.8

Finally, the fourth Umanak expedition (July-August, 1978) was designed to investigate hemostasis in Greenland Eskimos (19). The rationale for this last expe-

dition was based on experimental in-vitro evidence from Bang and Dyerberg (20-23) suggesting that eicosapentaenoic acid had an antithrombotic effect. This fact may explain the multiple reports of an increased bleeding tendency in Eskimos. Of interest is the observation made in 1915 by Peter Freuchen, the Danish arctic explorer (22): "An extremely frequent disorder (if it is proper to use this name for a condition which the people mostly disregard) is the frequent nose-bleedings. There is scarcely one individual of the tribe whose nose will not bleed spontaneously at least every fourth or fifth day... The frequency of nose-bleeding is probably connected with the nutrition which is exclusively of animal origin, because I myself, when living for longer periods as a hunter or traveling on expeditions, under which circumstances I never carry bread or other European provisions, get just as frequent nose-bleedings (but not so heavy) as the Eskimos. Under domestic circumstances this has never been the case".

The investigator's prediction was clearly verified by finding a significantly prolonged bleeding time in the Greenlanders, 8.1 min compared with 4.8 min in the Danish controls (Table 8). A marked decrease in platelet aggregation was also found and in half of the Eskimos examined, there were absent ADP and collagen-induced secondary phase platelet aggregation. The same fatty acid pattern found in food and plasma, was found in the analysis of the platelet membrane composition (Table 9).

Table 8 (Ref. 11)

Ivy bleeding time (BT) in Eskimos living in Greenland and in Denmark as compared to Danish controls.

	No.	BT (min.)
Greenland Eskimos	21	8.05 *
Danish living Eskimos	16	5.13 §
Danes	21	4.76

* $p<0.01$ as compared to Danish living Eskimos and Danes.
§ Not significantly different from Danes.

Table 9 (Ref. 11)

The content of polyunsaturated fatty acids in platelet lipids as per cent of total amount of fatty acids.

	18:2 n-6	20:4 n-6	20:5 n-3	22:5 n-3	22:6 n-3	Σ
Eskimos	3.9	8.5	8.0	3.3	5.8	29.5
Danes	8.2	22.1	0.5	1.0	1.5	33.3

III POLYUNSATURATED FATTY ACID BASICS

The three major groups of unsaturated fatty acids are shown in Table 10 (24). The omega number (N) represents the position of the first double bond counting from the methyl end of the fatty acid; the number after the C refers to the number of carbon atoms and that after the colon to the number of double bonds. Bioconversion apparently takes place primarily in the liver, members of a particular omega family can undergo further desaturation (towards the carboxyl group) and chain elongation but no interconversion from one omega series to another can occur in mammals. For example, linoleic acid (C18:2, N-6) can be converted to arachidonic acid (C20:4, N-6) and linolenic acid (C18:3, N-3) may be converted to eicosapentaenoic acid (C20:5, N-3) and docosahexaenoic acid (C22:6, N-3) in animals.

Table 10 (Ref. 24)

Major Families of Polyunsaturated Fatty Acids

Family designation	Parent fatty acid	Major metabolites	Characteristic structure	Principal sources
ω-9	C18:1 ω-9 oleic acid	C20:3 ω-9* eicostrienoic acid	$H_3C–C–C–C–C–C–C–C–\overset{9}{C}=$ C-RCOOH	Synthesis from acetate; animal and vegetable fats
ω-6	C18:2 ω-6 linoleic acid	C20:4 ω-6 arachidonic acid	$H_3C–C–C–C–C–\overset{6}{C}=$ C-R′COOH	Many vegetable oils
ω-3	C18:3 ω-3 linolenic acid	C20:5 ω-3 eicosapentaenoic acid	$H_3C–C–\overset{3}{C}=$ C-R″COOH	Some vegetable oils, leaves (18:3)
		C22:6 ω-3 docosahexaenoic acid		Marine oils (20:5, 22:6)

A fourth family of polyunsaturated fatty acids (ω-7) can be synthesized from palmitoleate (C16:1 ω-7), but only trace amounts of ω-7 polyunsaturated fatty acids are present in the tissues. The omega (ω) number indicates the location of the first double bond counting from the methyl end of the fatty acid (ω is the last letter of the Greek alphabet). An alternative nomenclature frequently used is the "n" system where "n" replaces the ω (e.g., 18:2 ω-6 = 18:2 n-6).
*Accumulates only in essential fatty acid deficiency.

Members of the omega-3 and omega-6 families are considered essential fatty acids since they cannot be synthesized de novo in animal tissues. The principal dietary sources of the omega-6 polyunsaturated fatty acid, linoleic acid, are vegetable oils

derived from plants, seeds and leaves. The omega-3 fatty acids found in marine fish oils are ultimately derived from the phytoplankton that synthesize eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Table 11 shows the most common dietary sources of polyunsaturated fatty acids (24). Linoleic acid is an essential nutrient as an obligatory precursor of arachidonic acid that in turn is a major component of cell membranes and principal substrate for prostaglandin and leukotriene metabolites (25). Polyunsaturated fatty acids of the omega-3 series are vital structural and physiological components of specialized membranes in brain, retinal cells and testis in mammals (26). The specific functions of tissue omega-3 fatty acids have not been established and there is little data on experimental models of omega-3 fatty acids deficiency. However, the presence of remarkably high levels of docosahexaenoic acid in human brain for example, in addition to the known inability of mammalian cells to synthesize omega-3 fatty acids, strongly suggests a dietary requirement for these polyunsaturated fatty acids.

Table 11

(Ref. 24)

Common Dietary Sources of Polyunsaturated Fatty Acids

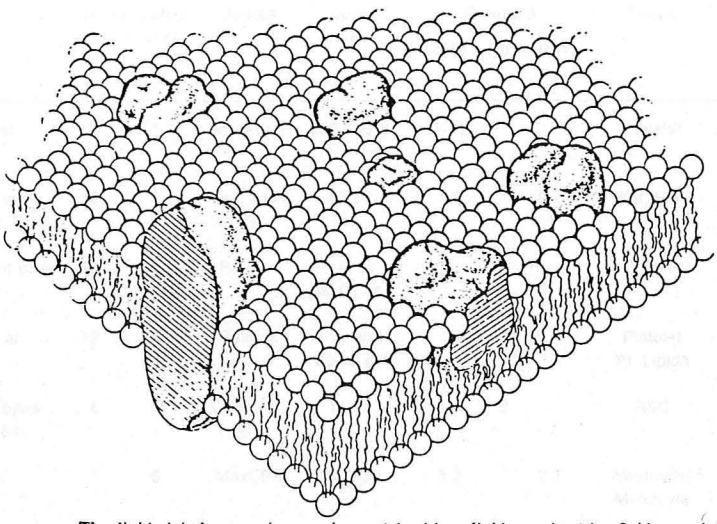
	Percent of total fatty acids*					
Source†	Linoleic	Arachidonic	Linolenic	Eicosapentaenoic	Docosahexaenoic	Saturated
Predominantly ω-6						
Safflower oil	73	—	0.5	—	—	9
Corn oil	57	—	1.0	—	—	13
Cottonseed oil	50	—	0.4	—	—	26
Sunflower seed oil	56	—	0.3	—	—	10
Peanut oil	29	—	1.0	—	—	19
Predominantly ω-3						
Linseed oil	15	—	55.0	—	—	13
Salmon oil	1	—	1.0	8	5	26
Cod liver oil	2	—	1.0	12	12	19
Channel catfish oil	6	2.0	0.7	4	9	26
Mackerel	2	2.0	1.0	10	16	35
Whale oil	1	4.0	—	3	7	19
Both ω-6 and ω-3						
Soybean oil	51	—	7.0	—	—	15
English walnut oil	55	—	11.0	—	—	11
Low in both ω-6 and ω-3						
Cow milk fat	2	—	1.0	—	—	62
Human milk fat	7	0.2	0.7	0.6	0.3	50
Lard	10	—	1.0	—	—	36
Chicken fat	17	—	1.0	—	—	33
Beef tallow	4	—	0.5	—	—	48
Egg yolk	11	6.0	0.2	—	—	53
Beef liver	10	6.0	0.5	—	—	39
Coconut oil	2	—	—	—	—	88
Olive oil	8	—	0.7	—	—	14
Cocoa butter	3	—	0.2	—	—	60
Palm oil	9	—	0.3	—	—	48

IV EFFECTS OF FISH OIL SUPPLEMENTS

1. CELL MEMBRANE LIPID COMPOSITION

Mammalian cell membranes consist of a phospholipid bilayer that are either partially or completely penetrated by intrinsic proteins. From a theoretical perspective, the cellular plasma membrane can be regarded as "a sea of lipids with icebergs of proteins" and any change in the lipid microenvironment can potentially modify membrane function. In the dynamic "fluid mosaic model", proposed by Singer and Nicolson (27), the fluid nature of the lipid bilayer permits some motion of small molecular probes (Fig. 2). Changes in the fatty acid composition of the membrane phospholipids can alter membrane fluidity and modulate the activity of th membrane-bound enzymes, immunological recognition of cells, receptor binding affinities, etc. (28-31).

