INTRODUCTION

AN ANTI-INFLAMMATORY FROM
THE SOUTHERN SEAS : LYPRINOL™

UN ANTI-INFLAMMATOIRE VENU
DES MERS DU SUD : LYPRINOL™

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Nous sommes ce que nous mangeons" - et buvons -, et la nature de nos nutriments et aliments conditionne nos fonctions physiologiques. L'eau nous est nécessaire. Les protéines, lipides et glucides également. Et les vitamines, les sels minéraux, les oligo-éléments, bien entendu. Mais la science alimentaire a progressé exponentiellement au cours des dernières décennies : les nutraceutiques et les aliments fonctionnels font désormais partie de notre environnement "santé à tout prix". Le prix, justement : retournant [ou le croyant] aux sources, très nombreux sont les gens qui veulent du "naturel". Ils oublient que l'amanite phalloïde, la ciguë, les bactéries du tétanos ou du botulisme, le VIH sont tous très naturels. La provenance naturelle signifie le plus souvent risque plutôt que santé. Et beaucoup de produits dits naturels écornent sérieusement le portefeuille.

Mais il y a des exceptions. Si, d'après certaines traditions, nous viendrions de la mer, il y a des océans des aliments qui apportent beaucoup sous un faible volume. Les acides gras poly-insaturés (AGPI) omega-3, et les caroténoïdes - entre autres - en font partie. La chaîne alimentaire veut que certains mollusques, placés là où il faut, engrangent et concentrent ces AGPI et les anti-oxydants. Perna canaliculus est la superbe, délicieuse moule verte de Nouvelle-Zelande. On la trouve dans certaines baies sans pollution, à l'eau assez fraîche, de l'île Sud de Nouvelle-Zelande, de Nouvelle-Caledonie, ou de l'Ouest du Mexique. Mais la zone de production est à la pointe Nord-Fst, et le long de la côte Est de l'Ile Sud de Nouvelle-Zelande. Là, dans le Marlborough Sound, et un peu plus au Sud, le Ministère de l'Agriculture et de Pêcheries de Nouvelle-Zelande a délimité une zone de production entièrement protégée et constamment surveillée. L'eau y est cristalline. Aucune industrie à l'horizon. Et très peu de maisons sur la côte : toutes doivent avoir une station d'épuration. S'il pleut, toute récolte de moules est arrêtée jusqu'à ce que les inspecteurs redonnent le feu vert. Les bateaux à moteur doivent croiser au-delà des 20 milles réglementaires, et le largage d'effluents est puni de prison. Chaque jour, plusieurs tonnes de moules sont ramassées, mises en cageots, et expédiées vers les marchés, poissonneries et grands restaurants du monde entier. Elles sont mangées crues et, à ce jour, aucun cas d'intoxication, empoisonnement ou allergie n'a été signalé.

On dit, là-bas, à Nelson ou à Christchurch, que les Maoris "maritimes", qui font de 30 à 40 moules leur petit déjeuner quotidien, ne souffrent ni d'arthrose, ni d'asthme. Et ce en contraste avec les Maoris "de l'intérieur" qui sacrifient aux joies du petit déjeuner "à l'anglaise" avec bacon, œufs, crêpes, et lait. Qui sait ? Mais cette rumeur légendaire a incité Jim et Bill Broadbent, des industriels Australiens entrepreneurs, à monter une usine de lyophilisation des moules à Nelson, à deux heures de camion des champs de myriculture. Cette usine est superbe, ultra-moderne, aux normes pharmaceutiques GMP. On y travaille en quasi-scapphandre, comme dans un bloc opératoire de transplantation d'organes. Les moules sont broyées, centrifugées, et leur purée orange est additionnée d'une dose déterminée d'acide tartreique pour éviter racrise et dégradation. La lyophilisation a lieu ensuite, et la poudre est mise en sacs. Cette poudre est vendue sous le nom de Seatone® dans...
de nombreux pays ; il faut en prendre 9 à 18 capsules par jour pour "lutter contre les rhumatismes". Mais si on retire - ce que fait la lyophilisation - l'eau des moules, on y laisse tout le sel (Seatone® est horriblement salée), les protéines et les glucides. D'où risque chez les hypertendus, risque allergique, ou d'intolérance digestive. Et la vision des 9-18 grosses capsules quotidiennes peut manquer de charme.

