SAFETY OF SEAL OIL AS A NUTRITIONAL SUPPLEMENT

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Preamble

Dr. Charles Mortimer (Bud) Harlow was born in Truro, Nova Scotia on 3 July 1908, and received his high school education at the Colchester County Academy located in that town. He attended Acadia University where he graduated with a B.Sc. in 1931, and then decided to pursue graduate studies at McGill University where he studied under the supervision of two distinguished doctors, J.B. Collip and Hans Selye. After being awarded a Doctor of Philosophy degree in 1938 by McGill, he entered medical school at that University, completing the M.D. program in 1941. During the Second World War, Dr. Harlow served as a Surgeon Lieutenant with the RCNVR and after the war continued his association with the Navy serving as a Consulting Pathologist of that service for many years. In 1950 he was appointed to the Faculty of Medicine at Dalhousie University where he taught laboratory medicine to medical students for twenty-eight years. Dr. Harlow also served as the Director of the Pathology Laboratories at Camp Hill Hospital and was a Consulting Pathologist to the Halifax Infirmary for many years.

While studying under Dr. J.B. Collip in the 1930s, Dr. Harlow became interested in the study of cholesterol in the vascular system and, when he took up his appointment at Dalhousie in the early 1950s, continued to study this important topic. Together with his colleagues he carried out research which demonstrated the value of fish in a person's diet in that it lowered cholesterol levels in the blood and therefore represented a preventative measure to reduce the incidence of heart disease.

Dr. Harlow was a popular speaker and frequently addressed groups interested in improving their health through diet. He is now retired and living in Halifax.

The 1950s and 1960s were a simpler world for lipid chemists. Modern chromatographic and spectroscopic techniques were just being introduced. Many scientists and researchers however became interested in the new idea that polyunsaturated fatty acids would reduce serum cholesterol and so improve longevity by reducing atherosclerosis. To paraphrase a remark of J. Sabine (1977), "anybody with 12 rats and a colorimeter plunged into blood lipid research." Several worked with fish oils and/or the unsaponifiables from fish oils (Peifer, 1967). Dr. C.M. Harlow had an ideal base for such research in Camp Hill Hospital (Harlow, 1961) and visited author Ackman at the Halifax Laboratory of the Fisheries Research Board of Canada to explore the concept of using fish oil in such research. At that time K. Karlsen operated a plant in Blandford, Nova Scotia for the production of seal oil from the blubber attached to seal pelts. The oil was easier to hydrogenate than many fish oils and was acceptable to the

margarine and shortening industry for hydrogenation and food use (Conacher et al., 1972; Mag, 1973). The fatty acid composition was known in some detail (Ackman et al., 1963) and after discussion it was agreed that it be used in Dr. Harlow's project.

Seal oil is made from the seal blubber. The latter is a layer of pure fat up to 5 cm in thickness. It has several physiological functions that are shared with sea lions, whales, manatees and other marine mammals. An advantage over fish oil was that the blubber sheet could be held in frozen storage until needed at Camp Hill Hospital. Then a simple procedure worked out in the author's laboratory was applied. Appropriate cubes of blubber could be cut out of the slab and any exposed surfaces trimmed clean. A cube could be dropped into a Waring Blendor™ half full of ice water, and in less than a minute the clear, virtually colourless and tasteless oil would be freed and float to the surface where it could be decanted. Since this simple procedure ensured a steady supply of very clean oil no concern existed over using antioxidants or about peroxides developing during oil storage. Dr. Harlow presented his results in public on only one occasion (Harlow et al., 1958) and the abstract from that meeting is reproduced as Figure 1. The patients under his care obviously found the freshly prepared seal oil quite acceptable, and Dr. Harlow observed no adverse effects with 26 ml of seal oil administered twice per day, to patients in his cardiac care program. After two months of dietary supplementation serum cholesterol was substantially reduced as stated in the abstract (Figure 1).

Dalhousie University
Medical Research Committee

Abstract of Clinical Research Meeting, November 13, 1957

*Serum Cholesterol Research to Marine Oil
in Cases of Atherosclerosis*

C. M. HARLOW, LEA STEEVES and A. MYRDEN

A marked decrease in the amount of ordinary fats in the usual American or Western European diet, without any change in the amount of calories or vitamins, lowers the serum cholesterol level. The fall is rapid in the first few days, but after a few weeks there is an approach to a new plateau (Keys). Such low fat diets usually contain less cholesterol and animal proteins, but the change in the serum cholesterol level in man does not depend upon this fact (Keys and Anderson). The responsible agent is clearly either in the quality of the fats or in the ratio of fat to carbohydrate calories.

It is tempting, then, to ascribe the effect of low fat diets to the decrease in animal fat, especially since some vegetable and marine oils given in large amounts may depress the serum-cholesterol level (Kinsell) (Bronte-Stewart). It appears that the effect of dietary fats on the serum cholesterol should be related to chemical composition rather than to origin.

For the past year we have conducted at Camp Hill Hospital a small Research project in which we investigated the effect of North Atlantic seal oil on a group of patients with atherosclerosis and hypercholesterolemia.

The group included cases of ischemic heart disease and peripheral atherosclerosis with claudication. All patients were placed on a low fat diet (5%) for two weeks to one month. During that time there was a definite lowering of the cholesterol level (340 mg% to 280 mg%). The patients were then given 26 cc. of seal oil twice a day. After a further two months, the cholesterol level decreased to around 200 mg%.