Fig. 2
(Ref. 27)



The lipid-globular protein mosaic model with a lipid matrix (the fluid mosaic model); schematic three-dimensional and cross-sectional views. The solid bodies with stippled surfaces represent the globular integral proteins, which at long range are randomly distributed in the plane of the membrane. At short range, some may form specific aggregates, as shown

Several studies have consistently demonstrated that it is possible to cause substantial changes in the polyunsaturated fatty acid composition of membrane phospholipids with dietary fish oil supplements (32-38). The pattern of platelet membrane lipid changes obtained with fish oil supplements is similar to the platelet membrane composition found in Greenland Eskimos (19,32-34). An increase in the omega-3 fatty acids, eicosapentaenoic and docosahexaenoic acids, at the expense of a decrease in the omega-6 derivatives, linoleic and arachidonic acid concentrations, have been documented in plasma lipid fractions (35,36), red cell membranes (33,37) neutrophil and monocyte membranes (38) and platelet membrane composition (32-34) after marine oil enriched diets. Table 12 summarizes some of the studies performed in normal human subjects.

Table 12

FISH OIL SUPPLEMENTS AND MEMBRANE COMPOSITION IN NORMAL SUBJECTS

Authors (year)	N	Duration (weeks)	Source	Dose (day)	Omega-3			Tissue	Membrane Composition			
					EPA	(g)	DHA		AA	Lin. Acid	EPA	DHA
Siess et al. (1980)	7	1	Mackerel	500-800 g	7-11		-	Platelet	↓	↓	↑	↑
Sanders et al. (1981)	12	6	Cod Liver Oil	20 cc	1.8		2.2	Plat, RBC	↓	↓	↑	↑
Goodnight et al. (1981)	11	4	Salmon	1 lb or 60-90 cc	-	10	-	Platelet	↓	↓	↑	↑
Harris et al. (1983)	12	4	Salmon	1 lb and 60-90 cc	-	20	-	Platelet PI. Lipids	↓	↓	↑	↑
Popp-Snidjers et al. (1984)	6	2	Cod Liver Oil	15 cc	-	3	-	RBC	NC	↓	↑	↑
Lee et al. (1985)	7	6	MaxEPA	18 caps	3.2		2.2	Neutrophil Monocyte	NC	NC	↑	NC

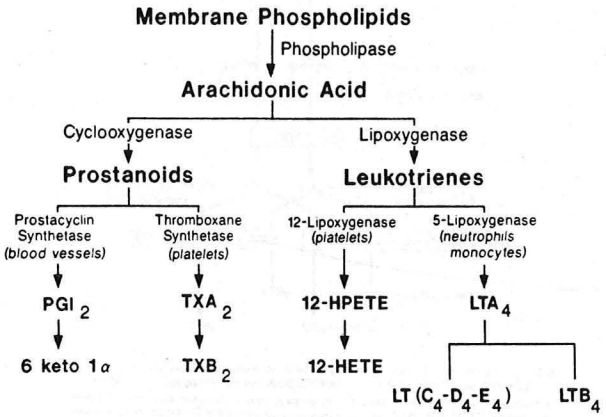
NC = no change

2. EICOSANOID METABOLISM

Eicosanoids, is probably a better term to define all the biologically active lipid mediators derived from the 20-carbon essential fatty acid, arachidonic acid. They are synthesized by almost every tissue and are involved in several physiological and pathophysiological mechanisms. The eicosanoids include, the classical prostanoid cyclooxygenase derivatives, thromboxane and prostacycline, as well as the lipoxygenase derivatives, leukotrienes B_4 and the leukotrienes C_4 - D_4 - E_4 (all components of the slow-reacting substance of anaphylaxis). Leukotriene B_4 is by far the most potent chemotactic and chemokinetic eicosanoid metabolite that also induces neutrophil aggregation, increases vascular permeability and promotes adhesion of neutrophils to vascular endothelism (39,40).

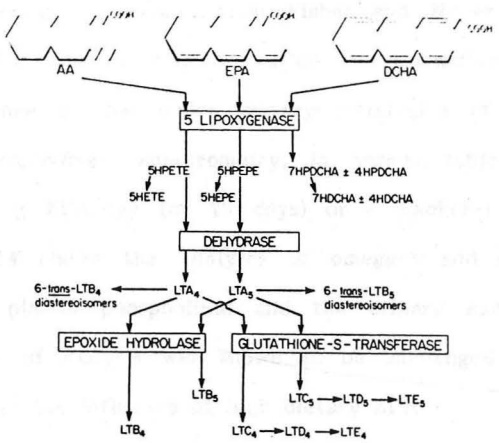
Arachidonic acid is an integral component of cell membranes, present only in the esterified form in the phospholipid fraction. Upon cell stimulation, arachidonate is hydrolyzed and released by a calcium-dependent mechanism involving phospholipase activity and is subsequently oxigenated by a particulate cyclooxygenase and/or a cytoplasmic lipoxygenase enzyme system. Unstable cyclic endoperoxides (PG G_2 and H_2) are further metabolized depending on the enzyme present in a given cell or tissue. Enzymes such as thromboxane synthetase in the platelet or prostacyclin synthetase in the endothelial cell, convert the endoperoxides to biologically active thromboxane A_2 (TxA_2) or prostacycline (PGI_2) (41). Cyclooxygenases seem to catalize the formation of the same endoperoxides in all tissues whereas lipoxygenase are more tissue-specific. Platelets have a 12-lipoxygenase pathway whereas neutrophils and monocytes, contain a 5-lipoxygenase enzymatic system (42). The major pathways of eicosanoid formation are illustrated in Fig. 3.

Fig. 3



Thromboxane A_2 induces platelet aggregation and is a potent vasoconstrictor whereas prostacyclin counterbalances these effects since it is a vasodilator and inhibits platelet aggregation (43). By and large, the cyclooxygenase and lipoxygenase derivatives of eicosapentaenoic acid are less biologically active than those derived from arachidonic acid. Marine EPA and DHA are competitive inhibitors for the cyclooxygenase enzyme system (44,45). Eicosapentaenoic acid is the substrate for prostanoids of the "3" series, thromboxane A_3 (TxA_3) which has attenuated vasoconstricting and platelet aggregating effects, and PGI_3 which has similar properties as prostacycline (PGI_2) (46,47). In addition, EPA is a preferred substrate as compared with arachidonate for product generation by the 5-lipoxygenase pathway resulting in increased synthesis of leukotriene B_5 (Fig. 4), which has markedly less chemotactic and aggregating activities for human neutrophils (40,48,49). EPA has also been shown to inhibit leukotriene B_4 formation (38,50).

Fig. 4 (Ref. 38)



Oxidative Metabolism of Arachidonic Acid (AA), Eicosapentaenoic Acid (EPA), and Docosahexaenoic Acid (DCHA) by the 5-Lipoxygenase Pathway. 5HPETE denotes 5-hydroperoxyeicosatetraenoic acid, 5HETE 5-hydroxyeicosatetraenoic acid, 5HPEPE 5-hydroperoxyeicosapentaenoic acid, 5HEPE 5-hydroxyeicosapentaenoic acid, 7HPDCHA 7-hydroperoxydocosahexaenoic acid, 4HPDCHA 4-hydroperoxydocosahexaenoic acid, 7HDCHA 7-hydroxydocosahexaenoic acid, 4HDCHA 4-hydroxydocosahexaenoic acid, and LT leukotriene.

Table 13 summarizes several studies that have consistently documented reduced platelet thromboxane B_2 formation (collagen stimulated) as well as increased formation of prostanoids of the "3" series, following the administration of dietary fish oil supplements (32,47, 51-54).