Les chercheurs Australiens, poussés par les frères Broadbent, ont démontré que les substances actives extraites de Perna canaliculus se trouvent dans la fraction lipidique des moules. Un procédé supercritique, utilisant le CO² liquide, permet l'extraction de cette seule fraction, sans utilisation de solvant. La société Flavex, en Allemagne, réalise cette opération et délivre une huile très visqueuse, orange, qui contient tous les principes actifs. Cette huile originelle est mélangée à de l'huile d'olive pharmaceutique (50 mg d'huile de moule/100 mg d'huile d'olive), additionnée d'une trace de vitamine E pour éviter tout risque de rancissement, et mise en capsule : voilà Lyprinol™. Un long chemin pour un produit strictement naturel, sans métaux lourds, pesticides, ou produit toxique, et qui ouvre des perspectives fascinantes pour le traitement des affections à composante inflammatoire.
Summary

The omega 3 polyunsaturated fatty acids have had a major impact on thinking in medicine in the last twenty years. The parent fatty acid in the omega 3 fatty acid family is alpha-linolenic acid (ALA) which is an essential fatty acid found in high concentrations in certain plant oils, such as flaxseed oil, walnut oil and canola oil. Several longer chain or derived omega 3 fatty acids are formed from alpha-linolenic acid and these are mainly found in fish, fish oils and from other marine organisms. The main marine omega 3 fatty acids are eicosapentaenoic acid (EPA), docosapentaenoic acid and docosahexaenoic acid (DHA). It is of interest that DHA is specifically localized in the retina and brain in humans and other mammals. The longer chain omega 3 fatty acids are rapidly incorporated into cell membrane phospholipids where it is regarded they influence the metabolism events within the cells. The mechanisms by which these changes occur include alteration in the fluidity of membranes such that there are subtle changes in receptor function, alteration in cell signalling mechanisms, membrane-bound enzymes, regulation of the synthesis of eicosanoids, and regulation of gene expression.

In this chapter, we report a comparison between the composition of the oil derived from the New Zealand Green Lipped Mussel (Lyrinol™) and two other oils rich in omega 3 fatty acids, namely flaxseed oil and tuna oil. The main lipid classes in Lyrinol™ were stear esters, triglycerides, free fatty acids, sterols and phospholipids while triglycerides were the main lipids in the other two oils. The main omega 3 fatty acids in Lyrinol™ were EPA and DHA, while in flaxseed oil and tuna oil the main omega 3 fatty acids were ALA and DHA, respectively. The main sterols in Lyrinol™ were cholesterol and desmosterol/brassicasterol, while in flaxseed oil and tuna oil the main sterols were beta-sitosterol and cholesterol, respectively.

Epidemiological observations, populations' studies and basic research indicate the possibility of influencing the outcome of cardiovascular disease, inflammatory disorders and neural function by ingestion of the omega 3 polyunsaturated fatty acids.

Keywords: Lyrinol - Fatty acids - Inflammation - Omega-3.

Résumé

Au cours des vingt dernières années, les acides gras polyinsaturés oméga-3 (n-3 PUFAs) ont eu un impact majeur sur notre manière d’envisager la médecine. L’acide gras originel de la famille oméga-3 est l’acide alpha-linolénique (ALA), acide gras essentiel retrouvé à haute concentration dans certaines huiles d’origine végétale, comme l’huile de lin, de noix, ou de colza. Plusieurs autres n-3 PUFAs à chaîne plus longue ou “branched” dérivent de l’ALA et sont présents dans les poissons, les huiles de poisson, et d’autres organismes marins. Les principaux n-3 PUFAs marins sont les acides éicosapentaénoïque (EPA), docosapentaénoïque et docosahexaénoïque (DHA). Il est intéressant de souligner que DHA est localisé spécifiquement dans la rétine et le cerveau humains, et d’autres mammifères.

Les n-3 PUFAs à chaîne longue sont rapidement incorporés dans les phospholipides des membranes cellulaires, et influencent le(s) métabolisme(s) cellulaire(s). Les mécanismes impliqués dans le déclenchement de ces variations comprennent l’altération de la fluidité des membranes avec modifications subtiles des fonctions de certains récepteurs, altérations des signaux cellulaires, des enzymes membranaires, de la régulation de la synthèse des éicosanoides et de l’expression des gènes.

Dans cet article, nous comparons la composition de l’huile extraite des moules vertes de Nouvelle-Zélande (Lyrinol™), et de deux autres huiles riches en n-3 PUFAs : l’huile de lin et l’huile de thon. Les principales classes lipidiques de Lyrinol™ sont les esters stéroliques, triglycérides, acides gras libres, stérols et phospholipides, alors que les triglycérides sont les principaux lipides des deux autres huiles. Les principaux n-3 PUFAs de Lyrinol™ sont EPA et DHA, alors que dans les huiles de lin et de thon ce sont ALA et DHA. Les principaux stérols de Lyrinol™ sont cholestérol et démostérol/brassicasterol, alors que dans les huiles de lin et de thon ce sont, respectivement, bêta-sitostérol et cholestérol.

Tant les observations épidémiologiques que les études de populations, ou les recherches fondamentales, suggèrent la possibilité d’influencer la survenue/évolution des maladies cardio-vasculaires, désordres inflammatoires, ou fonctions neurologiques par ingestion de n-3 PUFAs.

Mots-clés : Lyrinol - Acides gras - Inflammation - Oméga-3.
INTRODUCTION

The major constituents of marine lipids are the long chain omega 3 polyunsaturated fatty acids (PUFA). The interest in membrane PUFA developed at a great pace after two Nobel Prize winning discoveries in Medicine and Chemistry in the mid-1970s. Surprisingly, the omega 3 PUFA owe their popularity to epidemiological observations on the low rate of western lifestyle diseases in Greenland Eskimos.