It is interesting that we were able to lower the cholesterol level of 10 patients with atherosclerosis and hypercholesterolemia on a low fat diet and that a further lowering of the cholesterol could be obtained by adding 50 grams of seal oil (450 calories) to the low fat diet.

Fig 1　　Reproduction of the abstract of a 1958 presentation by C.M. Harlow on the impact of high doses of seal oil on human hematology.

Part of the new interest in the effects of polyunsaturated fatty acids on cholesterol (Harlow, 1961; Sabine, 1977; Peifer, 1967) soon drifted into the intensive use of liquid vegetable oils with a high content of linoleic acid, for example corn oil (Lands, 1986). Although the more widely used soybean oil had 50-60% linoleic acid (18:2n-6), it also had 10-12% alpha-linolenic acid (18:3n-3) which was less stable and not considered "essential" by many nutritionists. The simple shorthand notation of chain length, number of ethylenic bonds and position relative to the terminal methyl group define the two basic families of polyunsaturated fatty acids. "Omega-3" now a popular descriptive term, equates with n-3. The original rat work decades ago that defined linoleic acid (n-6) as "essential" was not thought applicable to the n-3 family of fatty acids until shown to be effective in the human neural system (Holman, 1992). In fact it has taken until this decade to have alpha-linolenic acid widely recognized for a biochemically important role in human nutrition (British Nutrition Foundation, 1992), However, very recent reviews elevate it to a more appropriate importance in human health (Cunnane, 1996). A section heading in a recent review on fats and fatty acids is simply "saturates, oleic acid and linoleic acid" (Katan et al., 1994) so alpha-linolenic acid is still not unequivocally accepted as a healthy food fat component despite lengthy or detailed review articles (Descomps, et al., 1995; Léger, et al., 1995; Mendy, 1995; Combe, 1996; Renaud, 1996) especially with reference to conversion to docosahexaenoic acid (DHA or 22:6n-3), and the popular Mediterranean diet (de Lorgeril et al., 1994).

Fish oils do not generally contain more than 1% alpha-linolenic acid, but instead have a total of 10-25% of the two higher "omega-3" fatty acids eicosapentaenoic (EPA or 20:5n-3) and DHA. These accumulate in marine life as shown in Figure 2 and then pass from the food eaten by the seals into the depot fat, but this fat is organized differently on the glycerol molecules. The first person to demonstrate this effectively was H. Brockerhoff of the Fisheries Research Board of Canada working in Halifax. All of his publications are conveniently listed in the references of a 1989 book chapter by Ackman and Ratnayake (1989). The essential difference between the fatty acid distribution on triacylglycerols can be summarized for the n-3 docosahexaenoic acid (DHA or 22:6n-3) as shown in Table 1. The same concept applies to the distribution of n-3 eicosapentaenoic acid (EPA or 20:5n-3) but a little less precisely as summarized in some detail in a book on triglycerides by C. Litchfield (1972).

Table I   Distribution of DHA on the glycerol molecules of two types of marine oils.

	Fish oils	Seal oils
sn-1	$y = 0.28x$	$y = 0.94x$
sn-2	$y = 2.06x$	$y = 0.22x$
sn-3	$y = 0.66x$	$y = 1.84x$

where y = mole % of 22:6 and x is the total of that fatty acid in the triacylglycerol.

This was an academic curiosity until the studies of the Danish group headed by Bang and Dyerberg on Greenland Eskimos showed that this population, subsisting largely on seal fats, did not have a high death rate from cardiovascular disease (Bang and Dyerberg, 1976; 1980; 1981; Bang et al., 1980; Dyerberg and Bang, 1978; Dyerberg et al., 1978; Ackman et al., 1980; Sinclair, 1980). Unfortunately seal oil was not available for medical research in 1970-90 and fish oil became the main vehicle for EPA