Table 13 FISH OIL SUPPLEMENTS AND EICOSANOID METABOLISM IN NORMAL SUBJECTS

Authors (year)	N	Duration (weeks)	Source	Dose (day)	Omega-3 EPA (g)	Omega-3 DHA	Platelets TXB_2 (Collagen Induced)	Platelets TXB_3 (Collagen Induced)	Urine Metabolites PGI_2-M	Urine Metabolites PGI_3-M
Siess et al. (1980)	7	1	Mackerel	500-800 g	7-11	-	↓	-	-	-
Brox et al. (1981)	10	6	Cod Liver Oil	25 cc	- 5	-	↓	-	-	-
Sanders and Hochland (1983)	10	2	MaxEPA	10 g	1.7	1.2	↓	-	-	-
Fischer & Weber (1983)	8	4	Cod Liver Oil	40 cc	4	-	↓	↓	-	-
Fischer & Weber et al. (1984)	6	3-4	Cod Liver Oil	40 cc	4	-	-	-	↑	↑
	3	3	Mackerel	750 g	10-15	-	-	-	↑	↑
von Schacky et al. (1985)	6	20	Cod Liver Oil	10-40 cc	- 2.8	-	↓	↓	NC	↑

NC = no change

Of special relevance is the report from Fisher and Weber (47) that provides the first direct evidence for the in vivo formation of prostaglandin I_3 in man. They demonstrated the presence of the major urinary metabolite of PGI_3, as measured by combined gas cromatography/mass spectrometry, in normal subjects that have ingested either cod liver oil (4 g EPA/day for 25 days) or a mackerel diet (10-15 g EPA/day for 3 days). Table 14 shows the analyses of omega-3 and omega-6 polyunsaturated plasma fatty acids in plasma phospholipid and the urinary excretion rate of PGI_2-M and PGI_3-M. Excretion of PGI_2-M was shown to be unchanged (cod liver oil) or even increased (mackerel) under the influence of high dietary EPA.

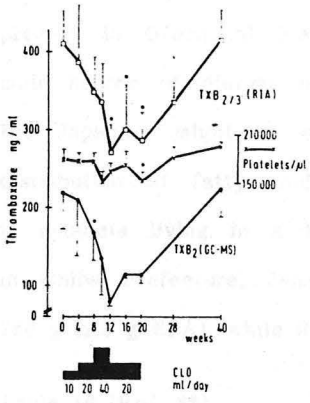
Table 14 (Ref. 42)

	Polyunsaturated fatty acid metabolism after intake of cod liver oil or mackerel				
	Control $n=6$	Cod liver oil Day 25 $n=6$	Control $n=3$	Mackerel Day 1 $n=3$	Mackerel Day 3 $n=3$
Rel.%					
C20:5ω3	0.7±0.3	6.5±1.2*	1.1±0.7	7.5±1.8*	12.2±4.7*
C22:6ω3	4.0±0.8	8.7±1.3*	4.1±1.5	5.0±1.2	6.2±0.8
C20:4ω6	9.8±1.9	7.9±1.2	8.7±2.6	7.2±2.4	6.8±1.1
C18:2ω6	23.2±2.1	14.1±2.4*	21.1±6.0	7.9±1.4*	5.6±1.9*
ng per 24 h					
PGI_2-M	146±39	162±52	121±51	192±27†	236±32†
PGI_3-M	Not detectable	83±25	Not detectable	114±32	134±48

The same authors (54) have further confirmed the above findings by measuring eicosanoid formation after long-term fish oil supplementation in 6 normal subjects given 10-40 cc of cod liver oil/day for 5 months. Thromboxane A_3 was formed in small amounts, whereas TxA_2 formation was reduced by 50% of the control values (Fig. 5). Prostaglandin I_3 was formed from EPA at rates 50% higher than PGI_2 which was basically unaffected (Fig. 6).

Fig. 5

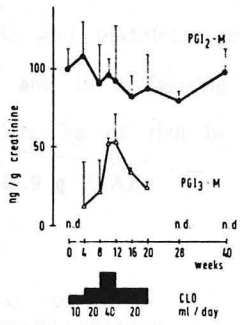
(Ref. 54)



Platelet count (platelets/μl) in blood and thromboxane formation in serum of clotted whole blood (ng/ml, mean±SEM) before, during, and after intake of dietary cod liver oil (CLO) in various dosages. Thromboxane (TX) $B_{2/3}$ (n = 6) was measured radioimmunologically (RIA), thromboxane B_2 (n = 3) was measured by combined gas chromatography/mass spectrometry (GC-MS). *, $P < 0.05$, paired t test as compared to time 0.

Fig. 6

(Ref. 54)



Endogenous prostaglandin I_2 and prostaglandin I_3 production as measured by the urinary metabolites prostaglandin I_2-M (PGI_2-M) and PGI_3-M (ng/g of creatinine, mean±SEM, n = 6) before, during, and after intake of dietary cod liver oil (CLO). The metabolites were measured by combined gas chromatography/mass spectrometry.

3. PLATELET FUNCTION and HEMOSTASIS

Changes in eicosanoid metabolism may explain the effects seen with fish oil supplements on platelet function and platelet-vessel wall interactions. A shift of prostanoid formation from the dienoic to the trienoic series may change the TXA/PGI balance towards a favorable less thrombogenic state (Table 15).

Table 15

PHYSIOLOGICAL PROPERTIES OF PROSTANOID METABOLITES

	ARACHIDONIC Ac	EICOSAPENTAENOIC Ac
Vessell Wall	PGI_2	PGI_3
	Anti-aggregatory	Anti-aggregatory
	Vasodilator	Vasodilator
Platelets	TXA_2	TXA_3
	Pro-aggregatory	Weak Pro-aggregatory
	Vasoconstrictor	Weak Vasoconstrictor

Indeed diets rich in fish oils may have an antithrombogenic effect as suggested by the findings of prolonged bleeding times and lower platelet aggregation commonly present in Greenland Eskimos (19) and Japanese fishermen (55). Since fish is the main source of dietary protein in Japan the low incidence of thrombotic disorders in the Japanese might be explained by the fish (EPA) rich diet. Table 16 shows the distribution of fatty acids in total plasma lipids and platelet aggregation with ADP in subjects living in a fishing village (Kawazu) and in a farming village (Kamagaya) in Chiba Prefecture, Japan. The average daily intake of fish in Kawazu was about 250 g (2.5 g EPA), while in Kamagaya it was 90 g (0.9 g EPA).

Table 16 (Ref. 55)

DISTRIBUTION OF FATTY ACIDS IN TOTAL PLASMA LIPID AND ADP CONCENTRATION THRESHOLD IN FISHING AND FARMING VILLAGE IN JAPAN

	Fishing (n=42)	Farming (n=43)	
Total plasma fatty acid (μg/ml)	3036±791	3125±829	
Fatty acids (%)			
16:0	21·4±1·8	22·5±1·9	
18:0	6·5±0·7	6·2±0·5	
18:1	19·6±2·6	20·7±2·9	
18:2	27·7±4·3	30·4±5·0	(p<0·005)
18:3	0·7±0·3	0·9±0·5	
20:3	0·9±0·3	1·1±0·4	
20:4 (AA)	6·8±1·3	5·8±1·4	(p<0·005)
20:5 (EPA)	3·8±1·6	2·3±1·2	(p<0·001)
22:6 (DHA)	7·1±1·4	4·5±1·5	(p<0·001)
EPA: AA ratio	0·58±0·26	0·41±0·27	(p<0·005)
ADP conc. threshold (μmol/l)	6·6±2·7	2·3±2·0	(p<0·001)

Results as mean±SD.

Table 17 summarizes several clinical studies (32-34,51,54,56-58) with dietary supplements from salmon, mackerel, cod liver oil or commercial preparations of fish oil that have shown bleeding times to be prolonged by 30% to 40% and also a diminished platelet aggregation in response to ADP or collagen.