The marine oils belong to the omega 3 PUFA family, which can be distinguished biochemically and physiologically from the other, more common family of PUFA, the omega 6 PUFA. There are two major types of omega 3 PUFA: alpha-linolenic acid is an 18 carbon fatty acid derived from plants, whereas fish, shellfish and marine omega 3 oils are the main dietary sources of the long-chain 20 and 22 carbon omega 3 PUFA. The main omega 6 PUFA is linoleic acid that is found in vegetable oils. The other two relatively common omega 6 PUFA are gamma-linolenic acid (found in Evening Primrose oil) and arachidonic acid (found in animal tissues such as muscle, liver, kidney and brain). The proportions of these omega 3 and omega 6 PUFA in various plant and marine oils are shown in table 1.

THE ESSENTIAL FATTY ACIDS

The two 18 carbon PUFA, linoleic and alpha-linolenic acids, are essential fatty acids (EFA), which means that like vitamins, they must be obtained in the diet. The essential nature of these PUFA was established in the 1930s (1). Linoleic acid was found to be the parent or precursor for a series of PUFA, now known as the omega 6 PUFA since linoleic acid itself is an omega 6 PUFA; likewise, alpha-linolenic acid is the parent fatty acid for the omega 3 PUFA. Linolenic acid is called alpha-linolenic acid in order to distinguish it from gamma-linolenic acid, an omega 6 PUFA, found in Evening Primrose oil (table 1). All fatty acids in the linoleic acid (omega 6 or n-6) PUFA family have their first double bond 6 carbons from the methyl end of the molecule. Similarly, all fatty acids in the alpha-linolenic acid (omega 3 or n-3) PUFA family have their first double bond 3 carbons from the methyl end (figure 1).

**Table 1** Fatty acid composition of edible fats and oils (g/100g fat or oil).

<table>
<thead>
<tr>
<th>Fat/oil</th>
<th>Sat*</th>
<th>Mono*</th>
<th>Poly*</th>
<th>18:2/18:3*</th>
<th>LCPn-3*</th>
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<tbody>
<tr>
<td>Coconut oil</td>
<td>87</td>
<td>6</td>
<td>2</td>
<td>20</td>
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<tr>
<td>Cocoa butter</td>
<td>60</td>
<td>33</td>
<td>3</td>
<td>28</td>
<td>0</td>
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<tr>
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<td>51</td>
<td>23</td>
<td>3</td>
<td>2</td>
<td>0</td>
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<tr>
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<td>49</td>
<td>37</td>
<td>9</td>
<td>46</td>
<td>0</td>
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<tr>
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<td>0</td>
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<tr>
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<td>45</td>
<td>10</td>
<td>0</td>
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<tr>
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<td>29</td>
<td>37</td>
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<td>31</td>
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<tr>
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<td>30</td>
<td>1</td>
<td>0.1</td>
<td>32</td>
</tr>
<tr>
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<td>29</td>
<td>27</td>
<td>37</td>
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<td>29</td>
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<tr>
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<td>26</td>
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<td>19</td>
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<td>15</td>
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<td>0</td>
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<tr>
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<tr>
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<tr>
<td>Canola oil</td>
<td>7</td>
<td>60</td>
<td>29</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Evening Primrose oil</td>
<td>7</td>
<td>9</td>
<td>78</td>
<td>150*</td>
<td>0</td>
</tr>
</tbody>
</table>

* Sat = Saturated, Mono = Monounsaturated, Poly = Polyunsaturated, 18 : 2/18 : 3 = ratio of linoleic acid to alpha-linolenic acid, LCPn-3 = long chain n-3 PUFA such as eicosapentaenoic acid, docosapentaenoic acid and docosahexaenoic acid.

- Contains no detectable alpha-linolenic acid.
- This oil contains 9% gamma linolenic acid.

**Figure 1** Structures of the essential fatty acids.

THE DOMINANCE OF THE OMEGA 6 PUFA IN THE FOOD SUPPLY

The content of omega 3 PUFA in the diet of westernised nations is considered to be low and there are several recommendations that the intake should be increased (2-4). The estimated intake of alpha-linolenic acid in USA and Canada is 1-2 g/head/day where the major vegetable oils used are reasonable sources of alpha-linolenic acid (soyabean oil and canola oil) [5].

In contrast, the diets of westernised nations are relatively enriched with the omega 6 PUFA linoleic acid at levels of approximately 11-16 g/day (2,5); the main source of this is from vegetable oils and derived products/foods such as margarines, salad dressings etc.
The consequence of ingestion of diets with this balance of omega 6 PUFA/omega 3 PUFA (a ratio of approx. 10 : 1) is that the tissue phospholipid fatty acid pools in our tissues become dominated by the omega 6 PUFA. This occurs since the total PUFA level in membranes is essentially constant and the extent of incorporation of omega 6 and omega 3 PUFA is determined by the ratio of omega 6 to omega 3 PUFA in the diet (6).