Origin of Marine Fatty Acids

| Phytoplankton |

EPA > DHA   18:2n-6   18:3n-3

Shellfish   Shellfish
Crustacea   Crustacea
Fish

                                          Energy

TG   PL
EPA > DHA   DHA > EPA

Human Diet   Seals   Seabirds

                            circulatory
DHA ⟷ EPA ⟶ Prostaglandin Group 3
                            system

Neurological systems? TXA_3 PGI_3
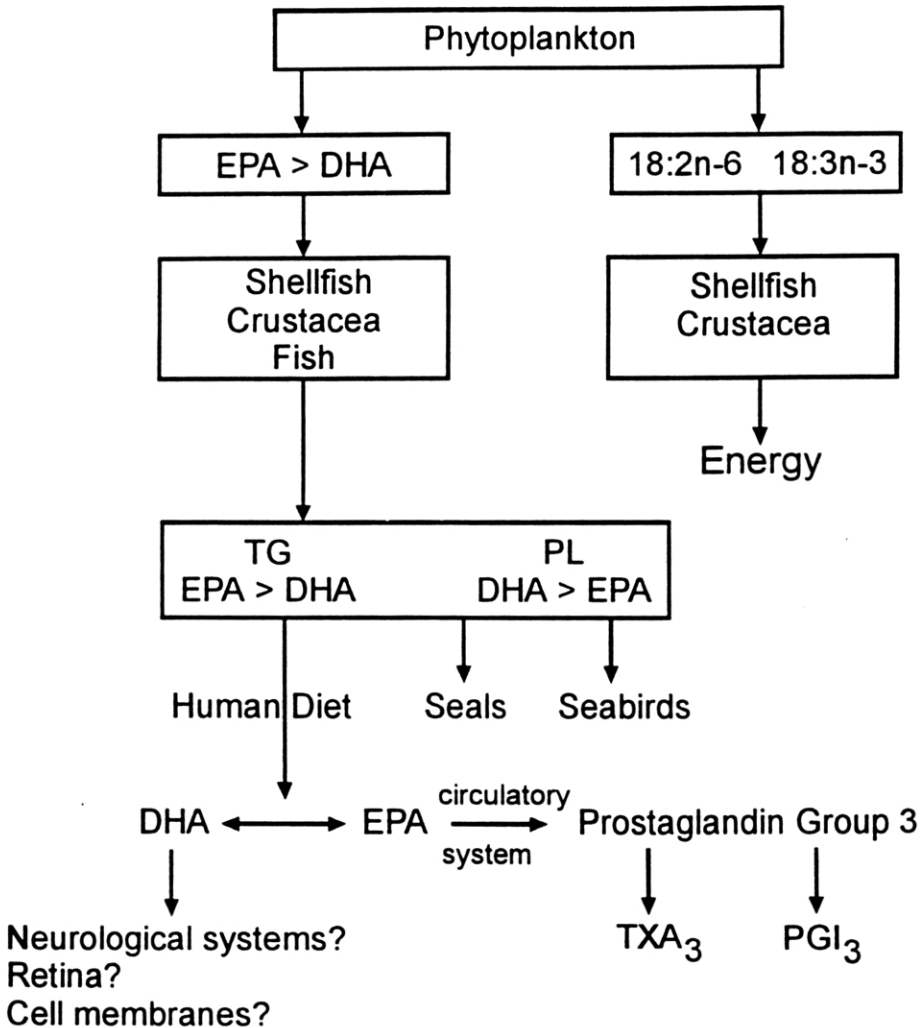Retina?
Cell membranes?

Fig 2   Outline of the utilization of different chain lengths of polyunsaturated fatty
acids produced by marine phytoplankton.

and DHA research. Initially most was supplied in refined form by the company British
Cod Liver Oils as MaxEPA™ (Ackman et al., 1989). It is ironic that cod liver oil was
widely given to children in large doses as a vitamin source without concern for safety,
or any known ill effects, for over a century. It has rapidly become clear that the safety
aspects of fish oils in human clinical trials are not a problem, although unlike the
situation in Dr. Harlow's era cumbersome examination of protocol procedures by
committees has became the normal procedure prior to effective research. Misgivings
about the safety of such products by North American regulatory agencies are difficult

to understand. Much the same attitude on safety could affect the introduction of seal oil into medical research and the burgeoning natural food for nutritional supplement markets.

The safety of seal oils was actually established thousands of years ago when seal fats became a staple of the diet of many aboriginal populations in several parts of the world, including the people today called the Inuit. Many ate the seal fat raw, others after rendering it to oil by some simple heat process. Some of these procedures are described in a book called *Kabloona* (De Poncins, 1941) by a writer who spent considerable time with the Inuit in Northern Canada.

At a biochemistry level of health interest Catherine Wo obtained her doctorate with a dissertation "The Nutritional Status of Alaskan Eskimos with Respect to Fatty Acids, Vitamin A and Vitamin E (Wo, 1973). An original communication from this work appeared in *Am. J. Clin. Nutr.* (Wo and Draper, 1975). Serum cholesterol ranged upwards from 200 mg/100 ml but only a few subjects exceeded 300 mg/100 ml. Vitamin E levels were perfectly adequate despite the highly unsaturated fatty acids of the diet. This work may not have been exciting to other researchers because the health of the Eskimos on a diet high in marine mammal fats was not abnormal and consequently it has attracted little attention.

Alaskan Eskimo plasma was recently investigated for n-3 polyunsaturated fatty acids (Parkinson et al., 1994). Fish and seal fat/meat were important in the diet and platelet functions and a few rare increases in bleeding times were considered as possibly due to a low regional intake of linoleic acid rather than to a high intake of seal fat or fish oil. Adipose tissue was not examined although potentially it "warrants consideration for use in clinical studies requiring precise documentation of long-term fatty acid consumption" (Leaf et al., 1995), subject to some caution (Connor et al., 1996).

A lower prevalence of impaired glucose tolerance was also examined in Alaskan peoples (Adler et al., 1994). The report was: "Consumption of seal oil at least five times per week was required to reduce risk", an important observation. Strangely, in this connection two 1996 papers on diabetes (Rivellese et al., 1996; Rossing et al., 1996) failed to mention the long-term effects observed with native populations and focused on either short term or long term studies with humans given fish oils (both with DHA>EPA). In well-controlled studies no benefit or deleterious effect was reported for blood glucose control in the fish oil groups. A multicenter study in Italy reviewed several other recent studies on diabetics and omega-3 fatty acids and decided that the "lack of effect of omega-3 fatty acids administration on glucose metabolism in patients with impaired glycaemia control is noteworthy. It eliminates concern about prescribing these products to diabetics" (Sirtori, 1996). The above emphasis on diabetes is solely because it is now an important pathological condition in some northern populations of Canada.

