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Review

Dietary n-3 polyunsaturated fatty acids and coronary heart disease-related mortality: a possible mechanism of action

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Abstract. Epidemiological and interventional studies indicate that dietary n-3 PUFA reduces mortality due to coronary heart disease (CHD). They act at a low dose, since one or two meals with fatty fish per week is sufficient to provide protection when compared with no fish intake. These fatty acids are effective in providing primary prevention in low- and high-risk subjects and secondary prevention. At high doses, dietary n-3 PUFAs have several beneficial properties. First, they act favourably on blood characteristics: they are hypocholesterolemic and hypotriglyceridemic; they reduce platelet aggregation; they exhibit antithrombotic and fibrinolytic activities; they reduce blood viscosity and they exhibit antiinflammatory action. Second, they reduce ischemia/reperfusion-induced cellular damage. This effect is apparently due to the incorporation of eicosapentaenoic acid in membrane phospholipids. Third, they reduce ischemia and reperfusion arrhythmias. All the effects exerted by n-3 PUFAs at high doses are incompatible with the beneficial action on CHD mortality in humans observed at low doses, where their main properties are related to circulation in the form of free fatty acids. Numerous experimental studies have indicated that low concentrations of exogenous n-3 PUFAs reduce the severity of cardiac arrhythmias. This effect is probably responsible for the protective action of n-3 PUFA on CHD mortality. Further studies are necessary to confirm this assumption in animals. Such studies should take account of the fact that only a low dose of n-3 PUFA (20 mg/kg/day) is necessary to afford protection. Furthermore, since the beneficial effect of n-3 PUFAs on CHD mortality is observed in fish eaters versus no-fish eaters, and since populations in industrialised countries consume excess n-6 PUFAs, control animals in long-term dietary experiments should be fed a diet with only n-6 fatty acids as a source of PUFAs.

Key words. Dietary n-3 polyunsaturated fatty acid; coronary heart disease; mortality; ischemia; blood properties; cellular damage; arrhythmia.

Introduction

Dietary lipids are known to modulate the incidence and severity of coronary heart disease (CHD). The level of lipids in the diet has a noticeable influence. Numerous epidemiological studies have reported a positive correlation between CHD mortality and the amount of lipids ingested [1–12]. Furthermore, several interventional studies have underlined the beneficial effects of low-fat diets [13, 14]. The type of dietary lipids is also of crucial importance in the prevention of CHD mortality. Saturated fatty acids exhibit detrimental effects, as demonstrated by numerous epidemiological investigations [1, 4, 8, 9, 12, 15–27]. Only two epidemiological studies have found no positive correlation between saturated fatty acid intake and CHD mortality [10, 28]. Interventional studies have

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been more controversial. In 1997, Oliver [14] reviewed the effects of low saturated fatty acid diets on CHD mortality. He indicated that only 5 of the 13 interventional studies performed in this field reported any beneficial effects of such diets. Only diets enriched with polyunsaturated fatty acids (PUFAs) were positively correlated with a reduced risk of CHD mortality. The authors concluded that reducing the amount of saturated fatty acids was not enough, whereas increasing the proportion of PUFAs was a prerequisite for the beneficial action of low-fat diets.

Natural PUFAs belong to two distinct families: the n-6 family comprising several fatty acids with the final double-bond located six carbons away from the terminal methyl group, and the n-3 family, which groups fatty acids with the final double-bond three carbons away from this group. The chemical structure of the main PUFAs is presented in figure 1. The dietary sources of n-6 PUFAs are numerous. They include different plant oils currently used in cooking such as corn, safflower and sunflower seed oils. These oils provide a high amount of linoleic acid (LA, 18:2 n-6), the precursor of n-6 PUFA. More unsaturated n-6 PUFAs such as arachidonic acid (AA, 20:4 n-6), are found in meats, mostly those of nonruminant animals. Dietary n-3 PUFAs are also found in plant oils and animal fats. The sole n-3 PUFA of plant origin is α-linolenic acid (α-LNA, 18:3 n-3), found in algae, soy oil and canola oil. Fish contains a high amount of more unsaturated n-3 PUFAs, such as eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3). Meat of nonruminant animals also contains a low level of n-3 PUFAs, but it is generally considered that n-3 PUFAs are of marine origin. Linseed oil contains a high amount of α-LNA. The diet in industrialised countries includes high levels of n-6 PUFAs and low levels of n-3 PUFAs [29]. This was well documented by the fatty acid composition of membrane phospholipids from human cardiac biopsies collected in Dijon (Burgundy, France) in the early 1980s [30]. The levels of LA and AA were high (19 and 23% total phospholipid fatty acids, respectively) and those of n-3 PUFAs were low (0.5, 1.6 and 5.1% total phospholipid fatty acids for EPA, docosapentaenoic acid and DHA, respectively). Furthermore, the intake of n-3 PUFAs varies considerably in the population, some people regularly eating different sources of n-3 PUFAs and others never eating fish.