Linoleic acid has been considered the main EFA for humans for a number of reasons. Following the discovery of the EFA, it was shown that of the two essential fatty acids, linoleic acid was the more effective of the two in curing the common clinical signs of EFA deficiency in the rat including the scaly skin, hair loss and reduced growth rate (7). This observation has subsequently been extended to other vertebrate species of terrestrial origin including man (7). Perhaps the factor that gave the greatest impetus to the current widespread use of the linoleic acid-rich vegetable oils and margarines was the discovery that these oils were associated with the lowering of plasma cholesterol levels (8). On the other hand, alphalinolenic acid may have been ignored because it oxidises more readily leading to off-flavours and odours in foods. Thus, food producers could see little benefit in including high levels of the omega 3 PUFA in foods.

Most terrestrial species show a requirement for the omega 6 PUFA as essential nutrients, however in fish and other marine species there is a requirement for the omega 3 PUFA. This is hardly surprising, since the marine food chain is dominated by the omega 3 PUFA (6).

**OMEGA 3 PUFA IN THE BRAIN AND RETINA**

Until the 1970s, the omega 3 PUFA were not considered to be significant for humans in physiological or biochemical terms. However, from this time, the omega 3 PUFA became a focus of research in relation to the structure and function of the mammalian brain (9,10). The structural lipids (phospholipids) of brain grey matter of many different mammals (n=31), as widely divergent in size and habitat as mice, humans and whales, were found to contain the same fingerprint pattern of PUFA. There were two main omega 6 PUFA (arachidonic acid and docosatetraenoic acid) and one main omega 3 PUFA (docosahexaenoic acid, DHA) (11). In contrast to the brain PUFA profile, the liver and muscle PUFA profile was extremely variable between these same 31 species (11).

Subsequently, these long-chain PUFA were shown to be located in specific membranes such as the cerebral cortex synaptosomes and synaptic vesicles and the photoreceptor outer segments in the retina (10,12). The specific location of high concentrations of these PUFA led to the speculation that they were playing an important structural and/or metabolic role in the nervous system. To test this hypothesis, rats, guinea pigs and primates were placed on diets containing adequate linoleic acid but negligible amounts of alphalinolenic acid for prolonged periods. This procedure led to a depletion of the retina and neural membranes of DHA, and under these circumstances there were alterations in the response of the eye to light (electroretinographic response) (12-14). The report of the effect of omega 3 deficiency on retinal function in 1972 was the first time that these fatty acids had been shown to influence an important physiological function in a mammal. Later studies in omega 3 deficient animals showed changes in behaviour of the animals, including the development of learning difficulties (15). These results are highly suggestive of an important role for omega 3 PUFA (especially DHA) in these specialised tissues and since this time scientists have searched for the mechanisms of action of DHA and other PUFA in neural membranes. It is now known that DHA plays a crucial role in:

- membrane fluidity (membrane order) which can influence the function of membrane receptors such as rhodopsin (16, 17),
- regulation of membrane-bound enzymes (Na/K-dependent ATPase) (18),
- signal transduction via effects on inositol phosphates, DAG and protein kinase C (18),
- regulation of the synthesis of eicosanoids derived from arachidonic acid (18),
- regulation of gene expression via the peroxisomal proliferation system (19),
- stimulation of neurite outgrowth in PC12 cells (20, 21), and
- selective accumulation of DHA by synaptic growth cones during neuronal development (20, 21).

The interest in omega 3 PUFA in human nutrition has remained high since milk is the main source of these important PUFA during the period of brain growth and development in infants. The human brain DHA (grey matter) content increases 20 fold between the last trimester and the first five months of life, at which point 3% of the brain grey matter is DHA (22). Breast milk contains arachidonic acid and DHA and an omega 6 to omega 3 PUFA ratio of about 3, whereas milk for mammals often only contain the parent 18 carbon EFA with omega 6 PUFA/omega 3 PUFA ratios substantially higher than human milk (23, 24). Recent studies have established that use of infant formulas can lead to low red blood cell, liver, muscle, adipose and brain levels of DHA (25-27). Furthermore, such formulas can
lead to reduced or impaired retinal function in formula-fed infants (28), particularly in pre-term infants (29). Supplementation of infant formula milks with DHA leads to maintenance of tissue DHA levels and similar retinal function to that of term infants fed human milk (28, 29). In peroxisomal disorders, in which the final assembly of DHA is faulty or missing, there is severe neurodegeneration, blindness and death at 2-4 years of age. Martinez et al (30) have reported that DHA replacement therapy resulted in rescue that was associated with increased myelination as determined by sequential MRI scans.