Seal Oil Production and Properties

The almost pure fat of seal blubber is easily rendered by mincing and steam cooking. Unlike fish oils made from whole fish or fish waste there is very little contamination by muscle phospholipid and protein, haem pigment, or other non-fat materials. Much of the information required for a full understanding of the physical properties has been recently summarized by Shahidi et al. (1996).

In Canada, in the past, the annual seal oil production was favoured by part of the margarine industry as a marine oil alternative to avoid putting the word "fish" on the label. After standard industrial refining (Conacher et al., 1972) it hydrogenated readily.

Raw seal oil was examined for Se, As, I and Br by cyclic instrumental neutron activation analysis (Elson et al., 1983). In µg/g the results were respectively 0.033 ± 0.002, 0.73 ± 0.01, 1.82 ± 0.06 and 2.01 ± 0.28. Sulfur was 12.4 ± 0.2 mg/kg by another technique (Wijesundera et al., 1988). Tocopherols are rather low for a marine oil, about 65 µg/g in 1996 production, and in these samples PCBs are in the range 0.8 - 1.0 ppm. The latter can however be reduced by half or more along with "fishy" aldehyde oxidation products, by a simple treatment with high vacuum (deodorization). This needs to be conducted below 185°C to preserve the *cis* configuration of ethylenic bonds. At more elevated temperatures that step can alter some bonds in the EPA and DHA to *trans* (Wijesundera et al., 1989). Since this effect is commonly observed in the linoleic and alpha-linolenic acids of refined vegetable oil, especially in the alpha-linolenic acid content (Chardigny et al., 1996), it should not lead to any greater risk than Canadians derive from their regular diets. Extension of alpha-linolenic acid *trans* isomers to C_{20} pentaenoic acids is possible (O'Keefe et al., 1990), but this has no known consequences in man.

Standard refining conditions for seal oil have been described (Conacher et al., 1972) and can be contrasted with active clay, bleaching, and H_3PO_4 only (Mag, 1973). The combination of active clay, filter aid, and phosphoric acid was applied successfully to seal oil, but deodorization was omitted, presumably until after hydrogenation in this case, but the hydrogenated seal oil product seemed to have desirable flavour and stability attributes when compared to the fish oils then in use.

The fatty acids of harp seal oil are easily concentrated to give 25.5% EPA, 25.3% DHA, 9.5% DPA, 4.1% stearidonic acid (18:4n-3) for a total of 64.4% omega-3 fatty acids in free fatty acid or ethyl ester form (Ratnayake et al., 1988). The oil itself can be enriched by simple crystallization at low temperature (Shahidi et al. 1996).

Seal Oil Compared to Fish oils

The digestibility of seal oil and fish oil is effectively the same, about 95%. M.S. Christensen has examined the details of the digestive process in a series of papers (Christensen et al., 1994; 1995a; 1995b; Christensen and Høy, 1996) with experiments on Wistar rats. In the opinion of a disinterested observer, J. Dyerberg, who experimented with humans on this topic, "the isometry of the n-3 FA in the glyceride molecule does not influence the assimilation of EPA plus DHA in man" (Dyerberg et al., 1995).

Another human study with a marine mammal oil fed to humans was conducted in 1988 and lasted 10 days, with finwhale oil and a herring oil control (Weaver et al., 1989). Serum and platelet phospholipids altered their fatty acid composition in parallel for the two oils. The authors noted an increase in n-3 docosapentaenoic (n-3 DPA, 22:5n-3) acid in the phospholipids during the ingestion of the whale oil, as this fatty acid was three times as important in that oil as in the herring oil. Otherwise they found no important differences. As will be discussed below, DPA is potentially a very beneficial n-3 fatty acid and is much more obvious in seal oils than in fish oils.

An extensive study comparing seal oil, whale oil, and cod liver oil administered to humans has recently been published (Østerud et al., 1995). A daily intake of 15 mL/day was provided as a supplement to the usual diet of healthy adult Norwegians. The study focused on blood properties and seems to show beneficial effects on several blood components thought to be associated with cardiovascular and thrombotic diseases. Unexpectedly, there were inexplicable and interesting differences among the effects of the three oils that were harmless but suggest that there should be further

study. A potential problem not often encountered in this type of research with humans is the already high intake of omega-3 fatty acids from food fish or cod liver oil in Norway. From this point of view the safety of additional supplements of omega-3 fatty acids in a ten-week trial suggests that there is no problem with an intake in excess of 300 mg/day (see below).

n-3 Docosapentaenoic Acid (DPA)

This fatty acid (22:5n-3) was not considered as important as EPA (20:5n-3) in the early 1980 period because a UK research company had already discovered that EPA led to the PGI_3 prostaglandin beneficial in man. DPA was also a minor component in the MaxEPA™ type fish oils (EPA>DHA) mainly employed for research on long-chain omega-3 fatty acids. Typical fish oil levels of DPA were ≤ 1%.

Two very recent studies have amplified our knowledge of the role of DPA in mammals. Amazingly, DPA was up to 10 times as effective as EPA in stimulating the migration of endothelial cells (Kanayasu-Toyoda et al., 1996). This suggests that it is a powerful anti-atherogenic factor. In another study (Bénistant et al., 1996) DPA was found to reduce prostacyclin production in endothelial cells. Even if acting only as an instant source of EPA inside the cells, this result should encourage further interest into the biochemistry of DPA. There is no reason to consider the DPA of seal oil as a problem. It is always present in fish lipids and fish oils, usually at 10% of DHA and if present in all human milk and thus transferred to human infants by nursing mothers (see below) it can hardly be harmful.

