Health Aspects of Fish and N-3 Pufa from Plant and Marine Origin: Summary of a Workshop

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Health aspects of fish and n-3 polyunsaturated fatty acids from plant and marine origin.

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An expert workshop reviewed the health effects of n-3 polyunsaturated fatty acids (PUFA), and came to the following conclusions.

1. Consumption of fish may reduce the risk of coronary heart disease (CHD). People at risk for CHD are therefore advised to eat fish once a week. The n-3 PUFA in fish are probably the active agents. People who do not eat fish should consider obtaining 200 mg of very long chain n-3 PUFA daily from other sources.
2. Marine n-3 PUFA somewhat alleviate the symptoms of rheumatoid arthritis.
3. There is incomplete but growing evidence that consumption of the plant n-3 PUFA, alpha-linolenic acid, reduces the risk of CHD. An intake of 2 g/d or 1% of energy of alpha-linolenic acid appears prudent.
4. The ratio of total n-3 over n-6 PUFA (linoleic acid) is not useful for characterising foods or diets because plant and marine n-3 PUFA show different effects, and because a decrease in n-6 PUFA intake does not produce the same effects as an increase in n-3 PUFA intake. Separate recommendations for alpha-linolenic acid, marine n-3 PUFA and linoleic acid are preferred.

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Descriptors: fish; marine n-3 PUFA; w-3 PUFA; alpha-linolenic acid; human; health; coronary heart disease

Introduction

This paper summarises the conclusions of an expert workshop on the health aspects of fish and n-3 polyunsaturated fatty acids (PUFA). The objectives of the workshop were to formulate scientifically sound conclusions on the health effects of (fatty) fish and the use of plant (alpha-linolenic acid: C18:3 n-3) and marine n-3 PUFA, mainly eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3) in products for health reasons. The discussions were held on the basis of questions sent to the participants beforehand, and on brief introductions by participants. The discussions were held in four sessions: fish and health, health effects of plant and marine n-3 PUFA, human experiments with n-3 PUFA, and conclusions and recommendations.

Fish and health

Does the consumption of fish prevent coronary heart disease (CHD)? What is the optimal intake (amount and frequency) of fish?

Several prospective observational studies and a randomised factorial trial showed that one serving of fish weekly may decrease the risk of fatal CHD by approximately 40% relative to no fish (Burr *et al*, 1989; Ascherio *et al*, 1995; Kromhout *et al*, 1995; Gillum *et al*, 1996; Daviglus *et al*, 1997). Higher intakes did not provide greater protection. The prospective studies had a comparable methodology, compared either fish with no fish or low doses of marine n-3 PUFA (fish oil) with no marine n-3 PUFA, and give a consistent picture of a reduction in CHD for the fish and marine n-3 PUFA groups. No inverse association between fish intake and fatal CHD has been found in a number of other prospective studies. This was probably due to the higher overall fish intakes (Lapidus *et al*, 1986; Morris *et al*, 1995). These latter studies do not contradict the hypothesis of beneficial effects of one serving of fish per week. They do strengthen, however, the conclusion that larger amounts of fish are not associated with increased benefits.

Does fatty fish or the EPA/DHA content of the diet have a specific role in the prevention of CHD?

Much of the fish consumed in the epidemiological studies was probably lean white fish. Combining data on the consumption of fish and the effects on CHD risk parameters with insights on mechanisms make it plausible, but not certain, that the very long chain n-3 PUFA were the fish components which caused the decrease in the risk of fatal CHD (Burr *et al*, 1989; Siscovick *et al*, 1995; Singh *et al*, 1997).

Does consumption of fish protect against cancer?

There is no proof that fish consumption decreases cancer risk. In animal experiments high doses of marine n-3 PUFA

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inhibited the development of chemically-induced breast and colorectal cancers in the promotion phase, but probably not in the initiation phase. Epidemiological studies on fish and marine n-3 PUFA and cancer show variable results, but most studies show an inverse relationship between fish intake and the risk of breast and colorectal cancer (Boutron *et al*, 1991; Neuget *et al*, 1993; Sasaki *et al*, 1993). These studies, however, provide little information on n-3 PUFA intakes. Recent epidemiological studies suggest an interaction between animal fat and fish from which might be hypothesised that marine n-3 PUFA protect against the effect of animal fat in colorectal cancer (Caygill *et al*, 1996). In trials with patients prone to development of colorectal adenomas marine n-3 PUFA have been found to 'normalise' hyperproliferation of mucosal crypt cells (Anti *et al*, 1992).