**OMEGA 3 PUFA AND CARDIOVASCULAR DISEASE**

Since 1976, there has been considerable interest in the marine omega 3 PUFA largely due to the observations on Greenland Eskimos by Dyerberg and Bang (31). The Greenland Eskimos had a low incidence of death from coronary heart disease and other diseases of affluence despite their diet being rich in fat - mainly seal fat (32). In addition, their plasma lipid levels were relatively low for such a high fat diet, their plasma fatty acid patterns were dominated by the omega 3 PUFA and they had a prolonged bleeding time compared with Danes living on a 'western' diet. Although cholesterol levels have predominated thought on coronary heart disease for many years, the extent of thrombosis is also extremely important in terms of occlusion of blood vessels. Thus, the possibility for the thrombosis tendency to be modulated beneficially by diet was particularly exciting. This potential advantage was partly explained by the discovery of two prostaglandin-like compounds at about the same time as the reports on the Greenland Eskimos were published. Thromboxane (TXA2) was discovered by Samuelsson and co-workers in 1975 (33) and shown to be synthesized by platelets resulting in an increased aggregation of platelets and constriction of blood vessels. Prostacyclin (PGI2), discovered by Vane and co-workers in 1976 (34), was largely produced by arterial endothelial cells and had the opposite actions to thromboxane - it is one of the most powerful agents known to prevent platelet aggregation and it promotes blood vessel dilation. The thrombosis tendency was seen as being controlled by the balance between the production of thromboxane and prostacyclin. Both these compounds are derived from arachidonic acid which is released from cell membrane phospholipids by the action of phospholipase A2. The eicosanoids derived from arachidonic acid are shown in figure 2.

The connection between bleeding time, the two eicosanoids (thromboxane and prostacyclin) and the Eskimos became apparent when it was appreciated that one of the main omega 3 PUFA in the platelet membranes of the Eskimos was eicosapentaenoic acid (EPA) - this was hardly detectable in platelets from Danes, consuming a typical western diet, where the main 20 carbon PUFA was arachidonic acid (31). It was shown that EPA was acting as a competitive inhibitor in the conversion of arachidonic acid to eicosanoids - the structures of the two PUFA are identical except for the presence of one extra double bond at the end distal to the carboxyl group. Besides being a competitive inhibitor of cyclo-oxygenase, EPA also acts as a substrate for the synthesis of eicosanoids. That is, whenever there was a reasonable amount of EPA as well as arachidonic acid in the membrane phospholipids, eicosanoids derived from both PUFA were produced. In the platelet, competition by EPA with arachidonic acid would mean a reduction in TXA2 production, less platelet aggregation and thus a lower thrombosis tendency (35).
The results from a number of different experiments have shown that marine omega 3 PUFA consumption leads to a reduced production of TXA2 and an overall elevation in the production of prostacyclin - that is, PG12 production is about the same or somewhat elevated and there is the appearance of a prostacyclin from EPA (i.e. PG13). Thus, following the ingestion of marine omega 3 oil, the overall balance between thromboxane and prostacyclin is shifted in favor of the latter (36). In physiological terms, this means a reduced thrombus tendency due to decreased platelet aggregation and increased vessel dilation. These results may explain the increased bleeding times observed in Eskimos, Japanese and westernised subjects who consume marine omega 3 oils. Vitamin E is also considered to reduce the production of pro-inflammatory eicosanoids by inhibition of the release of arachidonic acid from membrane phospholipids and to reduce platelet aggregation (37, 38).

The Japanese are another major group who consume high amounts of fish, however their diet is low in total fat in contrast to the Eskimos. Epidemiological studies reveal that there is also a low incidence of coronary heart disease in this population, however they are prone to a high incidence of stroke (39).

THE EFFECT OF OMEGA 3 PUFA ON PLASMA LIPID LEVELS, BLOOD PRESSURE, CARDIAC ARRHYTHMIA, HEART RATE VARIABILITY AND ATHEROSCLEROSIS

In addition to effects of marine omega 3 oils on thrombosis tendency, attention was also focussed on the reduced plasma lipid levels in the Eskimos. In both experimental animals and man, EPA and other long-chain 20 and 22 carbon PUFA effectively reduce the production and export of triacylglycerols (TAG) by the liver (40). This decrease has been attributed to increased hepatic mitochondrial and peroxisomal oxidation of omega 3 PUFA, with evidence that EPA and DHA operate via different mechanisms (41). Since raised TAG levels are considered an independent risk factor for coronary heart disease (42), the reduction of plasma TAG levels by marine omega 3 oils is another beneficial attribute of the omega 3 PUFA.

Normotensive and hypertensive subjects who have taken omega 3 PUFA in the form of marine omega 3 oils have shown lowered blood pressure in some, but not all, intervention trials (43, 44). A meta-analysis of 17 controlled clinical trials showed that supple-

mentation with doses generally more than 3 grams of marine omega 3 PUFA/day led to a clinically relevant reduction in blood pressure in individuals with untreated hypertension (44). The mechanism whereby these effects are mediated has not been elucidated, however the most attractive hypothesis involves an alteration in the balance between vasodepressor prostaglandins (reducing production of vasoconstrictive thromboxane and increasing vasodilatory prostacyclin). There may also be effects via vasoconstrictor response to stressor hormones and reduced blood viscosity (44).

Ventricular fibrillation is a major fatal complication of coronary heart disease. The introduction of omega 3 PUFA as marine omega 3 oils into the diet of rats or the acute infusion of EPA and DHA in dogs can reverse ventricular fibrillation, strengthening the rationale for the use of these fatty acids in preventing rhythm disorders (45, 46). The presence of omega 3 PUFA in the myocardial cell membranes electrically stabilizes the cell and prolongs the relative refractory period (47).