Although several studies with humans have examined the exchange among these three fatty acids it is sufficient to note that most were based on administering purified EPA or DHA. Remarkably, the DPA has been largely ignored although it is a quarter to a third of the DHA circulating in the plasma lipid subfractions of normals. This is shown for human controls in a study of the effects of fish oil (Leaf et al., 1995). It is also an important fatty acid among the omega-3 fatty acids of human milk, for example in Australia (Makrides et al., 1995) Table 2.

Table II   Fatty acids (w/w%) of breast milk supplied by mothers of fully breast-fed infants

Fatty acid	Week 6	Week 16	Week 30
18:2n-6	13.56	13.92	13.56
20:4n-6	0.45	0.40	0.39
18:3n-3	0.89	0.94	0.85
20:5n-3	0.07	0.07	0.06
22:5n-3	0.16	0.16	0.16
22:6n-3	0.26	0.21	0.19

Adapted from Makrides et al., 1995

As early as 1987 Innis and Kuhnlein (1987) reported from Canadian surveys that n-3 DPA was higher in the foods based on marine mammals available on Baffin Island than would be indicated from fish analyses. They cited similar observations in the food of Greenland Eskimos (4.6% 20:5n-3, 2.6% 22:5n-3, 5.9% 22:6n-3). They concluded "Our analyses of marine mammals and polar bear...draw attention to the complete pattern of ω-3 fatty acids in the Eskimo diet ... and the possibility of more complex interactions among the ω-3 fatty acids in influencing metabolism". In 1988 they reported on the breast milk of Inuit women consuming traditional foods (Innis and Kuhnlein, 1988). Compared to Vancouver women their n-3 fatty acids were as shown in Table 3. The two compositions obviously reflect diets but qualitatively are not very

different.

Table III   Comparison of omega-3 fatty acids (w/w%) in human milks from the Canadian north and a typical urban population.

	Inuit	Vancouver
20:5	1.1	0.2
22:5	0.8	0.4
22:6	1.4	0.4

Adapted from Innis and Kuhnlein (1987)

For infants DPA is clearly provided in Canadian maternal breast milk and so can be assumed to be perfectly safe. In fact all three omega-3 long-chain fatty acids shown are normally present in human milk, a material highly recommended for infants all over the world. This is further evidence that seal oil should be harmless in man despite the presence in the fatty acids of a modest percentage, usually 2-4%, of DPA (Ackman and Ratnayake, 1989). In fact research into the effect on adults of this fatty acid uniquely available to Canadians has started (Murphy et al., in press; Holub et al., unpublished results), and needs to be extended.

Long-Chain Omega-3 Intake

A large international company, Hoffman-LaRoche, have a self-affirmed GRAS status in the USA for their ROPUFA™ food additive fish oil. Their promotional material suggests that there is a "gap" in the current food intake of western civilization of 1 g per day of these long-chain omega-3 PUFA. Many authors have tried to define the needs of EPA and/or DHA in normals for a "preventive" role in maintaining cardiovascular health, as distinct from treating hypertriglyceridemia, for which MaxEPA fish oil is licensed by the National Health Service in the UK at a dose of several grams per day.

Most medical researchers have simply ignored DPA, assuming that it was merely an intermediate between EPA and DHA. The beneficial role of EPA in the wall of the vascular system has long been accepted (Lands, 1986). The role of DHA was more obscure until quite recently when it became apparent that it could be instrumental in suppressing "fibrillation" of the heart muscle (Weylandt et al., 1996). An elegant review of this new hypothesis has just been published (Nair et al., 1997). Although DHA is thought to be the main factor (Kang and Leaf, 1996) the precursor alpha-linolenic acid can also be influential in this respect (de Lorgeril et al., 1994; Renaud, 1996)

A recent study in humans (Conquer and Holub, 1997) confirms that in both omnivores and vegetarians the retroconversion of DHA to EPA over six weeks is limited to about 10% of the dietary intake of DHA in the longer term. A surprising feature of the fatty acid analyses of both serum and platelet total phospholipids was a modest reduction in the level of **both** 22:5n-6 (DPAn-6) and 22:5n-3 (DPAn-3) as DHA increased. The EPA increased slightly as a result of the retroconversion from DHA. Whether the observation on the two 22:5 fatty acids merely reflects their physical displacement by DHA or EPA in the phospholipids, or has metabolic consequences is not known. The observation that the DPA (n-3) exceeded DHA in the total phospholipids of human platelets in both omnivores and vegetarians on their regular diets is perhaps new evidence indicative of another role for this fatty acid in addition to that already proposed. The intake of 1.62 g/day of DHA, without any EPA, over six weeks is a novel

treatment compared to both the long history of fish oil experimentation with oils or concentrates with EPA>DHA, and the historical consumption of seal fats among Arctic populations. North Atlantic seal oil is well balanced as a source of both DHA and EPA in approximately equal proportions and provides much more DPA than fish oils. As is common in these circumstances, overloading a naturally balanced system in the body with one fatty acid at the expense of others may not be appropriate for good health if a more balanced proportion is historically proven safe. Overall this work provides further confirmation of the safety of longer-chain omega-3 fatty acid intakes.