Health effects of plant and marine n-3 PUFA

Does alpha-linolenic acid protect against CHD?
Epidemiological studies (Dolecek, 1992; Ascherio *et al*, 1996) and one clinical trial (De Lorgeril *et al*, 1994) suggest that intake of alpha-linolenic acid-rich food can decrease the risk of myocardial infarction and death. A relative deficiency of n-3 PUFA may be present in a large proportion of the population and might be a factor in CHD. In the Health Professional Follow-up Study the relative risk of CHD for a one energy-% increase in alpha-linolenic acid was 0.41 after adjustment for standard risk factors and intake of fibre and total fat (Ascherio *et al*, 1996). An increase in arterial compliance by dietary alpha-linolenic acid, which might decrease the work load of the heart, might contribute to a reduced risk in CHD by alpha-linolenic acid (Nestel *et al*, 1997). Arterial compliance is also increased by marine n-3 PUFA in diabetics.

Can the benefits of EPA/DHA be obtained through alpha-linolenic acid? If so, what is the conversion factor and on which criteria should this be judged?
The effects of EPA/DHA can not be fully reproduced by alpha-linolenic acid, even in high doses. Conversion of alpha-linolenic acid into EPA might play a role in the protection against CHD (Allman *et al*, 1995; Freese & Mutanen, 1997). However, the efficacy of alpha-linolenic acid in raising plasma EPA levels is low, alpha-linolenic acid is mainly oxidised, whereas EPA is incorporated into complex lipids. For every 10 g of dietary alpha-linolenic acid 0.5–1 g EPA has been found to be incorporated into complex lipids (Emken *et al*, 1990; Valsta *et al*, 1996). Supplementation with alpha-linolenic acid (10 g/d or more), however, minimally affected cellular phospholipid DHA levels (Mantzioris *et al*, 1994). The conversion ratio is a crude measure of conversion plus incorporation and depends on the dose of alpha-linolenic acid and the content of other PUFA in the diet (mainly linoleic acid). Further studies are required to obtain quantitative conclusions on the effect of dietary alpha-linolenic acid on tissue contents of EPA and DHA.

Is the conversion of alpha-linolenic acid to very long chain PUFA influenced by age or disease state?
There are very little quantitative data available on the effects of age and disease on the conversion of alpha-linolenic acid into very long chain PUFA (Bjerve *et al*, 1989; Nestel, 1992). Recent, unpublished data indicate that the amount of very long chain PUFA derived from alpha-linolenic acid in blood lipids does not differ between subjects over 60 y *vs* subjects under 35 y (Hornstra *et al*). Also, no difference has been demonstrated between term and pre-term infants in their ability to accumulate tissue EPA from dietary alpha-linolenic acid (Carnielli *et al*, 1996). In some diseases, for example insulin deficient diabetes, chronic alcoholism, and schizophrenia, the amount of EPA incorporated into tissue lipids from dietary alpha-linolenic acid may be decreased. In pregnancy-induced hypertension, the amount of very long chain PUFA in plasma and tissue phospholipids is increased (Al *et al*, 1995).

Does alpha-linolenic acid affect cancer risk?
Data on alpha-linolenic acid and cancer in humans are scarce (Dolecek, 1992). The results of animal studies are conflicting (Johnston, 1995). Increased intake of alpha-linolenic acid was associated with increased prostate cancer incidence (Giovannucci *et al*, 1993; Gann *et al*, 1994). This finding was recently confirmed in a case-control study in Finland (Harvei *et al*, 1997). However, other studies do not suggest an effect of alpha-linolenic acid intake on cancer mortality (Kolonel *et al*, 1988; Bougnoux *et al*, 1994). The reason for the observed relationship between the alpha-linolenic acid intake and prostate cancer is not clear.

Human experiments with n-3 PUFA

What is the outcome on blood lipids and lipoproteins?
Marine n-3 PUFA in doses of up to 7 g/d (average 3–4 g/d) lower fasting blood triglycerides by approximately 25% (Harris, 1997). VLDL lipids are most affected. There is evidence of a dose-response relation. The post-prandial triglyceride level is also lowered. LDL cholesterol increases by 4% in healthy subjects and by 7% in hypertriglyceridaemic patients (in addition to an increase in LDL particle size). HDL cholesterol increases by 3% in healthy people, but not in hypertriglyceridaemic patients. There are no indications for a modulating effect of linoleic acid on the effects of n-3 PUFA on blood lipids.

Dietary alpha-linolenic acid does not affect blood triglycerides, even at high intakes, and it lowers LDL cholesterol probably only when it replaces saturated fatty acids.