The aim of the present review was to establish which types of PUFAs would be the most protective in reducing CHD mortality, and to describe the possible mechanisms of action. We will review the effects of dietary n-6 and n-3 PUFAs in human studies from an epidemiological point of view, and also consider the results of interventional studies. We shall then try to assess the most probable mechanism of action for beneficial PUFAs, based on the numerous animal studies carried out in this field.

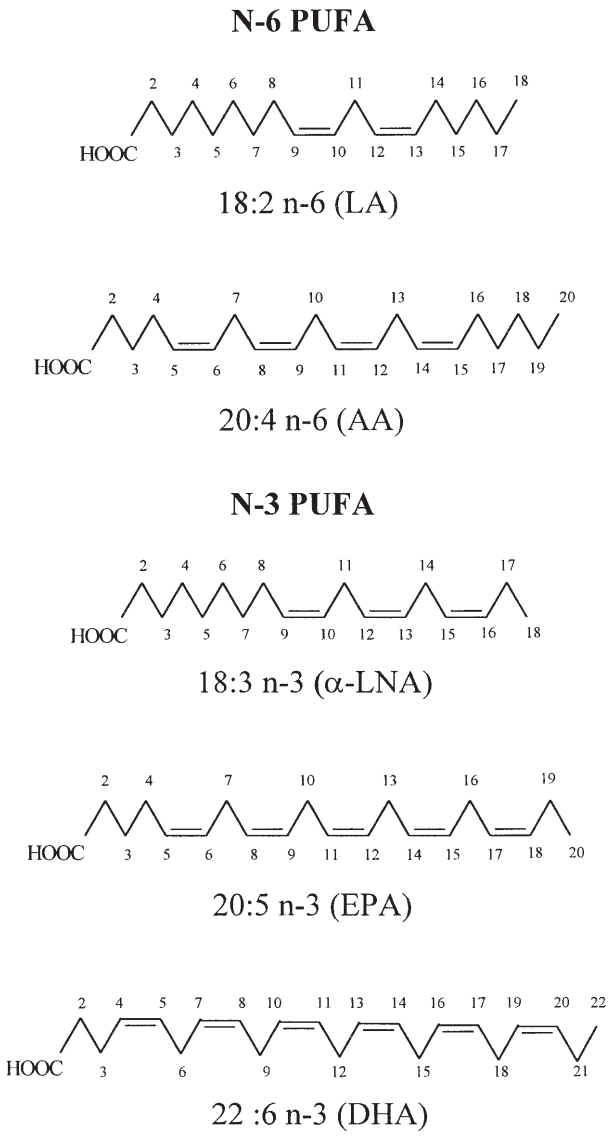

Figure 1. Structure of the main polyunsaturated fatty acids. PUFA, polyunsaturated fatty acids; LA, linoleic acid; AA, arachidonic acid; α-LNA, α-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

PUFAs and studies in humans

The effect of dietary n-6 PUFAs was studied extensively during the 1970s and 1980s. Numerous studies, performed in different industrialised countries, showed the beneficial effects on CHD mortality of replacing saturated fatty acids by such PUFAs [4, 6, 9, 18, 20, 22, 25, 26, 31–33]. However, some studies did not report any relationship between n-6 PUFAs and CHD mortality [19, 34]. Other epidemiological studies even showed a positive correlation between CHD mortality and the amounts of AA in plasma phospholipids [35] or LA intake, as measured by its content in adipose tissue [36]. Furthermore, sudden death from cardiac causes has been

associated with high levels of 20:4 n-6 or 18:2 n-6 in coronary phospholipids and adipose tissue, respectively [37–39]. These results clearly suggest the beneficial effects of replacing saturated fatty acids by n-6 PUFAs, but also that high levels may be detrimental under certain circumstances. This raises the question of dietary PUFAs belonging to the n-3 family. In the 1970s, several publications indicated that populations in Greenland and Japan exhibited a low incidence of sudden cardiac death [40–50]. This was associated with a high n-3 PUFA intake. Since these early studies, numerous epidemiological investigations have been carried out. Most have reported the protective effect of n-3 PUFAs [2, 28, 35, 39, 50–64], but others have not shown any correlation between the intake of n-3 PUFAs and CHD mortality [19, 62, 65–67]. The inconsistency of these results was explained by Siscovick et al. [68]. They showed that n-3 PUFAs act at a low dose. When compared with no fish intake, the intake of one or two meals including fish per week is protective in primary prevention. This has been confirmed [63], but it had already been demonstrated in 1985 and 1992 [53, 69]. Lean fish is not effective; only fatty fish affords protection [62]. This beneficial action is observed in normoglycemic subjects and not in hyperglycemic patients [55], but fish oil supplementation in type II diabetic patients increases low-density lipoprotein cholesterol [70]. Furthermore, high-risk subjects are more sensitive to the protective action of fish than low-risk subjects [64, 67]. All the interventional studies performed with n-3 PUFAs had a positive outcome, either in primary prevention [71] or after a first episode of CHD [72–77] when the diet contained fish, plant n-3 PUFA or the ethyl ether of EPA and DHA. Lastly, n-3 PUFAs can be recommended as primary prevention in low-risk and high-risk subjects and for secondary prevention [76, 78]. They reduce CHD mortality without decreasing the incidence of coronary events [71]. This clearly indicates that dietary n-3 PUFAs do not act by reducing the development of thrombosis and coronary atherosclerosis, although n-3 PUFAs decrease the synthesis of low- and very low [79] density lipoproteins [80]. They exert their beneficial effects downstream after vessel occlusion. Numerous investigations have been carried out to elucidate the mechanism of action of dietary n-3 PUFAs, and three main themes have been assessed (i) blood risk factors for coronary heart disease, (ii) severity of ischemia/reperfusion-induced cellular damage and (iii) severity of ischemia and reperfusion arrhythmia.