One of the best assessments of this problem of intake of n-3 fatty acids is that of Simopoulos (1989). She reports 300-400 mg per day as a desirable intake. As the conclusion from a NATO Advanced Workshop on Dietary ω3 and ω6 Fatty Acids these numbers appear to be the current acceptable figures. No doubt they were calculated solely on the basis of EPA and DHA, and inclusion of the DPA of seal oil in the total does not affect the conclusion that up to 2 g per day of seal oil with 200 mg/g of omega-3 fatty acids is in fact a daily intake of omega-3 long-chain fatty acids comparable to a capsule (1 g) of the popular MaxEPA™ products (nominally 300 mg of EPA + DHA per 1 g capsule). The seal oil provides DPA and DHA ≥ EPA, possibly an important difference from many commercial fish oils.

Conclusions

A variety of records, some going back thousands of years and some as recent as 1996, point to seal oil as perfectly safe for a role as a nutritional enhancer in our western society. The pioneering work of Dr. C.M. Harlow in this regard has been largely overlooked. There is no other practical source for the n-3 docosapentaenoic acid component, recently shown to be functionally beneficial in the endothelium of the circulatory system. It may be a part of the reasons for the extraordinary health benefits among Greenland Eskimos consuming high levels of seal oil.

References

Ackman, R.G. and Ratnayake, W.M.N. 1989. Fish oils, seal oils, esters and acids- are all forms of omega-3 intake equal?, In: *Health Effects of Fish and Fish Oils* (edited by R.K. Chandra) ARTS Biomedical Publishers, St. John's, Newfoundland, pp. 373-393.

Ackman, R.G., Burgher, R.D. and Jangaard, P.M. 1963. Systematic identification of fatty acids in the gas-liquid chromatography of fatty acid methyl esters: A preliminary survey of seal oil. *Canadian J. Biochem. Physiol.*, 41: 1627-1641.

Ackman, R.G., Eaton, C.A. and Dyerberg, J. 1980. Marine docosenoic acid isomer distribution in the plasma of Greenland Eskimos. *Am. J. Clin. Nutr.* 33: 1814-1817.

Ackman, R.G., Ratnayake, W.M.N. and Macpherson, E.J. 1989. EPA and DHA contents of encapsulated fish oil products. *J. Am. Oil Chem. Soc.*, 66: 1162-1164.

Adler, A.I., Boyko, E.J., Schrer, C.D. and Murphy, N.J. 1994. Lower prevalence of impaired glucose tolerance and diabetes associated with daily seal oil or salmon consumption among Alaskan natives. *Diabetes Care* 17: 1498-1501.

Bang, H.O. and Dyerberg, J. 1976. Composition of food consumed by Greenland Eskimos. *Acta Med. Scand.*, 200: 69-73.

Bang, H.O. and Dyerberg, J. 1980. Lipid metabolism and ischemic heart disease in Greenland Eskimos. *Adv. Nutr. Res.*, 3: 1-22.

Bang, H.O. and Dyerberg, J. 1981. The lipid metabolism in Greenlanders. *Meddelelser*

om Gronland, Man and Society, 2: 3-18.

Bang, H.O., Dyerberg, J. and Sinclair, H.M. 1980. The composition of the Eskimo food in North-western Greenland. *Am. J. Clin Nutr.,* 33: 2657-2661.

Bénistant, C. Achard, F., Slama. S.B. and Lagarde, M. 1996. Docosapentaenoic acid (22:5, n-3): metabolism and effect on prostacyclin production in endothelial cells. *Prostaglandins, Leukotrienes and Essential Fatty Acids* 55: 287-292.

British Nutrition Foundation. 1992. The Report of a Task Force *Unsaturated Fatty Acids: Nutritional and Physiological Significance,* Chapman and Hall, London, 211 pp.

Chardigny, J-M., Wolff, R.L., Mager, E., Bayard, C.C., Sébédio, J-L., Martine, L., and Ratnayake, W.M.N. 1996. Fatty acid composition of French infant formulas with emphasis on the content and detailed profile of *trans* fatty acids. *J. Am. Oil Chem. Soc.,* 73: 1595-1601.

Christensen, M.S., Høy, C-E. and Redgrave, T.G. 1994. Lymphatic absorption for n-3 polyunsaturated fatty acids from marine oils with different intramolecular fatty acid distribution. *Biochim. Biophys. Acta* 1215: 198-204.

Christensen, M.S. and Høy, C-E. 1996. Effects of dietary triacylglycerol structure on triacylglycerols of resultant chylomicrons from fish oil- and seal oil-fed rats. *Lipids,* 31: 340-344.

Christensen, M.S., Høy, C-E., Becker, C.C. and Redgrave, T.G. 1995a. Intestinal absorption and lymphatic transport of eicosapentaenoic (EPA), docosahexaenoic (DHA), and decanoic acids: dependence on intramolecular triacylglycerol structure. *Am. J. Clin. Nutr.* 61: 56-61.

Christensen, M.S., Mortimer, B-C., Høy, C-E. and Redgrave, T.G. 1995b. Clearance of chylomicrons following fish oil and seal oil feeding. *Nutr. Res.* 15: 354-368.

Combe, N. 1996. Lipid oxidation: analytical techniques. *OCL,* 3: 200-204.