FISH OIL SUPPLEMENTS AND PLATELET FUNCTION IN NORMAL SUBJECTS

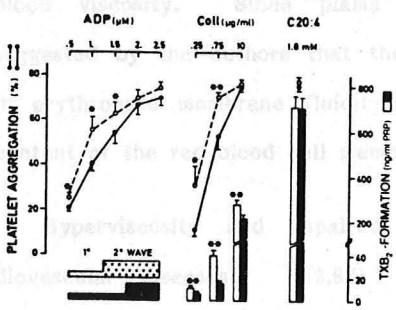
Table 17

Authors (year)	N	Duration (weeks)	Source	Dose (day)	Omega-3 EPA	Omega-3 (g)	Omega-3 DHA	Bleeding Time	Platelet Aggregation ADP	Platelet Aggregation Collagen
Siess et al. (1980)	7	1	Mackerel	500-800 g	7-11	-	-	-	↑	↑
Sanders et al. (1981)	12	6	Cod Liver Oil	20 cc	1.8		2.2	Prolong	↓	-
Goodnight et al. (1981)	11	4	Salmon	1 lb	-	10	-	Prolong	↓	-
Brox et al. (1981)	10	6	Cod Liver Oil	25 cc	-	5	-	NC	-	↓
Thorngren & Gustafson (1981)	10	11	Mackerel Salmon	-	2.3	-	-	Prolong	↓	↓
Nagakawa et al. (1983)	12	4	EPA caps	-	2	-	-	NC	↓	↓
Lorenz et al. (1983)	8	4	Cod Liver Oil	40 cc	4	-	6	Prolong	↓	↓
von Schacky (1985)	6	20	Cod Liver Oil	10-40 cc	-	2-8	-	-	-	↓

NC = no change

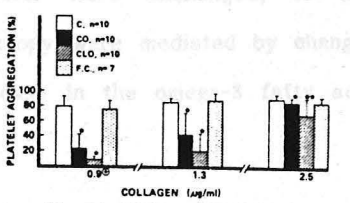
Some inconsistencies in the findings may be explained in part by differences in the nature of the dietary supplements, the amount of omega-3 fatty acids and the duration of therapy (59). In addition problems of compliance probably occurred in some of the studies, especially if one considers the unrealistically high dietary fish supplements that were used. Nevertheless, overall and irrespective of the methodology used, the findings consistently indicate less reactive platelets with decreased aggregation (Fig. 7,8) and also prolonged bleeding times (Fig. 9).

Fig. 7 (Ref. 58)



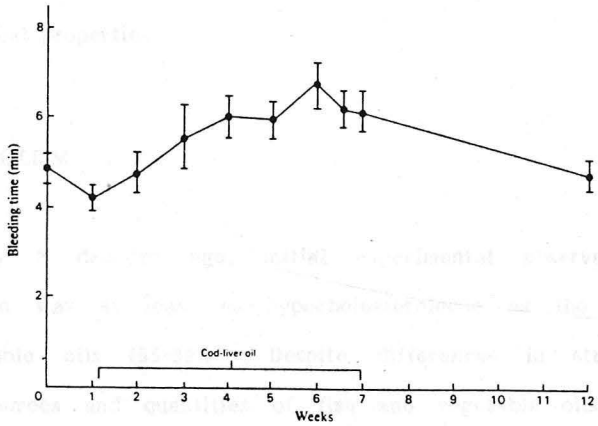
*Platelet aggregation in platelet-rich plasma (PRP) was stimulated with increasing concentrations of ADP and collagen (Coll) and with arachidonic acid (C20:4). Aggregation response measured as light transmission (left ordinate) is given as circles and associated thromboxane B_2 formation by vertical bars (right ordinate). The range of reversible (1° wave) aggregation on ADP is given by the small parts of the horizontal bars, and the range of irreversible (2° wave) aggregation on ADP by the dotted parts of the horizontal bars. Open symbols represent control data and closed symbols represent data after 25 days of cod liver oil supplement. Data are mean ± SEM; n = 8. *p < 0.05, **p < 0.01.*

Fig. 8 (Ref. 51)



Collagen induced platelet aggregation in 10 healthy male subjects before (C), after 6 weeks of daily dietary supplement of 25 ml corn oil (CO) or cod liver oil (CLO) and after a period of 4 months without any dietary addition (F.C.).

Fig. 9 (Ref. 33)



Change in bleeding time with cod-liver oil supplement in 12 male subjects. The points represent means ± SEM.

Finally, changes in the rheological properties of erythrocytes after omega-3 polyunsaturated fatty acid supplements have been reported (60,61). A reduction in whole blood viscosity and an increase in erythrocyte deformability was observed in normal subjects after 4 weeks of ingestion of 3.6 g of EPA (sardine oil capsules) in addition to the ordinary Japanese diet that contains 100 g of fish equivalent to 0.9 g EPA (60). The EPA content in erythrocyte membrane phospholipids markedly increased and was positively correlated with erythrocyte deformability. Cartwright et al. (61) used MaxEPA supplements (3 g of omega-3 fatty acids/day) and found a similar increase in erythrocyte deformability and a concomitant reduction in whole blood viscosity. Since plama viscosity and hematocrit were unchanged, it was suggested by the authors that the effects on blood rheology were mediated by changes in erythrocyte membrane fluidity, secondary to the increase in the omega-3 fatty acid content of the red blood cell membrane.

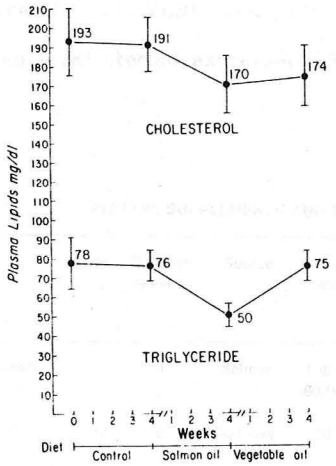
Hyperviscosity and impaired erythrocyte deformability have been found in cardiovascular diseases (62,63) and in diabetes mellitus (64). Thus, it would seem

possible that the protective cardiovascular effects of fish oils could possibly be contributed to by the combination of their platelet antithrombogenic effects and improved rheological properties.

4. LIPID METABOLISM

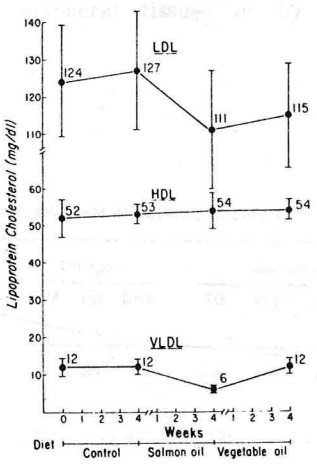
Approximately 3 decades ago, initial experimental observations indicated that fish oil ingestion was at least as hypocholesterolemic as the ingestion of polyunsaturated vegetable oils (65-69). Despite differences in study design, type of control diets, sources and quantities of fish and vegetable oils, the conclusion was that polyunsaturated fatty acids irrespective of the source, either fish or vegetable, were comparably hypocholesterolemic. However, if one considers that fish oils contain moderate amounts of cholesterol and vegetable oils contain none, and the fact that in all of those studies the daily intake of omega-6 fatty acids was consistently greater than the intake of omega-3 fatty acids, one can assume that on a gram for gram basis, the omega-3 fatty acids were considerably more hypocholesterolemic than linoleic acid (24). Over the years, however, more attention has been focused on vegetable oils like corn and safflower oil, rich in the omega-6 polyunsaturated fatty acid, linoleic acid (C18:2, N-6), which has been shown consistently to have marked hypolipidemic effects (70-72). Recently, Harris et al. (36) have demonstrated that in normal subjects, diets enriched with either omega-3 polyunsaturated fatty acids (from salmon oil) or with omega-6 polyunsaturated fatty acids (from a mixture of safflower and corn oil) produced similar reductions of plasma cholesterol and LDL levels (11%). However, only the salmon oil diet significantly reduced plasma triglyceride (33%) and VLDL levels (50%) (Fig. 10, Fig. 11).

Fig. 10 (Ref. 24)



Effects of control, salmon oil, and polyunsaturated vegetable oil diets upon plasma cholesterol and triglyceride levels in seven normal subjects. Diets contained 40% fat and 500 mg of cholesterol. Values reflect the mean (± SEM) lipid levels after 4 weeks of each diet.

Fig. 11 (Ref. 24)



Effects of control, salmon oil, and polyunsaturated vegetable oil diets upon plasma lipoprotein cholesterol levels in seven normal subjects. Diets contained 40% fat and 500 mg of cholesterol. Values reflect mean (± SEM) lipid levels after 4 weeks of each diet.

Table 18 summarizes most of the studies that have examined the effects of omega-3 polyunsaturated fatty acids upon plasma lipids and lipoprotein levels in normal subjects (33, 73-76). The most remarkable lipid-lowering effects of fish oil supplements have been the rapid and dramatic decreases in the levels of plasma triglycerides and VLDL. The mechanisms by which fish oils exert their hypolipidemic effects have not been completely established. However, turnover studies have indicated that a marked inhibition of hepatic synthesis in both VLDL apolipoprotein B and VLDL triglyceride is the most likely kinetic explanation for the profound hypotriglyceridemic effect of the omega-3 fatty acids (77). In addition, Illingworth et al. (78) studied the effects of dietary fish oil on LDL apoprotein B turnover, and found that fish oil significantly lowered the rate of LDL apo B synthesis in normal subjects. Since LDL apo B is derived almost entirely from VLDL apo B following the well established precursor-product relationship, this finding supports the hypothesis that fish oil mainly

inhibits hepatic VLDL synthesis. Other possibilities that have been mentioned, are increased removal of VLDL remnants by peripheral tissues or by the liver (24) and also enhanced fecal steroid excretion (79).