Potential mechanisms of action

Biochemical basis of the effects of n-3 PUFAs

N-3 PUFAs modulate several metabolic pathways because they constitute the substrates of different enzymes of lipid metabolism. Furthermore, they modify the activity of other enzymes via a change in membrane lipid environment or a modification in the gene expression of protein synthesis. N-3 PUFAs cannot be synthesised in the body and must be provided by the diet. After intestinal absorption, they are incorporated into chylomicrons and transferred to the liver, where they can be elongated and desaturated (fig. 2). Thereafter, they are released into the bloodstream and incorporated into different organs including the heart, lung, kidney, brain, adipose tissue, skeletal muscle and so on [81–85]. In the cellular environment, they can either be oxidised in the mitochondria or incorporated into complex lipids. The rate of β-oxidation is high [86, 87], which explains the rapid turnover in the body and the need for a constant dietary supply. They can be incorporated into triglycerides, phospholipids and cholesterol esters. Incorporation into phospholipids is of crucial importance to the regulation of cell metabolism, since these fatty acids can modify the physical properties of lipid domains in the membranes where proteins are inserted [88]. N-3 PUFAs compete with n-6 PUFAs for phospholipid fatty acid composition [89]. In the heart of rats fed only PUFAs of the n-6 family (10% sunflower seed oil) for 8 weeks, the level of n-6 PUFAs in membrane phospholipids is high. The LA, AA, 22:4 n-6 and 22:5 n-6 contents correspond to 19, 25, 3 and 6% total phospholipid fatty acids, respectively [90]. Although n-3 PUFAs are not present in the diet, DHA remains present (3% total phospholipid fatty acids), whereas EPA has disappeared. Substitution with a high level of dietary n-3 PUFAs (800 mg/kg/day) does not modify the proportion of saturated, monounsaturated and polyunsaturated fatty acids, but considerably alters the type of membrane polyunsaturated fatty acids [90]. The proportion of LA is not modified; but AA is greatly reduced (–45%), and its metabolites (22:4 and 22:5 n-6) disappear completely. They are replaced by a high proportion of DHA and a low proportion of EPA (18 and 2% total phospholipid fatty acids, respectively). Feeding lower amounts of n-3 PUFAs (360 mg/kg/day) for 6 weeks also results in similar levels of AA, 22:4 n-6, 22:5 n-6 and 22:6 n-3, but EPA (1% total phospholipid fatty acids) is incorporated to a lesser degree [91]. Lastly, the type of dietary n-3 PUFA plays a role. With a high level of dietary α-LNA (2400 mg/kg/day), membrane EPA reaches 2% of total phospholipid fatty acids, but DHA never exceeds 10% [92]. With only 350 mg/kg/day of dietary EPA, the proportion of DHA in cardiac phospholipids remains low (10% total fatty acids), but it reaches 21% of total phospholipid fatty acids with the same quantity of dietary DHA [91]. With this last dietary fatty acid, the incorporation of EPA into membrane phospholipids is nil. In summary, dietary DHA is highly incorporated into the cardiac membrane, but it is slowly retroconverted. α-LNA and EPA are less avidly incorporated into membrane phos-