Conacher, H.B.S., Page, B.D. and Chadha, R.K. 1972. Isomers of monoethylenic fatty acids in some partially hydrogenated marine oils. *J. Am. Oil Chem. Soc.* 49: 520-523.

Connor, W.E., Lin, D.S. and Colvis, C. 1996. Differential mobilization of fatty acids from adipose tissue. *J. Lipid Res.* 37: 290-298.

Conquer, J.A. and Holub, B.J. 1997. Dietary docosahexaenoic acid as a source of eicosapentaenoic acid in vegetarians and omnivores. *Lipids,* 32: 341-345.

Cunnane, S.C. 1996. Recent studies on the synthesis, β-oxidation, and deficiency of linoleate and α-linolenate: are essential fatty acids more aptly named indispensable or conditionally dispensable fatty acids? *Can. J. Physiol. Pharmacol.* 74: 629-639.

de Lorgeril, M. Renaud, S., Mamelle, N., Salen, P., Martin, J-L., Monjaud, I., Guidollet, J., Touboul, P. and Delaye, J. 1994. Mediterranean alphalpha-linolenic acid-rich diet in secondary prevention of coronary heart disease. *Lancet* 343: 1454-1459.

De Poncins, G. 1941. *Kabloona.* Garden City Publishing Company, New York, 339 pp.

Descomps, B., Michel, F., Fouret, G., El Boustani, S., Monnier, L. and Léger, C-L. 1995. Using stable isotopes to study bioconversion of polyunsaturated fatty acids in man. *OCL,* 2: 30-36. (*in French*)

Dyerberg, J. and Bang, H.O. 1978. Dietary fat and thrombosis. *Lancet,* 1978: 152.

Dyerberg, J., Bang, H.O., Stoffersen, E., Moncada, S. and Vane, J.R. 1978. Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis. *Lancet,* 1978: 117-119.

Dyerberg, J., Madsen, P., Møller, J., Aardestrup, I. and Schmidt, E.B. 1995. In: *Bioavailability of n-3 Fatty Acid Formulations in n-3 Fatty Acids: Prevention and*

Treatment in Vascular Disease (Edited by S.D. Kristensen et al.) Bi A Gi Publishers, Venora-Springer Verlag, London, pp. 217-225.

Elson, C.M., Ackman, R.G. and Chatt, A. 1983. Determination of selenium, arsenic, iodine and bromine in fish, plant and mammalian oils by cyclic instrumental neutron activation analysis. *J. Am. Oil Chem. Soc.*, 60: 829-832.

Harlow, C.M., Steeves, L. and Mryden, A. 1958. Serum cholesterol research to marine oil in cases of atherosclerosis. Abstract. *N.S. Med. Bull.*, 36: 127.

Harlow, C.M. 1961. High fish diet, obesity and blood cholesterol. *N.S. Med. Bull.* 40: 329-337.

Holman, R.T. 1992. A long scaly tale—The study of essential fatty acid deficiency at the University of Minnesota. In: *Essential Fatty Acids and Eicosanoids* (edited by A. Sinclair and R. Gibson), American Oil Chemists' Society, Champaign, IL, pp. 3-17.

Innis, S.M. and Kuhnlein, H.V. 1987. The fatty acid composition of Northern-Canadian marine and terrestrial mammals. *Acta. Med. Scand.*, 222: 105-109.

Innis, S.M. and Kuhnlein, H.V. 1988. Long-chain n-3 fatty acids in breast milk of Inuit women consuming traditional foods. *Early Human Dev.* 18:185-189.

Kanayasu-Toyoda, T., Morita, I. and Murota, S-I. 1996. Docosapentaenoic acid (22:5, n-3), an elongation metabolite of eicosapentaenoic acid (20:5, n-3), is a potent stimulator of endothelial cell migration on pretreatment in vitro. *Prostaglandins, Leukotrienes and Essential Fatty Acids* 54: 319-325.

Kang, J.X. and Leaf, A. 1996. Antiarrhythmic effects of polyunsaturated fatty acids. *Circulation*, 94: 1774-1780.

Katan, M.B., Zock, P.L. and Mensink, R.P. 1994. Effects of fats and fatty acids on blood lipids in humans: an overview. *Am. J. Clin. Nutr.* 60: 101S-122S.

Lands, W.E.M. 1986. *Fish and Human Health*. Academic Press Inc., Orlando, 170 pp.

Leaf, A., Connor, W.E., Barsted, L. and Sexton, G. 1995. Incorporation of dietary n-3 fatty acids into the fatty acids of human adipose tissue and plasma lipid classes. *Am. J. Clin. Nutr.* 62: 68-73.

Léger, C-L., Fouret, G., Bouvier, S., Sarda, P. and Descomps, B. 1995. Docosahexaenoic acid and retinal development. *OCL*, 2: 45-51. (*in French*)

Litchfield C. 1972. *Analysis of Triglycerides*. Academic Press, New York.

Mag, T. 1973. Clay-heat refining of edible oils. *J. Am. Oil. Chem. Soc.*, 50: 251-254.

Makrides, M., Simmer, K., Neumann, M., and Gibson, R. 1995. Changes in the polyunsaturated fatty acids of breast milk from mothers of full-term infants over 30 wk of lactation. *Am. J. Clin. Nutr.*, 61: 1231-1233.