Table 18

FISH OIL SUPPLEMENTS AND LIPID METABOLISM IN NORMAL SUBJECTS

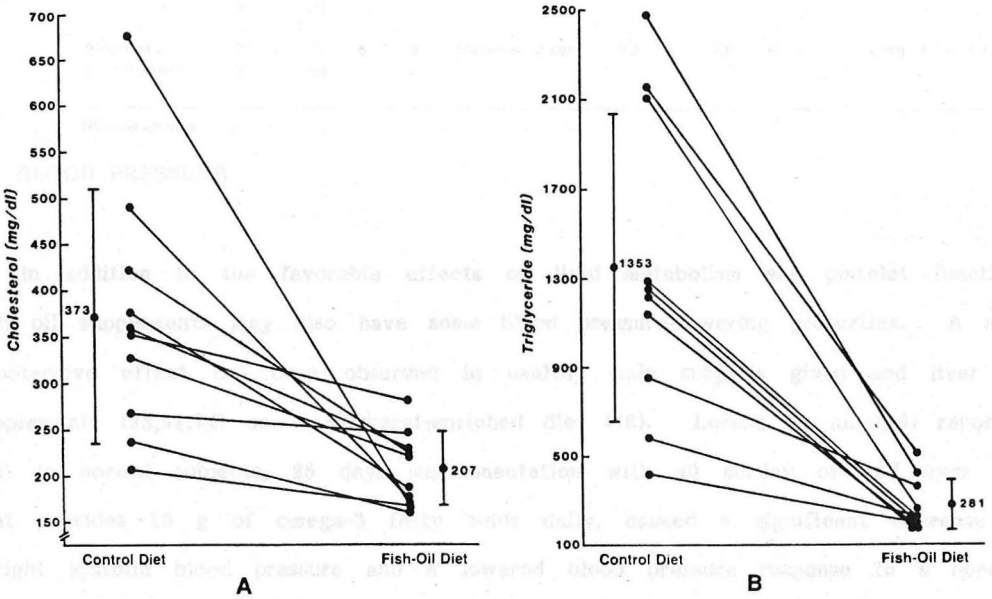
Authors (year)	N	Duration (weeks)	Source	Dose (day)	Omega-3			Lipid and Lipoproteins				
					EPA	(g)	DHA	TG	VLDL	Chol (%)	LDL	HDL
Harris & Connor (1980)	10	4	Salmon	1 lb and 60-90 cc	-	20	-	↓(40)	↓(40)	↓(17)	↓(15)	NC
Sanders et al. (1981)	12	6	Cod Liver Oil	20 cc	1.8		2.2	↓(22)	-	NC	-	↑(10)
Mortensen et al. (1983)	20	4	MaxEPA	10 g	-	4	-	↓(47)	↓(13)	NC	NC	NC
Sanders & Roshanai (1983)	5	3	MaxEPA	5 g	0.8		0.8	↓(14)	-	↓(4)	-	NC
	5	3	MaxEPA	10 g	1.6		1.6	↓(23)	-	↓(5)	-	↑(3)
	5	3	MaxEPA	20 g	3.3		3.2	↓(32)	-	↓(9)	-	↑(31)
Singer et al.	15	2	Mackerel	2 cans	2.2		-	↓(47)	-	↓(7)	↓(6)	↑(5)

NC = no change

In hyperlipidemic patients, the effects of omega-3 polyunsaturated fatty acids have been similar or even greater than those reported in normal subjects (80-83). Relatively small quantities of omega-3 fatty acids are much more hypolipidemic than large amounts of linoleic acid. Furthermore, fish oils have the unique property, not shared with the omega-6 fatty acids, of inducing profound hypotriglyceridemia. Recently, Phillipson et al. (80) have convincingly demonstrated the reduction of plasma lipids, lipoproteins, and apoproteins by dietary fish oil supplements in 20 patients with hypertriglyceridemia; 10 with Type IIb and 10 with Type V. These patients were studied on three separate diets differing primarily in fatty acid composition and fat content, for periods of 4 weeks each. The control diet contained a fatty acid mixture typical of a low-fat therapeutic diet (polyunsaturated to saturated fat ratio of 1.4), the fish oil diet was enriched with omega-3 fatty acids

(from salmon oil or a commercially available fish oil, MaxEPA), and the vegetable oil diet was high in the omega-6 fatty acid, linoleic acid (from a mixture of corn and safflower oil). In the Type IIb patients, the fish oil led to reductions of 27% in plasma cholesterol and 64% in plasma triglyceride levels and the VLDL-triglyceride levels dropped from 216 to 55 mg/dl, as compared with the control diet. Significant reductions in apolipoprotein B and C-3 levels during the period of fish oil diet paralleled the declines in LDL and VLDL levels. The vegetable oil diet had much less effect. With fish oil, the Type V group had marked decreases in their plasma cholesterol level from 373 to 207 mg/dl (45%) and triglyceride levels from 1353 to 281 mg/dl (79%) (Fig. 12). VLDL levels were dramatically lowered but LDL cholesterol rose from 84 to 125 mg/d, suggesting an improved clearance of VLDL. Apolipoproteins B, C-3 and E all decreased significantly. In contrast, the vegetable oil diet caused a rapid and significant rise in plasma triglyceride levels in this group of patients.

Fig. 12 (Ref. 80)



Changes in Levels of Plasma Cholesterol (A) and Triglyceride (B) in 10 Patients with the Type V Phenotype (Control versus Fish-Oil Diet).

To convert cholesterol and triglyceride values from milligrams per deciliter to millimoles per liter, multiply by 0.026 and 0.0113, respectively. The mean change was −1072 mg per deciliter ($P<0.001$) for triglyceride and −166 mg per deciliter ($P<0.01$) for cholesterol.

The potential therapeutic value of dietary fish oil supplements in the management of hyperlipidemia is examined in Table 19 that summarizes the only studies as yet available on the effects of omega-3 fatty acids on plasma lipid and lipoprotein levels in hyperlipidemic patients (80-83).

Table 19 FISH OIL SUPPLEMENTS IN HYPERLIPIDEMIC SUBJECTS

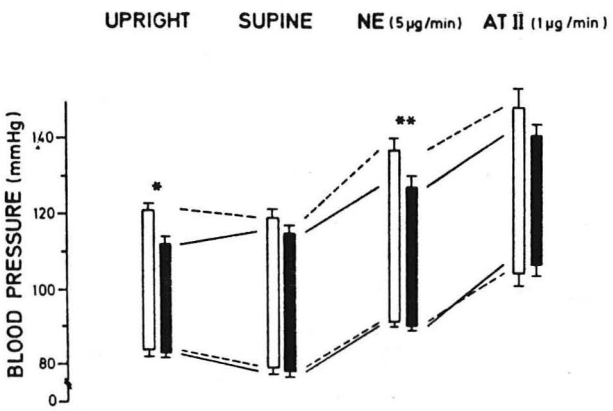
Authors (Country, year)	Phenotype	(n)	N	Duration (weeks)	Source	Dose (day)	Omega-3 EPA (g)	Omega-3 DHA (g)	Lipid and Lipoproteins TG	VLDL	Chol (%)	LDL	HDL
Phillipson et al. (US, 1985)	IIb	(10)	20	4	Salmon or MaxEPA	-		20	↓(64)	↓(75)	↓(27)	↓(12)	↓(17)
	V	(10)							↓(79)	↓(85)	↓(45)	↓(48)	↓(13)
Sanders et al. (U.K, 1985)	IV IIb/IV IV/V	(14) (4) (2)	20	4 Double-Blind	MaxEPA	15 g	2.7	1.9	↓(26)	-	NC	-	↓(12)
Simmons et al. (Australia, 1985)	IIa IIb IV V	(9) (8) (7) (1)	25	12 Double-Blind	MaxEPA	6 g 16 g	1.1 2.9	- -	↓(33) ↓(58)	↓(27) ↓(55)	NC ↓(5)	↓(5) ↓(9)	↓(5) ↓(7)
Singer et al. (G.D.R., 1985)	IV V	(5) (3)	8	2	Mackerel	2 cans	2.2	2.8	↓(71)	-	↓(20)	↓(19)	↓(14)

NC = no change

5. BLOOD PRESSURE

In addition to the favorable effects on lipid metabolism and platelet function, fish oil supplements may also have some blood pressure-lowering properties. A mild hypotensive effect has been observed in healthy male subjects given cod liver oil supplements (33,58,74) or a mackerel-enriched diet (76). Lorenz et al. (58) reported that in normal subjects, 25 days supplementation with 40 ml/day of cod liver oil, that provides 10 g of omega-3 fatty acids daily, caused a significant decrease in upright systolic blood pressure and a lowered blood pressure response to a norepinephrine infusion (Fig. 13). No changes in plasma catecholamines, renin or urinary aldosterone were found.