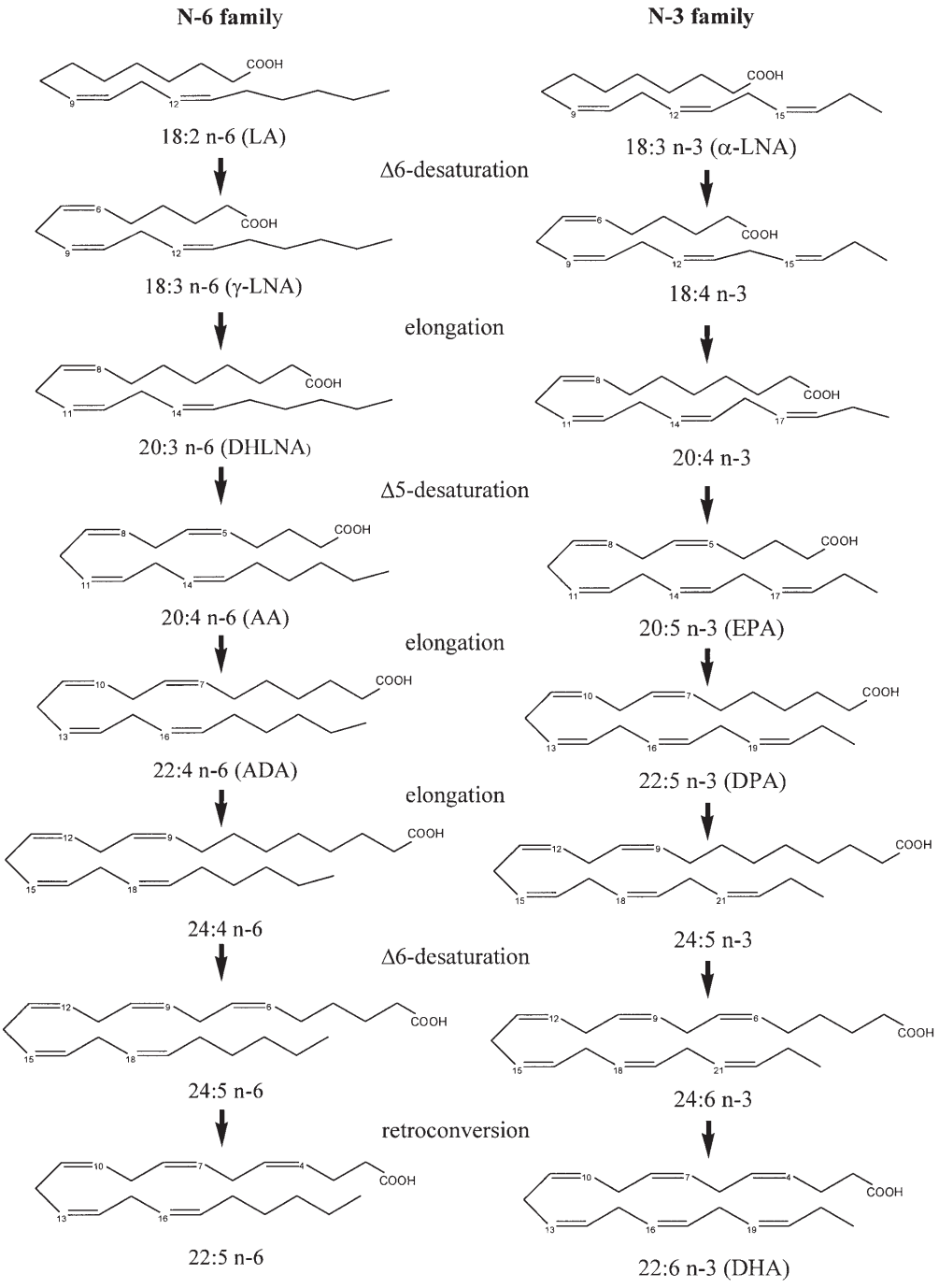

Figure 2. Elongation and desaturation of polyunsaturated fatty acids in the n-6 and n-3 families. LA, linoleic acid; γ-LNA, γ-linolenic acid; DHLNA, dihomo-γ-linolenic acid; AA, arachidonic acid; ADA, adrenic acid; α-LNA, α-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.

pholipids, but they can be elongated and desaturated in DHA. Furthermore, in long-term dietary experiments, the equilibration of fatty acid proportions in cardiac phospholipids is generally reached after 4–6 weeks of diet.

The two families of PUFAs are also involved in eicosanoid synthesis. When incorporated into phospholipids, PUFAs can be released as free fatty acid through the action of the calcium-dependent phospholipase A2. Thereafter, they can enter the cyclooxygenase pathway through which a broad spectrum of prostaglandins is synthesised. AA and EPA compete for the cyclooxygenase. They contribute to the synthesis of series 2 (from AA) and series 3 (from EPA) prostaglandins. Modification of prosta-

glandin synthesis induced by n-3 PUFAs is characterised by a reduction in thromboxan B_2 and prostaglandins I_2 and E_2 [93–101], and an increase in thromboxan A_3 and prostaglandin I_3 and E_3 [102–104]. Thereafter, prostaglandins are released and act locally via specific receptors modulating cyclic AMP or inositide triphosphate (IP_3) formation [105, 106]. Series 2 and series 3 prostaglandins do not always have the same effect. For example, thromboxan A_2 is proaggregatory and vasoconstrictory [107], whereas thromboxan A_3 is ineffective [108].

