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REVIEW

About the controversies of the cardioprotective effect of n-3 polyunsaturated fatty acids (PUFAs) between animal studies and clinical meta-analyses: a review with several strategies to enhance the beneficial effects of n-3 PUFAs

Luc Demaison[1] · Thibault Leger[1] · Catherine Vergely[2] · Luc Rochette[2] · Kasra Azarnoush[3]

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Abstract

Several meta-analyses describing the effect of n-3 polyunsaturated fatty acids on the survival rate of the victims of an acute coronary event do not clearly support a beneficial impact of these fatty acids. Yet, animal studies consistently show n-3 PUFA–induced protection against ischemia-reperfusion-induced myocardial injuries. The impact on reperfusion arrhythmias of these PUFAs is more controversial. The literature shows the anti-arrhythmic properties of circulating n-3 PUFAs. However, when these fatty acids are incorporated in the cardiac membrane, they protect the myocardial tissue vis a vis cellular damage but they can be either pro- or anti-arrhythmic during reperfusion, depending on the severity of tissue injuries. The latter elements can explain the lack of beneficial effect observed in the meta-analyses, but a proper use of n-3 PUFAs may provide advantages in terms of survival rate. This review discusses the different results obtained in humans and animals and presents several strategies to enhance the beneficial effects of n-3 PUFAs.

Keywords N-3 PUFAs · Heart · Infarction · Cellular damages · Arrhythmias · Cardiac death

Introduction

In industrialized societies and numerous emerging countries, a large portion of the population eats what is called a Western diet. This diet is characterized, among other things, by a too large n-6 polyunsaturated fatty acid (PUFAs) supply and a too low n-3 PUFAs intake [1].

Several meta-analyses have indicated that cardiovascular disease–related mortality and sudden death are decreased by n-3 polyunsaturated fatty acids (PUFAs) [2–4]. However, other meta-analyses have not observed any beneficial effects on these endpoints [5, 6]. The discrepancies can result from the nature of the chosen n-3 PUFAs, the doses used, and/or the duration of the treatments. They can also result from the selection of clinical trials included in the different meta-analyses, some of them being considered reliable and others not credible. Thus, the cardioprotective effect of n-3 PUFAs on survival after a cardiac event is far from being obvious in the light of the published meta-analyses [7].

Cardiac-related mortality mainly results from three factors: the incidence of coronary events, the severity of myocardial damages, and the seriousness of arrhythmias. Several meta-analyses [4, 8] showed that n-3 PUFAs are inoperative in preventing the occurrence of myocardial infarction which mainly arises through atherosclerosis and/or thrombosis. This lack of beneficial effect is explained by the amount of n-3 PUFAs administrated which seems to be too low to be efficient. Indeed, a recent long-term randomized clinical trial conducted in Japan [9]

✉ Luc Demaison
luc.demaison@inra.fr

[1] INRA, UNH, Unité de Nutrition Humaine, Université Clermont Auvergne, CRNH Auvergne, 63000 Clermont-Ferrand, France

[2] Research team Pathophysiology and Epidemiology of Cerebro-Cardiovascular Diseases (PEC2, EA7460), University of Bourgogne Franche-Comté, UFR des Sciences de Santé, Dijon, France

[3] Heart Surgery Department, G. Montpied Hospital, Clermont-Ferrand University Hospital, Clermont-Ferrand, France

indicated that the use of much higher n-3 PUFAs doses led to a significant protection against atherosclerosis and a reduction of the incidence of coronary events. That study is encouraging as regards a reduction of cardiac mortality, but other cardiac events, notably myocardial tissue survival and ventricular arrhythmias during and after ischemia, must be examined.

The protection of myocardial tissues is an important factor in that it may explain a possible beneficial effect of n-3 PUFAs. A meta-analysis highlighted the possible protective action of n-3 PUFAs from cardiac failure [10]. The study showed that PUFAs of the n-3 series increased the left ventricular ejection fraction in a subgroup of patients with dilated cardiomyopathy, although the improvement was not noticed in the overall studied population of cardiac failure patients. Regarding acute myocardial infarction (AMI), a meta-analysis showed that a small quantity of fish oil reduced the risk of AMI but increasing the dose of fish oil (FO) did not reduce this risk any more [3]. In contrast, the same small quantity of FO reduced coronary heart disease–related lethality, but increasing the dose improved survival. This suggests that, in addition to its effects on AMI incidence, FO is also able to protect the myocardium. The observation was confirmed by Wen et al.'s meta-analysis [4] indicating that patients with coronary heart diseases were subject to lower sudden and cardiac deaths without any alteration of coronary event incidence. Finally, it seems that cardiac patients can be protected either through the preservation of the myocardial cell viability or the reduction of lethal ventricular arrhythmia occurrence.