The overall protective effects of the long chain omega 3 PUFA on heart function may also be related to improved heart rate variability. Decreased heart rate variability has been in fact strongly associated with increased risk of sudden death, and in a randomized trial, evaluating the effects of marine omega 3 oil vs. placebo in post-infarction patients, significant increases in heart rate variability were observed in the omega 3 PUFA-treated patients (48). Patients with higher fish intakes have also been shown to have greater heart rate variability.

Thus there are a number of mechanisms by which the omega 3 PUFA from marine omega 3 oils and seaweeds may exert physiological effects which may partly explain both epidemiological and clinical observations associated with a reduced risk for cardiovascular disease. In addition to the effects of omega 3 PUFA on plasma lipid levels, thrombosis tendency, blood pressure and incidence of cardiac arrhythmia, there is evidence that these fatty acids reduce the adhesion of blood cells to the endothelium, reduce blood viscosity and inhibit the development of atherosclerosis (35, 49).

INTERVENTION TRIALS USING OMEGA 3 PUFA (CARDIO-VASCULAR DISEASE)

An important question is do marine omega 3 oils make any real difference to whether we will suffer from fewer adverse cardiac events. The first study to prospectively...
explore the cardioprotective effect of omega 3 fatty acids in a secondary prevention population was the Diet and Reinfarction Trial. Burr et al (50) studied 2,013 men who had survived a heart attack. Half were advised to eat oily fish twice a week or to take marine omega 3 oil capsules, in an amount equivalent to the omega 3 fatty acid content of the fish diet, while the other half were advised only to eat a prudent diet. Survival over the subsequent 2 years was followed. The group advised to consume oily fish showed a 29% reduction in overall, 2-year mortality, compared with the control group. These results pointed to a possible protective effect for omega 3 fatty acids during ischemia and reperfusion, as already indicated by animal studies.

Another prospective, randomized clinical trial using omega 3 fatty acids was reported by Singh et al (51). Patients presenting with suspected myocardial infarctions (n=360) were randomized to placebo, marine omega 3 oil (2 g of EPA+DHA per day) or mustard seed oil (containing 2.9 g of α-linolenic acid per day). After one year, CHD events were significantly reduced in both omega 3 fatty acid groups. Von Schacky and colleagues (52) recently reported a small but statistically significant reduction in angiographically-determined CHD progression in a study which provided 6 g of omega 3 fatty acids for 3 months followed by 3 g/d for 21 months or placebo in 223 patients. There were 7 cardiovascular events in the control group and 2 in the omega 3 group (p = 0.10).

The most recent test of the effects of marine omega 3 fatty acids on CHD morbidity and mortality was the GISSI-Prevention Trial (53). This study was conducted in Italy and included 11,324 patients with known CHD. In a factorial design, 2830 of the patients were assigned to take vitamin E (300 mg/d); another 2836 were given 850 mg of omega 3 fatty acids daily; another 2830 given both and the final 2828 received neither. After 3.5 years of follow up, an intention-to-treat analysis revealed that total mortality in the patients given omega 3 fatty acids was 20% lower than in those patients not so treated, and the incidence of sudden cardiac death was reduced by 45%. Vitamin E showed a beneficial trend but it was not statistically significant. These results were achieved despite the fact that over 25% of patients reported that they stopped taking the capsules. The finding of the GISSI-Prevention study provides strong support for the use of marine omega 3 fatty acids in secondary prevention of acute coronary syndromes. The mechanism by which marine omega 3 fatty acids protect against cardiac death are not known with certainty, but it may relate to their ability to prevent cellular damage during periods of ischemic stress.

OMEGA 3 PUFA AND INFLAMMATORY DISEASES

Thus far, we have focussed on the effects of the omega 3 PUFA on the cardiovascular system and the potential for these PUFA to influence the development of one of the major causes of death in western countries. Another major area of interest relates to the effect of these PUFA on inflammatory diseases such as rheumatoid arthritis, psoriasis, IgA nephropathy, colitis and asthma. The basis of the relationship between marine omega 3 oils and inflammatory diseases stems from the original observations on the Greenland Eskimos where such diseases occur at a low incidence compared with the Danish population (32). The biochemical basis for the observations may be linked with another series of compounds derived from arachidonic acid. These compounds are known as leukotrienes (54) and the initial enzymatic step in their formation from arachidonic acid involves the 5-lipoxygenase; a series of leukotrienes is formed from this point as illustrated in figure 3. These issues will be discussed in considerable detail in other Chapters in this volume, however suffice it to say that the ingestion of marine omega 3 oils down-regulate the production of pro-inflammatory species (55, 56). Furthermore, clinical trials with marine omega 3 oils in the prevention of inflammatory conditions such as arthritis have led to improvement in the condition of the subjects in all trials (55). The clinical signs most affected were an improvement in tender joints, morning stiffness, grip strength and interval to fatigue onset. In these studies the production of leukotriene B4 from stimulated neutrophils was significantly decreased. It has also been shown in studies of rheumatoid arthritis patients who have taken marine omega 3 oils, that there is a decreased production of other pro-inflammatory mediators thought to be affected by the levels of leukotrienes, such as interleukin-1, platelet activating factor and tumour necrosis factor. Although the dose of marine omega 3 oil had to be quite high to achieve results (about 5 g/day of EPA plus DHA), there is considerable interest in the combined use of marine omega 3 oil and conventional anti-inflammatory drugs to reduce the pain and inconvenience caused by rheumatoid arthritis.