Dietary n-3 PUFAs also act on numerous metabolic pathways either by direct interaction with protein synthesis or by modulation of the membrane lipid environment and physical properties. At high doses, fish oil is a peroxisome proliferator via peroxisome-proliferator-activated-receptor (PPAR) activation [109], but not at low doses [110]. Gene expression is responsible for the n-3 PUFA-induced increase in uncoupling proteins-3 and acyl coenzyme A (acylCoA) oxidase [111], reduction in the sodium channel [112] and restoration of Na,K-ATPase in the heart of diabetic animals [113]. Numerous cardiac enzymes are modulated by dietary n-3 PUFAs. They include enzymes of the sarcolemma (α-adrenergic [114, 115] and β-adrenergic [116–119] functions), mitochondria (oligomycin-sensitive ATPase [120], carnitine palmitoyltransferase I [121], pyruvate dehydrogenase [122, 123]), sarcoplasmic reticulum (Ca-ATPase [124, 125]), cytosol (phosphodiesterase [126]) and membrane compartments (phospholipase A and lysophospholipase [127]). This modulates the function of the different organelles [128–133]. All these changes are of interest, but it is difficult to explain the beneficial action of n-3 PUFAs on CHD mortality from these basic biochemical observations.

Blood risk factors

When administered at a dose higher than 130 mg/kg/day for several weeks, n-3 PUFAs exhibit a wide range of beneficial actions on blood properties. These actions include (i) a hypolipidemic effect [46, 49, 134–139] characterised by cholesterol-lowering activity [88, 134, 140–149] and hypotriglycemic activity [134, 142, 143, 145, 146, 149–153]; (ii) decreased platelet aggregation [46, 49, 88, 97, 135–137, 139, 147, 154]; (iii) antithrombotic activity [49, 147] and an increased thrombolytic effect [138, 140, 142, 143, 147, 152]; (iv) a reduction in blood viscosity [88, 137, 155]; (v) a slight hypotensive effect [135, 137, 147, 150]; (vi) a favourable change in eicosanoid production responsible for vasodilator and antithrombotic properties [88, 147]; and (vii) an antiinflammatory action [135]. Conversely, when they are administered at a dose which is low but sufficient to reduce CHD mortality in humans, they lose a high proportion of their properties. They no longer exert any hypocholesterolemic or hypotriglyceridemic effects [156, 157], and they lose their antiaggregation and fibrinolytic properties [156, 157]. Their beneficial action on CHD mortality thus occurs through a different mechanism. At the dose known to reduce CHD mortality (4 g of fish oil per day or two fish-containing meals per week), they only contribute to reducing postprandial triglyceride levels [158–160]. Although excess plasma triglycerides act by inducing activated factor VII and by favouring thrombosis [161], coronary occlusion, arrhythmias and sudden death, a low fish intake reduces CHD mortality without modifying the incidence of coronary events. N-3 PUFAs can reduce atherosclerosis and thrombosis at a high dose, but not at a low dose known to reduce CHD mortality.

Cell damage during ischemia and reperfusion

The effect of n-3 PUFAs on ischemia and reperfusion-induced cellular damage has been studied extensively in different experimental models, including the whole animal, the isolated heart perfused according to the Langendorff method or working procedure and cultured cardiomyocytes. All the in vivo studies were carried out with high doses of n-3 PUFAs (800–2500 mg/kg/day) administered for a duration of 4–8 weeks. Although some studies were inconclusive [162–164], the vast majority reported a beneficial effect of either marine or plant n-3 PUFAs. Long-term dietary enrichment with n-3 PUFAs reduced infarct size [165–167], improved the recovery of mechanical cardiac activity during the reestablishment of coronary flow [90, 91, 168–173], prevented the release of creatine kinase or lactate dehydrogenase [90, 91, 169–171, 174, 175], favoured the recovery of action potential during reoxygenation [176, 177] and improved cell survival during hypoxia [178, 179]. These studies thus clearly indicated that n-3 PUFAs reduce the cellular damage triggered by ischemia and reperfusion. N-3 PUFAs exhibit their beneficial effects once incorporated into membrane phospholipids, since they do not provide protection when administered as free fatty acids, unlike n-6 PUFAs [180]. Exogenous DHA may even be deleterious during ischemia and reperfusion [181]. The majority of studies agreed that EPA is responsible for the beneficial effects of n-3 PUFAs when compared with DHA [91, 179, 181, 182], although some investigations also showed a protective effect of DHA [178, 183]. The higher the level of EPA in cardiac phospholipids, the greater the protection during ischemia and reperfusion [91]. The mechanism of action of n-3 PUFAs during ischemia and reperfusion probably involves both cellular calcium homeostasis and the intracellular pH value. During ischemia, the activation of anaerobic glycolysis contributes to decreasing the cytosolic pH. Excess acidosis, which is known to disrupt anaerobic glycolysis, hinders the production of sufficient energy, which in turn favours an ac-