The involvement of ventricular arrhythmias in the potential n-3 PUFAs protective effect has also been investigated in humans, and the results published until now in meta-analyses seem to be clear. PUFAs of the n-3 family do not protect the heart against severe ventricular arrhythmias in patients with implantable cardioverter defibrillators [5, 11]. Thus, it seems that n-3 PUFAs, although not protective against fatal arrhythmias, improve myocardial cell viability during AMIs. The phenomenon is still to be demonstrated however, i.e., by studying fibrosis and/or apoptosis in human cardiac tissues directly after the occurrence of a coronary event. Nevertheless, as it will be developed further, animal studies perfectly enlightened the studies performed in humans and solutions do exist in order to potentiate the beneficial effects of n-3 PUFAs.

This review aims at explaining the discrepancies between the cardiac effects of n-3 PUFAs in the human literature as compared to the studies performed with laboratory animals. Two aspects will be taken into consideration: the influence of n-3 PUFAs on cell viability after a coronary event and their effects on the occurrence and severity of reperfusion arrhythmias.

Cardioprotection by n-3 PUFAs

Numerous animal studies showed that n-3 PUFAs protect the myocardial tissue during ischemia-reperfusion episodes. The observation was ascertained by a reduction of the infarct size [12–17], an improved recovery of mechanical functions after reperfusion [13, 14, 17–24], a lower release of myocardial enzymes such as lactate dehydrogenase and creatine kinase [17, 19, 22, 24–26], and a lower acidosis and lactate release [22] during the ischemia-reperfusion episodes. This consensus, obtained either in in vivo models or ex vivo isolated hearts, has found only few contradictors. Ku et al. [27] found that dietary eicosapentaenoic acid (C20:5 n-3 or EPA) was efficient in improving the recovery of the cardiac mechanical function after cold storage only when it was administrated in association with a cholesterol-rich diet; Huggins et al. [28] did not mention any cardioprotective effect of fish oil in female mice subjected to global ischemia-reperfusion; and finally, Hlavackova et al. [29] published that a n-6 PUFAs–rich diet decreased the infarct size in male rats compared with a fish oil–rich diet. These contradictory pieces of information, although not insignificant, cannot however counteract the numerous studies describing the positive effect of n-3 PUFAs in ischemia-reperfusion. This is all the more important as such a n-3 PUFAs–induced cardioprotection has been shown after coronary artery bypasses in humans [30]: it was demonstrated by improved myocardial lactate and oxygen extractions and reduced troponin T and creatine kinase releases after the surgery.

A possible explanation of the n-3 PUFAs-related cardioprotective effect during ischemia-reperfusion is the beneficial effect of these fatty acids on the recovery of the coronary flow during reperfusion. As soon as 1993, Yang et al. [24] observed an improvement of this recovery in fish oil–fed rats. The ascertainment was confirmed by Sergiel et al. [31], although these authors noted just a tendency. Taken together, these observations indicate that the coronary arteries, mainly the micro-vessels, take part in the effects of n-3 PUFAs by favoring the coronary flow and improving cardiac mechanical performances. However, several studies performed in cultured cardiomyocytes reported that n-3 PUFAs also act directly on the contractile cells [23, 32]. The n-3 PUFAs–induced cardioprotection thus seems to result from a valuable action on all the cardiac cell types.