What is the outcome on neural development?
Both very long chain n-3 and n-6 PUFA are important for neural development (Innis, 1991; Makrides *et al*, 1996). An important issue still is the adequacy of alpha-linolenic acid in infant food as a precursor for DHA, in particular for pre-term infants (Salem *et al*, 1996). In these infants, formulas enriched with DHA were found to improve visual acuity and neuro-mental development. These effects disappeared at somewhat later age (Carlson *et al*, 1993; Innis *et al*, 1996), but were of longer duration in other studies (Uauy *et al*, 1996). An increased occurrence of sepsis or necrotising enterocolitis at enteral feeding with supplemented marine n-3 PUFA cannot be ruled out (Carlson *et al*, 1996). Although DHA levels in brain autopsy tissue from babies fed formulas have been found to be lower than in breast-fed babies, it is not clear whether supplementation of formulas with marine n-3 PUFA has beneficial effects on brain development.

Due to the difficulties in establishing the physiological consequences of DHA shortage in vivo, the question whether the addition of DHA alone or in combination with arachidonic acid to infant formulas confers a physiological benefit to the infant has not yet been clarified. The same holds true for supplementation with alpha-linolenic acid at realistic doses to pregnant or lactating mothers in relation with the health of the new-born (Al *et al*, 1997). It is also uncertain to what extent the ratio of linoleic acid to alpha-linolenic acid and/or the absolute amounts of linoleic acid and alpha-linolenic acid in the mothers' diet are of relevance to the health status of the foetus and breast-fed infant.

What is the outcome on lipid peroxidation?
Oxidised LDL has been implicated in atherosclerosis. Oxidation of LDL leads to formation of thiobarbituric acid-reactive substances (TBARS). However, TBARS reflect both the content of PUFA and their oxidizability in LDL-particles. Moreover, appreciable amounts of TBARS may be present in foods and absorbed into the blood stream. Therefore, TBARS (in fact a rather unspecific method) should no longer be used as a measure for the oxidizability of LDL-particles *in vivo*.

Oxidizability of LDL is measured in vitro and may not reflect oxidation sensitivity of LDL in vivo. Linoleic acid-enriched LDL particles are oxidised *in vitro* faster than LDL particles enriched with saturated or monounsaturated fatty acids (Reaven *et al*, 1994). Inconsistent *in vitro* results have been published with respect to both plant and marine n-3 PUFA (Suzukawa *et al*, 1995; Brude *et al*, 1997). Both n-6 and n-3 PUFA, however, should be regarded as sensitive to oxidation *in vivo*.

Since consumption of n-3 PUFA can be associated with a higher susceptibility to in vitro oxidizability of LDL, this should be accompanied by adequate amounts of antioxidants such as α -tocopherol (Muggli, 1994). The established beneficial effects of plant and marine n-3 PUFA on other risk factors for CHD likely outweigh any increased risk from oxidative changes.

What is the outcome on diabetes?
The effects of dietary n-3 PUFA on CHD risk factors in diabetics (type II) are not much different from those in non-diabetics (Axelrod *et al*, 1994). In some studies marine n-3 PUFA induced a small increase in blood glucose levels in diabetics (Vessby *et al*, 1992). Marine n-3 PUFA may reduce the insulin response (secretion) to glucose, but no consistent effect of marine n-3 PUFA on insulin sensitivity has been found. A possible interaction with the n-6 PUFA in the diet has not been adequately studied. Moderate doses of marine n-3 PUFA, and also fish consumption (Feskens *et al*, 1991), may be beneficial in diabetes, for example by an effect on triglycerides, but the use of large doses of marine n-3 PUFA should not be encouraged.

What is the outcome on blood pressure?
Intake of marine n-3 PUFA can reduce blood pressure in hypertensive but less commonly in normotensive subjects. A minimum daily amount of 3 g may be needed for a significant reduction (Appel *et al*, 1993; Morris *et al*, 1993). Typically, 5–6 g n-3 PUFA daily reduced systolic and diastolic blood pressure by 3.4 and 2.0 mm Hg, respectively, which can be expected to reduce risk for both stroke and CHD. In epidemiological studies no relation could be detected between fish intake and blood pressure. The limited data on alpha-linolenic acid show variable effects (Salonen, 1991; Nestel *et al*, 1997).