cumulation of intracellular calcium and magnesium [184, 185]. This accumulation of the two cations acts on mitochondrial metabolism, contributing to respiratory uncoupling and a further decrease in energy production [186]. When compared with n-6 fatty acid-rich mitochondria, n-3 fatty acid-rich mitochondria are more resistant to calcium- and ADP-magnesium-induced respiratory uncoupling [131]. Furthermore, excess AA favours intracellular acidosis during hypoxia [187]. N-3 PUFAs decrease levels of AA in the blood and cardiac phospholipids [188], which may partly prevent acidosis during hypoxia. The two phenomena may result in some protection during ischemia, since energy production may be better preserved, and calcium and magnesium accumulation reduced. When coronary flow resumes, cytosolic calcium and magnesium concentrations rapidly return to physiological levels, but because of the reestablishment of respiratory activity and membrane potential, mitochondria continue to accumulate calcium for a long time [185]. Preventing mitochondrial calcium accumulation with ruthenium red at reperfusion favours the recovery of mechanical activity [189]. All interventions that reduce intracellular calcium concentrations at the end of ischemia improve the recovery of mechanical activity [190]. When compared with n-6 fatty acids, n-3 PUFAs minimise mitochondrial calcium accumulation during reperfusion [122, 172, 191]. This beneficial effect is probably responsible for the lower levels of superoxide anion measured in n-3 fatty acid-rich hearts during ischemia and reperfusion [192]. It may contribute to hindering the opening of the permeability transition pore, respiratory uncoupling and apoptosis. Consequently, n-3 PUFAs should act by reducing cellular and mitochondrial calcium accumulations during ischemia and reperfusion. The reasons for this effect are not known. Several mechanisms may be responsible: (i) reduction in the production of inositol triphosphate [115, 193] or cyclic AMP [194] that could reduce intracellular calcium accumulation; (ii) decreased phospholipase A2 activity and increased lysophospholipase activity [127], leading to a lower accumulation of the detergent lysophospholipids; (iii) a favourable change in myocardial eicosanoid production. The production of series-2 prostaglandins is reduced by n-3 PUFAs during the reoxygenation of cultured cardiomyocytes [195]. This may be protective, mainly because these fatty acids reduce the production of the vasoconstrictor thromboxan A_2 [171, 196]. One argument in favour of the beneficial action of n-3 PUFA-derived prostaglandins is that the beneficial effect of fish oil during reperfusion is abolished by indomethacin [170]. However, DHA is as effective as EPA in decreasing thromboxan A_2 production during reperfusion [182], and this fatty acid does not provide protection against ischemia- and reperfusion-induced cellular damage. Series-3 prostaglandins, although produced at low concentrations from EPA, may also be protective; (iv) another mechanism may be involved. EPA, the protective fatty acid, increases the synthesis of nitric oxide by human endothelial cells. This could explain the improved maintenance of coronary flow during reperfusion induced by fish oil observed in certain experiments [163]. However, the primary mechanism of action responsible for the protective action of n-3 PUFAs during ischemia and reperfusion is still to be determined.

Effects on ischemia and reperfusion arrhythmias

Effect of n-3 PUFAs incorporated into cardiac phospholipids

Since the early 1980s, several investigators have studied the effects of long-term treatments with different dietary fatty acids on the incidence and severity of arrhythmia occurring during or after ischemia or under conditions of adrenergic stimulation. The main finding has been that PUFAs (mainly n-3 PUFAs) markedly reduce the severity of arrhythmia after adrenergic stimulation [197–199], during ischemia [200–203] and during reperfusion [193, 204, 205]. PUFAs in the n-3 series are more effective than n-6 PUFAs in decreasing ischemia and reperfusion arrhythmia [203, 204]. The beneficial antiarrhythmic action of n-3 PUFAs is observed at high doses (330–2100 mg/kg/day with diets given for 8–16 weeks). It is due to their incorporation into cardiac phospholipids, since all studies were carried out in isolated papillary muscle, atrium or perfused heart. These substances reduce ventricular fibrillation. Two mechanisms of action are suggested: (i) the intervention of prostaglandin synthesis, since blocking the cyclooxygenase pathway by indomethacin or aspirin contributes to suppressing the antiarrhythmic properties of n-6 and n-3 PUFAs [200, 203, 204, 206]; (ii) involvement of the inositide phosphate pathway [207]. N-3 PUFAs reduce inositol triphosphate production during α-adrenergic stimulation [115] and postischemic reperfusion [193]. The involvement of inositol triphosphate in the antiarrhythmic effect of n-3 PUFAs is strengthened by the fact that EPA and DHA are preferentially incorporated into phosphatidyl inositol [208]. Finally, the two mechanisms of action (prostaglandins and inositol triphosphate) may act synergistically by reducing cytosolic calcium spikes and preventing arrhythmia. A parallel seems to exist between the antiarrhythmic properties of n-3 fatty acids and their ability to reduce ischemia and reperfusion-induced cellular damage, a lower degree of damage explaining the lower severity of arrhythmia. All studies on reperfusion arrhythmias were performed under conditions of low-severity ischemia, when the severity of reperfusion arrhythmias is positively related to the severity of ischemia-induced cellular damage. However, the curve of reperfusion arrhythmia severity as a function of ischemic intensity is