Fig. 13 (Ref. 58)



*Blood pressure in the upright and supine positions and during norepinephrine (NE) and angiotensin II (AT II) infusion. Systolic and diastolic blood pressure correspond to the upper and lower ends of the bars, respectively. Open bars give control data and closed bars indicate data after 25 days of cod liver oil supplement. Values are mean ± SEM; n = 8. *p < 0.05; **p < 0.01.*

Similar results have also been described using commercially available preparations of mackerel or herring, when given in a crossover fashion for 2 weeks to normal subjects (76), hyperlipidemic (83), or mild hypertensive patients (84). A slight (by 8%) but significant reduction in systolic blood pressure was consistently found only with the mackerel diet which contained higher EPA (2.2 g vs 1.0 g/day) and DHA (2.8 g vs 1.8 g/day) concentrations. Table 20 (85) shows the blood pressure results obtained by Singer et al. using the same study design but in different patient populations (76,83,84). In normotensive subjects, a markedly lower systolic and diastolic blood pressure was observed at the end of the period on the mackerel diet whereas, in hypertensive and hyperlipemic subjects only systolic blood pressure was significantly decreased. Of interest also is a Japanese report on the effects of capsules containing fish oils (1.6 g EPA and 1.0 g DHA/day) in end stage renal failure patients on chronic hemodialysis (86). It was shown that in addition to the hypolipidemic effects, the diastolic blood pressure decreased at the end of the 13 week study.

Table 20 (Ref. 85)

Systolic (SBP) and diastolic blood pressure (DBP) before and at the end of mackerel and herring diet and after 3 months control in normotensive (n = 15), hypertensive (n = 14) and hyperlipemic subjects (n = 8)

	Before	At the end	After 3 months
Mackerel			
Normotensives-SBP	128 ± 14***	113 ± 11	121 ± 11
-DBP	80 ± 9**	73 ± 10	73 ± 6
Hypertensives-SBP	152 ± 12**	140 ± 11	145 ± 12
-DBP	93 ± 12	89 ± 10	95 ± 11
Hyperlipemics-SBP	144 ± 23**	131 ± 20**	141 ± 21
-DBP	95 ± 13	93 ± 15	95 ± 14
Herring			
Normotensives-SBP	124 ± 12	120 ± 13	124 ± 13
-DBP	75 ± 8	73 ± 6	78 ± 8
Hypertensives-SBP	146 ± 14	137 ± 11	144 ± 11
-DBP	92 ± 10	91 ± 6	94 ± 9
Hyperlipemics-SBP	144 ± 18	140 ± 26	144 ± 22
-DBP	94 ± 16	93 ± 17	98 ± 16

Values are mean ± SD: *** $p < 0.001$, ** $p < 0.01$.

The mechanism for this mild hypotensive effect is largely unknown. One can only speculate that it may be related to changes in lipid composition and/or the fluidity of cell membranes at the receptor sites for vasoactive hormones or neurotransmitters (28,31). Alternatively, prostaglandins of the 3-series may have a beneficial effect on the vessel wall tone (87). The blood pressure-lowering effect of fish oils is certainly unimpressive and needs to be confirmed with better designed controlled studies. If this effect is proven real, it may be of some significance in the overall beneficial impact of the marine fatty acids on the cardiovascular system.

6. MACROVASCULAR DISEASES

Descriptive epidemiologic data in Eskimos suggest that fish consumption may protect against cardiovascular diseases (5-11). As mentioned above, a protective effect against atherosclerotic vascular disease may be directly related to their high fish intake. The average daily marine consumption of the Eskimos is estimated to be about 400 g of meat from the arctic mammals, seals and whales (3,16,17). The

consumption of fish has not been directly determined but has been estimated to be about one fifth of the marine meat (18). That is very similar to the average (100 g) daily fish intake of the Japanese, who also have a low prevalence of cardiovascular diseases (55,88). To further the previous findings of decreased platelet aggregation in subjects living in a Japanese fishing village as compared with those in a farming village (55), Kagawa et al. carried out mass spectroscopic analyses of eicosapolyenoic acids of serum lipids in subjects living in Kohama, a fishing island in Okinawa known to have the lowest incidence of cardiovascular diseases in Japan (88). As expected from the higher fish intake in Kohama (147 vs 92 g/day), the total amount of eicosapolyenoic acids in the islanders was higher than in people living in mainland Japan. The low mortality from cardiovascular diseases in Okinawa is shown in Table 21.

Table 21 Ref. 88) Comparison of diseases in mainland Japan and Okinawa.

(International classification)	Mainland	Okinawa
Total population[a]	114,511,000	1,076,000
Mortality per 10^5 population[d]		
Hypertensive diseases (B27)[a]	16.4	7.2
Ischemic heart diseases (B28)[a]	39.8	20.2
Cerebral vascular diseases (B30)[a]	146.2	79.3
Cerebral infarction (B30b)[a]	63.8	18.9
Senility without psychosis (B45a)[a]	24.4	44.7
Total death[a]	607.6	478.9
Incidence of diseases at 50–59 yrs of age (% of total population)[b]		
Hypertension	23.2	8.2
Heart diseases	9.5	2.3
Diabetes mellitus	2.6	1.2
Longevity (male)[c]		
Life expectancy at 65 yrs of age	13.8	15.7
Ratio of 90^+-year population to 60^+-year population	0.65	1.60

More recent epidemiological studies, the Zuthphen study in the Netherlands and the Western Electric Company in Chicago (89-90), have suggested that even a much smaller intake of fish may contribute to a reduction in the incidence of coronary heart disease. In the Zuthphen longitudinal study, information about con-

sumption of 852 men aged 40-59 years without coronary heart disease at entry, was collected by the cross-check dietary history method obtained from the participants and their wives. An inverse dose-response relationship was observed between fish consumption in 1960 and death from coronary heart disease during 20 years of follow-up. This relationship persisted after multiple logistic-regression analyses. Cardiovascular mortality was more than 50% lower among those who consumed at least 30 g of fish/day than among those who did not eat fish (89). The risk ratios for death from coronary heart disease adjusted for other risks factors are shown in Table 22. The explanation for these findings is largely unknown. It seems unlikely that the low fish intake had any obvious effect that could be measured on eicosanoid metabolism, platelet function and lipid and lipoprotein levels.

Table 22 (Ref. 89)

Adjusted Risk Ratios of Death from Coronary Heart Disease (CHD) to Fish Consumption in 1960 among 852 Middle-Aged Men Who Were Free of CHD at Entry.*

Follow-up Period	No. of Deaths from CHD	Fish Consumption (g/day)				
		0	1–14	15–29	30–44	≥45
1960–1970	27	1.00	0.65	0.82	0.00	0.47
1971–1980	51	1.00	0.67	0.51	0.64	0.41
1960–1980	78	1.00	0.64	0.56	0.36	0.39
95% confidence limits for 1960–1980			0.32–1.26	0.27–1.15	0.14–0.93	0.13–1.15

*Risk ratios were adjusted according to the following logistic model: CHD = age + fish consumption + systolic blood pressure + serum total cholesterol + cigarette smoking + subscapular skinfold thickness + physical activity + energy intake + dietary cholesterol + prescribed diet + occupation.

The findings of the Dutch study prompted the authors of the Western Electric Study to investigate the question of fish intake in their study population (84). Previously this study had shown that the lipid composition of the diet was associated with the serum cholesterol level and the risk of coronary death in a cohort of middle-aged men employed by the Western Electric Company in Chicago during 1957 (91). Table 23 shows the reanalysis of the data that supports the European study. They also found that consumption of fish at entry was inversely associated in a graded manner with the 25-year risk of death from coronary heart disease (90).

Table 23 (Ref. 90)

Twenty-five-Year Risk of Death, by Amount of Fish Consumed, among Middle-Aged Men Who Were Free of Coronary Heart Disease at Entry.