What is the outcome on thrombosis/haemostasis?
Because direct effects on arterial thrombosis can not be measured, surrogate endpoints have to be used to estimate effects on thrombotic risk. In endothelial cells (*in-vitro* studies) marine n-3 PUFA reduced the levels of mRNA coding for adhesion molecules and increased prostacyclin synthesis, whereas effects on nitric oxide release were inconsistent. Data on effects of marine n-3 PUFA and alpha-linolenic acid on coagulation and fibrinolysis are incomplete and inconsistent. Marine n-3 PUFA, but not alpha-linolenic acid, might reduce the level of circulating platelet aggregates. Summarising, there are insufficient data on the extent to which the anti-CHD effects of marine n-3 PUFA are mediated by changes in haemostasis (Knapp, 1997).

What is the outcome on arrhythmias?
In animal models alpha-linolenic acid, EPA, DHA, and other PUFA reduced the susceptibility to ventricular fibrillation and in cardiac myocytes they had antiarrhythmic activity (Charnock, 1994; Leaf & Kang, 1996). Effects on ion channels play a role. Epidemiological and clinical studies also suggest antiarrhythmic effects from the consumption of fish and marine n-3 PUFA (Siscovick *et al*, 1996; Christensen *et al*, 1997; Singh *et al*, 1997). The evidence warrants controlled clinical trials on the effect of marine n-3 PUFA on ventricular fibrillation (the main cause of sudden death from acute heart attacks). No human data are available on the prevention of primary cardiac arrest by alpha-linolenic acid.

What is the outcome on immune function and inflammation?
Marine n-3 PUFA moderately, but reproducibly, decrease pain and morning stiffness in rheumatoid arthritis (Kremer, 1996) and may also have beneficial effects in renal transplantation (fewer complications) and in inflammatory bowel disease. Their effect on immunologic and inflammatory diseases is probably due to the decrease in (effective) immune and inflammation mediators (eicosanoids and cytokines) (Endres & Von Schacky, 1996). High dietary amounts of alpha-linolenic acid may also modestly decrease eicosanoid and cytokine synthesis, but do not have a clear effect on immunologic and inflammatory responses. There is no solid evidence that marine n-3 PUFA is effective in asthma and only weak evidence in psoriasis. There are no epidemiological data for fish and arthritis and there is only one study on alpha-linolenic acid and arthritis which showed that alpha-linolenic acid was ineffective. Due to effects of n-3 PUFA on immune and inflammation mediators, an increased intake of EPA might lead to a suppression of immune and inflammation responses, and consequently, to a decrease in host resistance to infections. However, no adverse effects of marine n-3 PUFA on infection in humans have been reported.

Recommendations and conclusions

Are there scientific reasons to increase the current intake of n-3 fatty acids?

There is sufficient scientific evidence to support the view that marine n-3 PUFA can be beneficial for health, especially for those who are at high risk for CHD. The meeting therefore agreed with the general recommendation to eat fish at least once a week. Fatty fish is preferable because of its high content of n-3 PUFA. People who do not eat fish could consider consuming marine n-3 PUFA equivalent to the amount obtained from fatty fish, namely 200 mg EPA plus DHA daily. This is equivalent to 10–40 g of fatty fish per day. Pregnant women and neonates may require, in addition to linoleic acid and alpha-linolenic acid, some intake of very long chain n-3 PUFA to cover the needs for optimal growth and development.

Should there be separate recommendations for plant and marine n-3 PUFA?

Separate recommendations for plant and marine n-3 PUFA may be needed due to their different physiologic effects. The meeting concluded that there are no scientific reasons to deviate from a recommendation for alpha-linolenic acid of one energy-%. Because the impact of an advice on intake of n-3 PUFA could be significant, solid scientific underpinning through large-scale prospective
studies and randomised controlled clinical trials is required. In addition, nutritional studies done so far with realistic amounts of EPA and DHA should be repeated with alpha-linolenic acid.

Is the use of the n-3/n-6 ratio scientifically sensible?

Experience with the polyunsaturated/saturated fatty acid ratio suggests that the use of a n-3/n-6 ratio will not be helpful. For instance, one might increase the n-3/n-6 ratio by lowering of the dietary linoleic acid level, but this does not have the same effect as increasing the n-3 PUFA level. The background of the use of the ratio is that the absolute amounts of n-3 PUFA in the Western diet might be too low and that the conversion of alpha-linolenic acid to DHA can be decreased by increasing the dietary amount of linoleic acid. Although the latter may be true, the absolute dietary amount of linoleic acid as such is important for CHD and not the ratio to n-3 PUFA.

Furthermore, alpha-linolenic acid and marine n-3 PUFA have distinct physiological effects and can not replace each other. However, the n-3/n-6 ratio is being discussed widely and is therefore difficult to ignore. The meeting concluded that there should be separate recommendations for alpha-linolenic acid, marine n-3 PUFA and linoleic acid and that the n-3/n-6 ratio should not be used.

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