bell shaped. When the severity of ischemia exceeds a certain degree, the severity of reperfusion arrhythmias becomes negatively related to ischemic intensity. This can lead to conflicting results. In a recent but as yet unpublished study of severe local ischemia in an isolated, perfused working rat heart, the severity of reperfusion arrhythmias was increased by membrane n-3 PUFAs as compared with n-6 PUFAs. This can be explained by the n-3 PUFA-induced lowering effect on the severity of ischemia- and reperfusion-related cellular damage. For severe ischemia, the severity of reperfusion arrhythmias can remain positively related to the intensity of ischemia in n-3 PUFA-fed animals because of the protective effect of these PUFAs. Conversely, it can become negatively related in n-6 PUFA-fed animals because of the severity of ischemia. Further investigations are therefore necessary to clearly estimate the effects of membrane n-3 PUFAs on reperfusion arrhythmias. Finally, n-3 PUFAs may also act as exogenous free fatty acids.

Exogenous effect of n-3 PUFAs

As early as 1981, Murnaghan [209] reported that exogenous PUFAs increase the arrhythmia threshold in isolated perfused rabbit heart. The results of this study stimulated considerable interest in the problem. Numerous subsequent studies reported the antiarrhythmic effect of free PUFAs. Most of them were performed in cultured cardiomyocytes in which arrhythmias were induced by ouabain [210, 211], β-stimulation [212], lysophospholipids or palmitoylcarnitine [213]. PUFAs of the n-3 series are particularly effective in reducing these arrhythmias, whereas saturated and monounsaturated fatty acids are ineffective. PUFAs act as free fatty acids rather than being incorporated into membrane phospholipids [214], since their beneficial effects are eliminated by washout with albumin. More important, they act at a low dose (5–10 µM). The antiarrhythmic effect of exogenous PUFAs has been confirmed in the whole animal, since the infusion of fish oil emulsion, pure EPA, DHA or α-LNA in the ischemic dog contributes to suppressing ventricular fibrillation without modifying the fatty acid composition of membrane phospholipids [215, 216]. Exogenous PUFAs, but not saturated or monounsaturated fatty acids, decrease cell excitability and increase the refractory period [217, 218]. Using patch-clamp techniques, several investigators have reported interesting effects on the different ion currents constituting the action potential. Inward sodium [219–221] and calcium [221, 222] currents are reduced by exogenous PUFAs, and the voltage-dependent potassium current and transient outward current are also decreased [221, 223, 224]. The inhibition of I_{Na} is responsible for a PUFA-induced reduction in cell excitability [225]. The order of efficacy for inhibiting I_{Na} is as follows: DHA > EPA > α-LNA. PUFAs of the n-6 family (AA and LA) are much less effective, whereas saturated and monounsaturated fatty acids have no effect [219, 226]. PUFAs bind directly to the sodium channel [227], thus reducing its ability to be opened [112, 220]. A similar pattern of binding is observed between PUFAs and L-type calcium channels [228], but the efficacy of n-6 and n-3 PUFAs in inhibiting I_{Ca} is similar. No specific interaction has yet been demonstrated between PUFAs and potassium channels, but the inhibition of outward potassium currents may be responsible for the PUFA-induced increase in the refractory period. Finally, all these inhibitions (I_{Na}, I_{Ca} and I_K) may be responsible for the antiarrhythmic effect of exogenous PUFAs, although it is too soon to know which is responsible for the antiarrhythmic properties of n-3 fatty acids. However, the antiarrhythmic action of exogenous DHA and EPA seems to arise through increased membrane fluidity [226], since benzyl alcohol also increases membrane fluidity and is active in preventing arrhythmia [229].

Most of the studies on plasma lipids have been focused on blood risk factors (total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides). The data concerning the concentration of free n-3 PUFAs in plasma are rare. This is due to the fact that free fatty acid concentrations are considerably modulated by numerous physiological parameters (feeding status, stress, and so on). Hartog et al. [230] indicated that the concentration of total free fatty acids is close to 100 µM in pigs fed a normolipidic diet containing 9% lipids. Furthermore, the proportion of n-3 PUFAs reaches 11% of total plasma fatty acids in humans fed 85 mg/kg/day of fish fatty acids for 3 weeks [145]. Feeding low doses of fish or fish oil could make it possible to detect free n-3 PUFA concentrations close (5–10 µM) to those effective in reducing arrhythmia severity in cultured cells. This is peculiarly true under stress conditions such as myocardial infarction, since adrenergic stimulation favours free fatty acid release from the adipose tissue. This last tissue might be of crucial importance in constituting a store of n-3 PUFAs easily released into the plasma. Japanese people with a low rate of CHD-induced mortality exhibit a proportion of n-3 PUFAs in adipose tissue that is five times higher than in British people [231]. The store in the adipose tissue could also contribute to buffering the decrease in plasma n-3 PUFA concentrations occurring between each dietary supply. This underlines the importance of the duration between each ingestion and suggests that a moderate supply once or twice per week may be more protective than a one-off ingestion, even if the latter is massive.