Fish Consumption	No. of Men at Risk	Deaths, by Cause								Total Deaths	
		Coronary Heart Disease		Other CVR Diseases*		Malignant Neoplasms		Other Causes			
g/day		no.	%	no.	%	no.	%	no.	%	no.	%
0	205	42	20.5	15	7.3	13	6.3	14	6.8	84	41.0
1–17	686	128	18.7	39	5.7	72	10.5	25	3.6	264	38.5
18–34	779	121	15.5	31	4.0	78	10.0	38	4.9	268	34.4
>35	261	34	13.0	16	6.1	27	10.3	14	5.4	91	34.9
Total	1931	325	16.8	101	5.2	190	9.8	91	4.7	707	36.6
P value for trend		0.008		0.264		0.318		0.965		0.051	

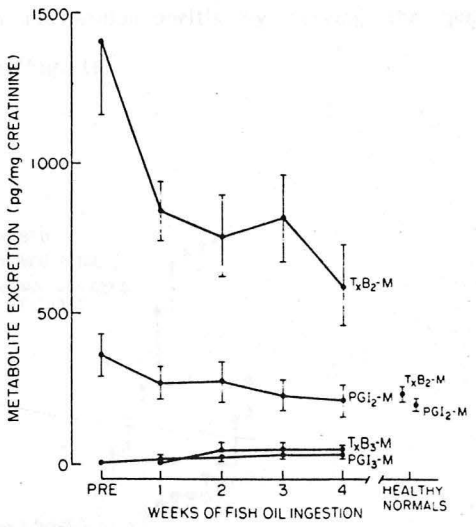
*CVR denotes cardiovascular-renal.

There is limited experimental evidence suggesting that fish oil supplements may give some protection against the acute ischemic lesions that result from vascular injury or occlusion. Culp and coworkers have tested the effects of menhaden (fish) oil supplements in acute cerebral and myocardial infarctions in cats and dogs respectively (92,93). Cerebral ischemia was produced by ligation of the left middle cerebral artery of cats fed either a basal cat chow or the basal diet supplemented with menhaden oil for 18-24 days. It was found that the neurological deficit and the volume of brain infarction in the fish oil treated group was less than that of the control group (92). In an experimental model of electrically induced myocardial infarction, mongrel dogs were fed standard dog chow and half of them also received menhaden oil supplements for 36-45 days prior to infarction (93). Thrombosis and subsequent infarction was induced by constant electrical stimulation of the left circumflex coronary artery in ambulatory dogs that were monitored by telemetry. In the fish oil-fed dogs the size of infarction was 3% of the left ventricle as compared to 25% in the control animals. These experiments need to be interpreted with extreme caution, however. It still remains to be established whether fish oils can also protect the intima from developing the typical atherosclerotic lesions. Of particular interest, however, is the recent report that cod liver oil supplements prevented inti-

mal hyperplasia in autogenous vein grafts used for arterial bypass (94). Fourteen mongrel dogs received 28 segments of 1 cm size undistended jugular vein interposed between bilaterally divided femoral arteries. All the animals were fed a 2% cholesterol diet but only half of them were supplemented with cod liver oil (EPA 1.8 g/d) from 1 week before to 6 weeks after the operation. Mean intimal thickness was measured from multiple vein graft cross sections with a Zeiss computerized image analyzing system that used a mean of 140±11 measurements from each graft. Marked intimal hyperplasia occurred in the control group and increased from 4.3 to 86.4 um whereas in the fish oil-fed group, inhibition of intimal hyperplasia was manifested with reduced intimal thickening from 4.0 to 24.8 um. These provocative findings need to be considered for future clinical studies in the prevention of intimal hyperplasia in vein grafts used for myocardial revascularization.

Clinical evidence for the effects of fish oil in cardiovascular diseases is even more scanty. Saynor et al. (95) recently reported, in an uncontrolled and poorly designed study, that fish oil supplements may be beneficial in patients with angina. An heterogeneus population of 107 patients were given MaxEpa (3.8 g/d) for 2 years. In addition to the hypolipidemic effects, the subset of patients with angina reported a reduction in anginal episodes and a substantial reduction in the consumption of nitroglycerin tablets. Patients with peripheral vascular diseaes have also been shown to benefit from fish oil supplementation (96,97). Woodcock et al. (96) demonstrated a reduction in whole blood viscosity after 7 weeks of EPA 1.8 g/day in a double blind randomized study. Recently Knapp et al. (97) showed a significant reduction of TXA_2 in these type of patients, that coincided with formation of inactive TXA_3 and PGI_3 (Fig. 14) after 4 weeks of 10 g of EPA/day. Neither of these two studies were designed to assess clinical efficacy on the peripheral vascular condition and the limited number of patients studied would not permit this evaluation.

Fig. 14 (Ref. 97)



Urinary Excretion of Metabolites of Thromboxanes and Prostacyclins before (PRE) and during Ingestion of 50 ml of Fish Oil Daily for Four Weeks in Six Patients with Atherosclerosis. Values are means ±SEM. TxB_2-M denotes 2,3-dinor-thromboxane B_2, TxB_3-M denotes 2,3-dinor-thromboxane B_3, PGI_2-M denotes 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$, and PGI_3-M denotes 2,3-dinor-6-keto-17-ene-prostaglandin $F_{1\alpha}$. Excretion rates of the four metabolites at the fourth week of the study were significantly different from pretreatment values: TxB_3-M, $P<0.001$; PGI_3-M, $P<0.001$; TxB_2-M, $P<0.01$; and PGI_2-M, $P<0.05$. The values for TxB_2-M and PGI_2-M in age-matched controls (healthy normals) are those of Reilly and FitzGerald.

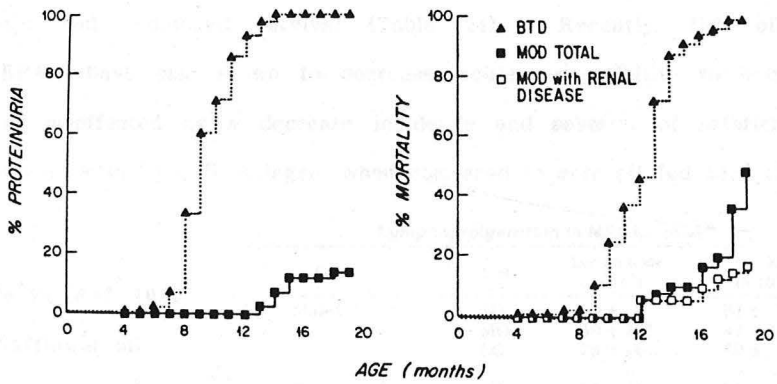
7. AUTOIMMUNE DISEASES

Considerable evidence is available to support the role of prostaglandins and leukotrienes in the pathogenesis of inflammation and immune reactions (98-100). Therefore, dietary supplements with fish oils, that have the ability to influence both tissue lipid composition (32-38) and eicosanoid synthesis (44-50) derived from omega-3 polyunsaturated fatty acids, may represent a "natural immusuppressive agent".

Indeed a salutary effect of marine omega-3 fatty acids has been demonstrated in experimental animal models of autoimmune diseases (101-105). A menhaden oil diet, rich in EPA, protected (NZB x NZW)F_1 mice (murine model for systemic lupus erythematosus) from autoimmune nephritis (101-102). Only 15% of mice treated with the fish oil diet from weaning, had died with severe renal disease at 19 months, versus 98% of control animals on a beef tallow diet (Fig. 15). The menhaden oil diet also protected these mice from renal disease when it was instituted at 4-5 months of age. Recently, the same authors (103) have further characterized the protective

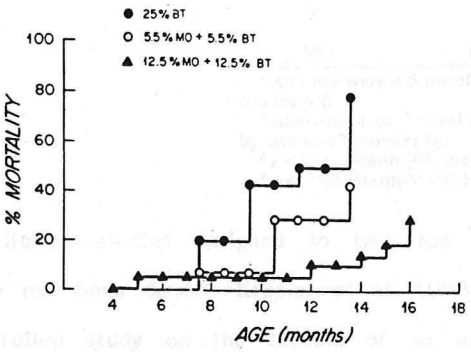
effect of dietary fish lipids on murine lupus glomerulonephritis by varying the quantities and timing of the marine oil administration (Fig. 16).

Fig. 15 (Ref. 102)



Effects of a diet formulated with beef tallow (BTD) or menhaden oil (MOD), instituted at weaning, on the development of proteinuria and mortality in female NZB×NZW/F_1 mice. For both proteinuria and total mortality, $P < 0.005$.

Fig. 16 (Ref. 103)



Effects of Diets on Mortality of Female NZB/W Mice. Mice were fed the diet containing 12.5 wt% MO + 12.5 wt% BT beginning at age 5 weeks and continuing throughout the experiment until age 16 months (▲, Group G). The other two diets were 5.5% MO + 5.5% BT (○, Group H) and 25% BT (●, Group I) and both of these diets were started simultaneously at age 6 months after these animals were fed lab chow ad libitum until age 6 months.