OMEGA 3 PUFA, MAJOR DEPRESSION AND SCHIZOPHRENIA

Changes in dietary fat consumption during the last century, including the lower omega 3 PUFA intake, may have contributed to an increased prevalence of cardiovascular disease (57). These same changes in
Horrobin proposed that since prostaglandins are derived from membrane PUFA, defective phospholipase A2 or cyclo-oxygenase could lead to a prostaglandin deficiency that might be sensitive to dietary interventions with essential omega 3 PUFA. There have been numerous small-scale studies that have examined various aspects of the membrane hypothesis with some evidence to support alterations in membrane phospholipids. The results have reported higher cerebrospinal fluid levels of prostaglandins, depletion of PUFA in red cell membranes, altered responses to a niacin challenge test (increased PGE2 production), increased phospholipase A2 activity and decreased synthesis and increased breakdown of membrane phospholipids. These results have provided the rationale for treatment of symptoms of schizophrenia with omega 6 PUFA, omega 3 PUFA and PGE1. In contrast to the omega 6 studies that have yielded negative results in 3 of 4 studies, all published studies of omega 3 PUFA treatment report positive results. Treatment with intravenously administered PGE1 has shown some benefits in a study of 7 subjects.

Depression and schizophrenia are currently the subject of intensive research investigations using marine omega 3 PUFA around the world.

**PLANT AND MARINE SOURCES OF OMEGA 3 PUFA**

The main focus of attention on omega 3 PUFA has been on marine sources of these fatty acids since the marine food chain is dominated by omega 3 PUFA. Fish, shellfish and other fish products such as fish eggs (roe) or marine omega 3 oils such as cod liver oil, tuna oil and menhaden oil are the main sources of omega 3 PUFA in our diet.

The other source of omega 3 PUFA in our diet is alphalinolenic acid, found in some vegetable oils such as rapeseed oil (canola oil), linseed or flaxseed oil, walnut oil and soybean oil, and in low amounts in baked beans and most green leafy vegetables. There is considerable debate about the effectiveness of the conversion of alpha-linolenic acid to EPA and DHA in humans by the pathway shown in figure 4.
\[ \alpha\text{-linolenic acid} \\
18:3 (\Delta^9,12,15) \\
\downarrow \Delta\text{-6-desaturase} \\
18:4 (\Delta^9,12,15) \\
\downarrow \Delta\text{-5-desaturase} \\
20:4 (\Delta^8,11,14,17) \\
\downarrow \Delta\text{-5-desaturase} \\
20:5 (\Delta^5,8,11,14,17) \\
\downarrow \downarrow \Delta\text{-6-desaturase} \\
22:5 \leftrightarrow 24:5 \leftarrow \leftrightarrow 24:6 (\Delta^6,9,12,15,18,21) \\
\downarrow \\
22:6 (\Delta^4,7,10,13,16,19) \\
\] 
DHA

Figure 4: Pathway for metabolizing ALA (18 : 3n-3) to DHA (22:6n-3). Solid arrows denote metabolic reactions, while the dashed arrows indicate reoxidation reactions that take place in either mitochondria or peroxisomes. Pathway described in reference 80.

The evidence available indicates that the conversion of alpha-linolenic acid to EPA occurs in most people, but that the process is slow and much less efficient than the direct incorporation of EPA and DHA from the diet into tissues (69). Vegetable oil sources rich in alpha-linolenic acid have not shown the beneficial effects in lowering plasma lipid levels or reducing blood pressure compared with marine sources of omega 3 PUFA (40). There is some evidence that vegetable oils containing alpha-linolenic acid may reduce thrombosis tendency (6) and the incidence of coronary heart disease (51, 70).

The consumption of marine omega 3 PUFA in USA and Australia is between 0.1 to 0.2g/day of EPA plus DHA (5), although measurement of plasma levels suggests an intake at the lower end of the range (71). This figure is substantially lower than the intakes of the Eskimos and Japanese of 10 and 1 g/head/day, respectively. Most of the clinical trials to date have used very high levels of marine omega 3 oils containing between 3 to 10 g/day of the omega 3 PUFA. The extent of incorporation of omega 3 PUFA from the diet can vary depending on the total fat intake and the types of other fatty acids being consumed. High levels of linoleic acid can interfere with the incorporation of omega 3 PUFA into tissue pools and also slow the rate of conversion of alpha-linolenic acid to EPA and DHA. Thus a practical way of increasing the omega 3 content of tissues is to decrease the linoleic acid content of the diet at the same time as increasing the omega 3 PUFA content (72).