Studies in the whole animal

Several models, including rats, dogs, pigs, nonhuman primates and humans, have been used to assess the effects of dietary PUFAs during ischemia and reperfusion. These are the models of choice because both arrhythmia and sudden cardiac death can be quantified. In addition,

the effects of both circulating free fatty acids and fatty acids incorporated into membrane phospholipids were taken into account. The numerous studies performed in whole animals with a long-term dietary supply ranging from 4 weeks to 1 year can be divided into two groups: those that utilised large quantities of lipid supplement and those using only moderate quantities of supplement. Several conclusions may be drawn from the first group: (i) when compared with low-fat diets, supplementation with saturated fatty acids decreased the arrhythmia threshold [232] and increased ventricular fibrillation during ischemia and reperfusion [233–237] and sudden cardiac death [235]; (ii) monounsaturated fatty acids were as effective as saturated fatty acids in promoting ischemia and reperfusion arrhythmias [238]; (iii) compared with saturated fatty acids, n-6 PUFAs decreased ischemic arrhythmias [233–240] and mortality [235, 238], but not reperfusion fibrillation [234, 235, 238]; (iv) compared with n-6 fatty acids, n-3 PUFAs increased the arrhythmia threshold during normoxia and ischemia [241] and were more effective in reducing ischemic arrhythmia [232, 235, 242], reperfusion arrhythmia [175, 234, 235, 238] and the rates of sudden cardiac death [175, 232]; (v) n-6 fatty acids could prevent the beneficial effects of n-3 PUFAs [243]. In the second group of studies, where lipid supplements were utilised at a low dose, the beneficial effects of n-3 PUFAs were less obvious. Although Gudbjarnason et al. [35, 244] reported that fatal ventricular fibrillation was associated with a high AA-to-DHA ratio in humans, several studies in patients with acute myocardial infarction or tachycardia indicated that fish oil supplements had no effect [245, 246]. Rats eating the meat of cows fed fish oil did not seem to absorb a sufficient amount of n-3 fatty acid to prevent arrhythmia [247]. Conversely, McLennan et al. demonstrated that a low DHA intake (230 mg/kg/day for 5 weeks) prevented ischemic arrhythmias [248], which was not the case for EPA. Such an antiarrhythmic effect was even observed with lower n-3 PUFA intake (75–100 mg/kg/day for 4–10 months) [175, 241, 249, 250]. The antiarrhythmic effect of low doses of n-3 fatty acids was rapid and did not require their incorporation into membrane phospholipids [215, 216].

Conclusion and new insights

PUFAs of the n-3 series can prevent CHD-related mortality in humans. This effect is mainly observed when subjects consume low doses of these fatty acids, as compared with people who never eat fish. N-3 PUFAs exhibit several beneficial activities: at high doses, they display numerous favourable actions on blood properties, decrease ischemia- and reperfusion-induced cellular damage and abolish reperfusion arrhythmia in the case of mild ischemia; at low doses, they lose their beneficial effects on blood characteristics (except for a hypolipidemic effect on postprandial triglycerides) and ischemia- and reperfusion-induced cellular damage, but levels of circulating free n-3 fatty acids need to be increased. This last assumption needs to be verified in the whole animal and in humans. Since n-3 PUFAs act at low doses in humans, it is unlikely that this is due to a favourable change in the fatty acid composition of the membrane. The effect on ischemia- and reperfusion-induced cellular damage is therefore unlikely. It is believed that their mechanism of action results from an increase in circulating free n-3 fatty acids, which contributes to reducing ischemia- and reperfusion-induced arrhythmias. These considerations impose two new criteria on the evaluation of the true mechanism of action of dietary n-3 PUFAs: (i) n-3 fatty acids must be present as circulating free fatty acids, and their levels must remain low (equivalent to one meal with fish per week, i.e. 20 mg of n-3 fatty acids/day/kg); (ii) the control group must be devoid of n-3 fatty acids and contain n-6 fatty acids as the sole source of PUFAs. If these criteria are adhered to, it may be possible to determine whether circulating n-3 PUFAs reduce ischemia and reperfusion arrhythmias. Such an experiment could be performed in an isolated heart model perfused with two media that differ in terms of their PUFA composition. The hearts would be subjected to local ischemia followed by reperfusion. If ischemia and reperfusion arrhythmias are reduced by circulating n-3 fatty acids, we would be sure that n-3 fatty acids act as circulating free fatty acids. However, other mechanisms may also be involved, and experiments in the whole animal must be performed to clarify them. Whatever the mechanism studied, the animals should be fed according to the dietary criteria described above.

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