A similar fish oil diet, reduced cyclooxygenase metabolites and suppressed autoimmune lupus in MRL-1 pr mice (104). This is a predictable fulminant model of systemic lupus erythematosus regulated by a single autosomal gene and characterized by massive

T cell lymphoproliferation, increased expression of Ia surface antigens and fatal immune complex glomerulonephritis. Administration of fish oil but not vegetable oil decreased lymphoid hyperplasia, prevented an increase in macrophage surface Ia expression, reduced the formation of immune complexes, delayed the onset of renal disease and prolonged survival (Table 24). Recently, fish oil supplements with MaxEPA have also shown to decrease mouse susceptibility to collagen-induced arthritis as manifested by a decrease incidence and severity of arthritis in B10 RIII mice immunized with Type II collagen, when compared to corn oil fed mice (105).

Table 24 (Ref. 104)

SO=Safflower oil

MO=Menhaten oil

LC=Lab Chow

Lymphoproliferation in MRL-lpr mice[a]

Sex	Diet	Lymph Node ($\times 10^7$)	Spleen ($\times 10^7$)
Males	SO	90 ± 8	81 ± 9
	MO	59 ± 12[b]	47 ± 10[b]
	LC	75 ± 16	69 ± 9
Females	SO	90 ± 24	71 ± 9
	MO	29 ± 6[b]	45 ± 14[b]
	LC	44 ± 6	60 ± 5

[a] Values at 4-5 mo = mean ± SEM (n = 4-5).
[b] $P < 0.05$ (Mann-Whitney U test).

Renal disease in MRL-lpr mice[a]

Diet	n	Histology	IgG[b]
SO	8	3.0 ± 0.3[c]	2.0 ± 0.5[d]
MO	10	0.4 ± 0.2	0

[a] All mice were 4.5 mo of age. Results are expressed as means ± SEM. Grades = 0-4.
[b] Glomerular peripheral capillary loop deposits of IgG, as determined by immunofluorescence.
[c] $P < 0.01$ (Mann-Whitney U test).
[d] $P < 0.05$ (Mann-Whitney U test).

Human studies designed to test the effect of fish oils on autoimmune diseases have not been done. Kremer et al. (106) recently reported a 12-week, double masked controlled study on the effects of an experimental diet high in polyunsaturated fat and low in saturated fat, with a daily supplement (1.8 g) of EPA. A very modest improvement in morning stiffness and the number of tender joints were found in the experimental group. On follow-up evaluation at 1-2 months after stopping the diet, the experimental group had deteriorated significantly in all clinical parameters. Of

interest also, is the preliminary report of 20 Japanese patients with IgA nephropathy divided in 2 groups; one was treated with fish oils (1.6 g EPA and 1.0 g DHA/day) for a year and the other served as controls with no fish oil supplements. At baseline both groups had similar clinical characteristics and after one year, renal function (evaluated by the reciprocal of the serum creatinine level) had not changed in the EPA group (1/1.8 to 1/2.1 mg/dl) whereas the control subjects significantly deteriorated (1/1.7 to 1/3.17 mg/dl). In fact, two of the control patients were begun on chronic hemodialysis (107).

Despite the encouraging immunosuppressive effects of fish oils in marine lupus, no studies are as yet available in human SLE or other autoimmune conditions.

8. DIABETES MELLITUS

As previously discussed, diabetes mellitus in Eskimos is exceptionally and most intriguingly uncommon (67). Differences in genetic susceptibility to the disease are probably important, but except for an early crude study in 1972 reporting an increased frequency of HLA-A9 type in Greenland Eskimos (108), no additional information is available. The prevalence of 0.05% amongst the Greenland Eskimos for both insulin dependent and non insulin dependent diabetes can be regarded as negligible. It is even much lower than the western prevalence of Type I insulin dependent diabetes (0.2-0.3%) which only constitutes 10-15% of all cases of diabetes (109). It is tempting to speculate that environmental and/or dietary factors may account for this extraordinary low prevalence of diabetes. Although there have been no studies assessing the overall effects of omega-3 polyunsaturated fatty acids on glucose homeostasis in diabetes mellitus, one might hypothesize that changes in tissue lipid composition (32-38) could favorably influence the activity of membrane-bound enzymes

and receptors (28-31). Possibly changes in eicosanoid metabolism could also have a beneficial metabolic effect (110).

Autoimmune Type I insulin dependent diabetes is practically non-existent in Eskimos. It is most provocative, but perhaps scientifically naive,to speculate that the high fish oil consumption in Greenland Eskimos may provide a natural immunosuppressive state that prevents the development of Type I insulin dependent diabetes. The pathogenic process leading to IDDM is probably an autoimmune destruction of the beta cells occurring in genetically susceptible individuals, triggered by unknown environmental agents (111,112). Evidence is available in experimental models (BB/W rat) that immunosuppressive agents can prevent IDDM. Human immunointervention studies with corticosteroids and azathioprine have been inconclusive, however (113-116). Recently the London-Ontario group in Canada have shown in an open uncontrolled study that cyclosporin, if given early at onset of IDDM, remission and insulin independency can be induced in more than 50% of diabetic patients (117). These features persist as long as the cyclosporin treatment is maintained. It seems obvious that the immunosuppressive therapy was probably given too late, when the pancreatic damage was far advanced and probably irreversible. Nevertheless, even if this form of therapy does not act on the primary pathogenic mechanism, the cascade of immune events is interrupted, resulting in some objective clinical evidence of remission. The Cyclosporin/Diabetes French Study Group recently confirmed the above findings in a multicenter double-blind randomized study on 122 patients with newly diagnosed Type I IDDM (118). After 9 months of follow-up they found an increase rate of complete remission of the diabetes in the cyclosporin group as compared with the placebo treated group (24.1 vs 5.8%).

Administration of omega-3 polyunsaturated fatty acids may prove to be beneficial as a "natural" and "harmless" immunosuppressive approach that could abate the classi-

cal mononuclear cell infiltration of pancreatic islets via changes in leukotriene metabolism and hopefully preserve or restore beta cell function in Type I insulin dependent diabetes mellitus.

V FISH OIL SUPPLEMENTS: SAFETY AND DOSE RELATED EFFECTS

Eskimos constitute the best historical and demographic precedent that attests to the safety of a diet high in omega-3 polyunsaturated fatty acids. Some fish oils contain high levels of cetoleic acid (C22:1, N-11) which is an isomer of erucic acid (C22:1, N-9). Erucic acid is found in rapeseed oil and when given in high concentrations can cause transient myocardial lipidosis and fibrosis in several species of experimental animals (119). However, neither erucic nor cetoleic acids have ever been shown to have any detrimental effect in humans (24).

Diets rich in polyunsaturated fatty acids may interfere with absorption and increase the requirements of Vitamin E (120). Pigs fed mackerel oil diets develop an asymptomatic discoloration of the adipose tissue known as "yellow fat disease" that has no clinical repercusions (121). Signs of Vitamin E deficiency (loss of deep tendon reflexes, posterior column dysfunction and ataxia, that occur in patients with abetalipoproteinemia or severe fat malabsorption) have not been reported in Eskimos or in fish oil feeding studies. Furthermore, the commercially available fish oil preparations like MaxEPA contain supplemental Vitamin E to prevent autooxidation.

Despite the increased bleeding tendency, inhibition of platelet aggregation, and occasionally mild thrombocytopenia, no clinical bleeding tendency of any relevance has been observed in any of the studies discussed.

No evidence is available to suggest an increased incidence of malignant neoplasias in individuals who consume a diet high in polyunsaturated omega-3 fatty acids.

In fact, fish consumption has been correlated with a low incidence of breast cancer in Greenland and in Japan (122,123). The incidence of nasopharyngeal and salivary gland carcinoma in Greenland and gastric carcinoma in Japan are among the highest in the world (124,125), however.

Fish oil supplements have been given in a wide range of different concentrations in the many studies reviewed in this rounds. As can be interpreted from Tables 12, 13, 17-19, there appears to be a dose related effect. A conclusion can be made that a consistently effective dose is 3 g/day of EPA or 5-6 g of omega-3 fatty acids a day. This can be obtained as a commercial preparation or alternatively from eating fatty fish. Half a pound of salmon, for example, would provide approximately 30 g of fish oil equivalent to 4-6 g of omega-3 fatty acids. Further studies, however, will be required to establish the minimal effective amounts of fish oil and fish meat that result in optimal effects.

VI CONCLUSION

What you have heard today may be a very important story: The lipid and antithrombogenic effects of marine omega-3 polyunsaturated fatty acids may impact favorably on atherogenesis. It was once said by an annonymous writer that "What passes for knowledge is often no more than organized ignorance". Only considerable additional investigation will allow us to know if the fish oil story holds up. If it does, it may provide a harmless multifactorial attack on many significant disease processes.

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