Mendy, F. 1995. The need for polyunsaturated fatty acids in the first months of life. *OCL*, 2: 36-45. (*in French*)

Murphy, M., Wright, V., Ackman, R.G. and Horackova, M. *in press*. Diets enriched in menhaden fish oil, seal oil, or shark liver oil have distinct effects on the lipid and fatty-acid composition of guinea pig heart. *Molecular and Cellular Biochemistry*, in press.

Nair, S.S.D., Leitch, W.J., Falconer, J. and Garg, M.L. 1997. Prevention of cardiac arrhythmia by dietary (n-3) polyunsaturated fatty acids and their mechanism of action. *J. Nutr.*, 127: 383-393.

O'Keefe, S,F., Lagarde, M., Grandgirard, A., and Sebedio, J.L. 1990. *Trans* n-3 eicosapentaenoic and docosahexaenoic acid isomers exhibit different inhibitory effects on arachidonic acid metabolism in human platelets compared to the respective cis fatty acids. *J Lipid Res.*, 31: 1241-1246.

Østerud, B., Elvevoll, E., Barstad, H., Brox, J., Halvorsen, H., Lia, K., Olsen, J.O., Olsen, R.L., Sissener, C., Rekdal, Ø and Vognild, E. 1995. Effects of marine oils

supplementation on coagulation and cellular activation in whole blood. *Lipids,* 30: 1111-1118.

Parkinson, A.J., Cruz, A.Z., Heyward, W.L., Bulkow, L.R., Hall, D., Barstaed, L. and **Connor, W.E.** 1994. Elevated concentrations of plasma ω-3 polyunsaturated fatty acids among Alaskan Eskimos. *Am. J. Clin. Nutr.* 59: 384-388.

Peifer, J.J. 1967. Hypocholesterolemic effects of marine oils. In: *Fish Oils their Chemistry, Technology, Stability, Nutritional Properties, and Uses* (edited by M.E. Stansby) The AVI Publishing Company, Inc., Westport, CT, pp. 322-361.

Ratnayake, W.M.N., Olsson, B., Matthews, D. and **Ackman, R.G.** 1988. Preparation of omega-3 PUFA concentrates from fish oils via urea complexation, *Fat Sci. Technol.,* 90: 381-386.

Renaud, S. 1996. Secondary prevention of heart attacks through diet: the role of alpha-linolenic acid. *OCL,* 3: 169-172. (*in French*)

Rivellese, A.A., Maffettone, A., Iovine, C., Dimarino, L., Annuzzi, G., Mancini, M., and **Riccardi, G.** 1996. Long-term effects of fish oil on insulin resistance and plasma lipoproteins in NIDDM patients with hypertriglyceridemia. *Diabetes Care* 19: 1207-1213.

Rossing, P., Hansen, B.V., Nielsen, F.S., Myrup, B., Hølmer, G. and **Parving, H-H.** 1996. Fish oil in diabetic nephropathy. *Diabetes Care* 19: 1214-1219.

Sabine, J. 1977. *Cholesterol.* Dekker, New York, 489 pp.

Shahidi, F., Wanasundara, P.K.J.P.D. and **Wanasundara, U.N.** 1996. Seal blubber oil: A novel source of ω3 fatty acids. *J. Food Lipids,* 3: 293-306.

Simopoulos, A.P. 1989. Summary of the NATO advanced research workshop on dietary ω3 and ω6 fatty acids: Biological effects and nutritional essentiality, *J. Nutr.* 119: 521-528.

Sinclair, H.M. 1980. Advantages and disadvantages of an Eskimo diet. In: *Drugs Affecting Lipid Metabolism* (edited by R. Fumagalli, D. Kritchevsky and R. Paoletti) Elsevier/North Holland Biomedical Press, Amsterdam, pp.363-370.

Sirtori, C.R. 1996. Omega-3 fatty acids do not impair diabetes control. CVD/Lipids dialog. Issue 5, Dec. 1996, 1-4.

Weaver, B.J., Piché, L.A., Ackman, R.G. and **Holub, B.J.** 1989. The effect of positional placement of EPA in ingested triglyceride on EPA accumulation in human platelet and plasma phospholipids. In: *Health Effects of Fish and Fish Oils,* (edited by R.K. Chandra) ARTS Biomedical Publishers, St. John's, Newfoundland, pp. 581-590.

Weylandt, K.H., Kang, J.X. and **Leaf, A.** 1996. Polyunsaturated fatty acids exert antiarrhythmic actions as free acids rather than in phospholipids. *Lipids,* 31: 977-982.

Wijesundera, R.C., Ackman, R.G., Abraham, V. and **deMan, J.M.** 1988. Determination of sulfur contents of vegetable and marine oils by ion chromatography and indirect ultraviolet photometry of their combustion products. *J. Am. Oil Chem. Soc.* 65: 1526-30.

Wijesundera, R.C., Ratnayake, W.M.N. and **Ackman, R.G.** 1989. Eicosapentaenoic acid geometrical isomer artifacts in heated fish oil esters. *J. Am. Oil Chem. Soc.,* 66: 1822-1830.

Wo, C.K.W. 1973. *The Nutritional Status of Alaskan Eskimos with Respect to Fatty Acids, Vitamin A and Vitamin E.* Ph.D. Thesis, University of Illinois at Urbana-Champaign.

Wo, C.K.W. and **Draper, H.H.** 1975. Vitamin E status of Alaskan Eskimos. *Am. J. Clin. Nutr.* 28: 808-813.