There have been some concerns about the adverse effects of ingestion of large doses of marine omega 3 oils owing to the peroxidation of the highly unsaturated PUFA in these oils (73). Although the supplements are generally encapsulated, the potential for increased oxidation in the body once they have been absorbed must be considered. The intake of antioxidants, such as vitamin E, should be considered in such circumstances (74).

The Lipid Composition of Lyprinol™, an Oil Derived from the New Zealand Green Lipped Mussel

There have been a number of studies that have suggested that the lipid fraction from the New Zealand Green Lipped mussel is strongly anti-inflammatory by comparison with other more common marine oils (75, 76). We have conducted a number of studies to examine the composition of the oil from this mussel (Lyprinol™) that is sold as a 1:2 mixture of mussel lipids: olive oil with added vitamin E at 1.5 mg/kg final product. In the data presented here, we have compared the lipid composition of flaxseed oil, tuna oil and Lyprinol™. The main lipid classes in these natural oils were separated by thin layer chromatography and the results are shown. The main class of lipids in each of the oils was triacylglycerols, however the TLC pattern for Lyprinol™ was distinct in showing the obvious presence of steryl esters, free fatty acids, sterols and polar lipids. The tuna oil also showed the presence of sterols, while the flaxseed oil showed the presence of partial glycerides.

The fatty acids from the three oils were analysed (as methyl esters) by capillary gas liquid chromatography on a polar liquid phase (50m BPX70 column, SGE Australia). The main fatty acids in flaxseed oil were palmitic acid (16 : 0), stearic acid (18 : 0), oleic acid (18 : 1n-9), linoleic acid (18 : 2n-6) and alpha-linolenic acid (18 : 3n-3), with 18:2:n-6 accounting for approx. half of the total fatty acids. In tuna oil, the main fatty acids were palmitic acid (16 : 0), palmitoleic acid (16 : 1n-7), stearic acid (18 : 0), oleic acid (18 : 1n-9), EPA (20 : 5n-3) and DHA (22 : 6n-3), with
DHA accounting for nearly a third of all fatty acids. The Lyprinol™ fatty acid profile included palmitic acid (16:0) and its individual components, linoleic acid (18:2n-6), oleic acid (18:1n-9), and EPA (20:5n-3) and DHA (22:6n-3), with oleic acid accounting for nearly half of all fatty acids (Table 2).

The sterols were analyzed by capillary gas liquid chromatography on a non-polar liquid phase (50m BPX5 column, SGE Australia). The main sterols in Lyprinol™ were cholesterol, desmosterol, brassicasterol, and 22-trans-dehydrocholesterol, while cholesterol was the main sterol in tuna oil and beta-sitosterol and campesterol were the main sterols in flaxseed oil.

These data illustrate that three different sources of omega 3 fatty acids, two of which were of marine origin, have very different lipid classes, fatty acid and sterol compositions. Preliminary studies indicate that the anti-inflammatory material is in the fatty acid fraction of Lyprinol™ (75) (Table 3).

**Table 3**: The main sterols in three natural oils (% of total sterols).

<table>
<thead>
<tr>
<th>Sterol</th>
<th>Flaxseed oil</th>
<th>Tuna oil</th>
<th>Lyprinol™</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trans-22-dehydrocholesterol</td>
<td>0.4</td>
<td>0.2</td>
<td>10.9</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>4.0</td>
<td>98.5</td>
<td>31.8</td>
</tr>
<tr>
<td>Desmosterol/brassicasterol</td>
<td>3.1</td>
<td>0</td>
<td>23.1</td>
</tr>
<tr>
<td>24-methylene cholesterol</td>
<td>0.7</td>
<td>0.1</td>
<td>7.0</td>
</tr>
<tr>
<td>Campesterol</td>
<td>25.3</td>
<td>0.1</td>
<td>1.7</td>
</tr>
<tr>
<td>Stigmasterol</td>
<td>5.1</td>
<td>0.1</td>
<td>0.8</td>
</tr>
<tr>
<td>Zearin-cholesterol</td>
<td>51.1</td>
<td>0</td>
<td>6.4</td>
</tr>
</tbody>
</table>

**CONCLUSION**

The interest in the omega 3 PUFA and the recognition that many western diets contain low levels of these PUFA has led to numerous recommendations to alter the balance of linoleic acid to omega 3 PUFA in our diets (77). Current evidence would suggest that the ratio in many western diets is of the order of 10:1 (5). Some have argued that the ideal ratio should be based on that found in human milk or that of the food selected by primitive man and by wild animals that give figures of less than 3:1 (78, 79). In any event, it is clear that reducing the ratio in our diet will lead to changing it more towards what it is likely to have been during our past. This has the advantage of having an evolutionary precedent, a diet referred to as a Diet of Evolutionary Adaptiveness (79).

**REFERENCES**

4. International Society for the Study of Fatty Acids and Other Lipids, workshop held at NIH April 1999, Bethesda, MD (reported in the ISFAL newsletter).


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