The extended researches about n-3 PUFAs as cardioprotective agents [33, 34] did not neglect the question of the efficient fatty acid responsible for the cardioprotective effect. N-3 PUFAs represent a family of several fatty acids including alpha-linolenic (C18:3 n-3 or ALA), eicosapentaenoic (C20:5 n-3 or EPA), docosapentaenoic (C22:5 n-3 or DPA), and docosahexaenoic (C22:6 n-3 or DHA) acids (Fig. 1). ALA is essential, since it cannot be

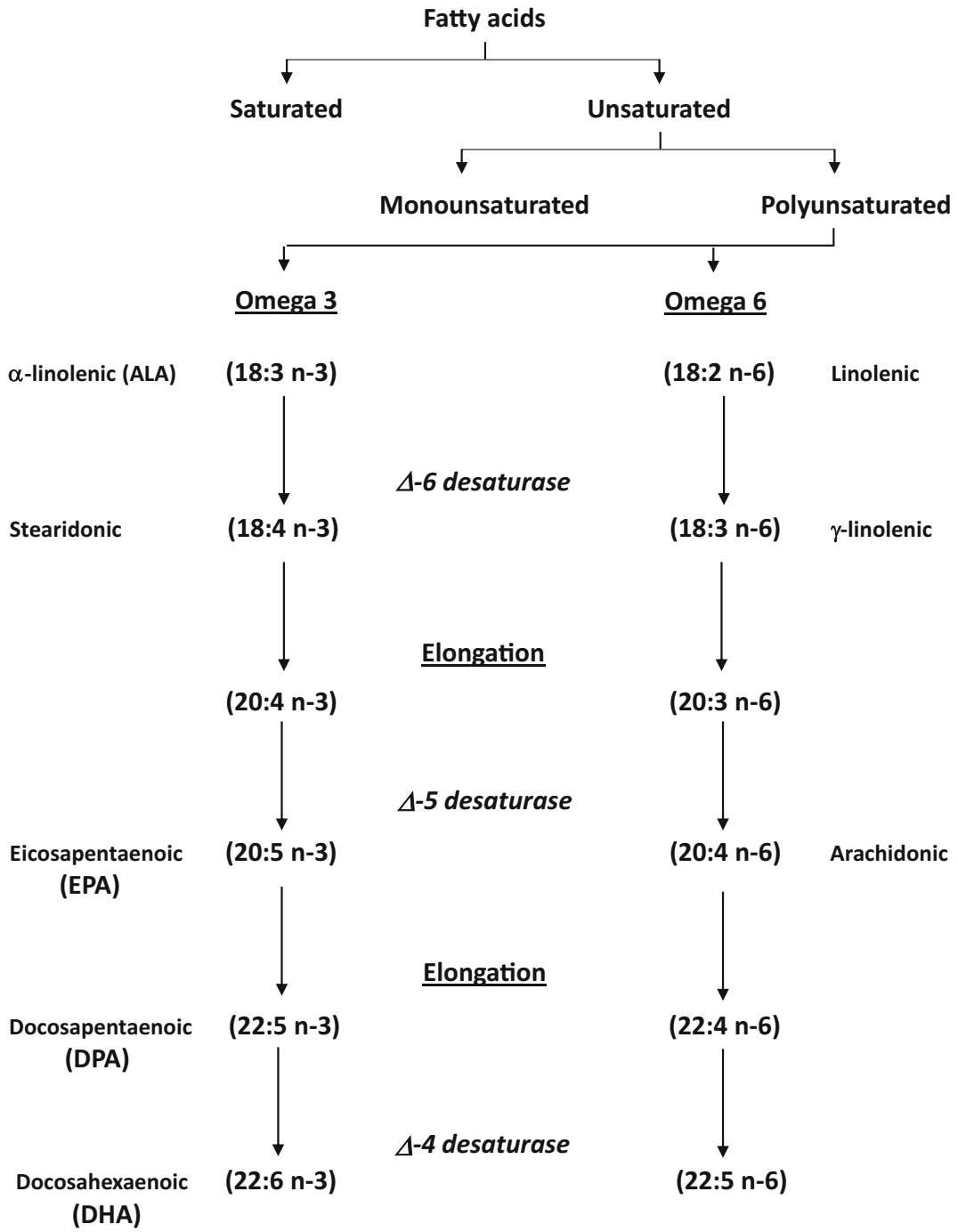

Fig. 1 Schematic representation of the polyunsaturated fatty acid metabolism

synthesized by the organism. It is found in large quantities in vegetable oils such as linseed, canola, hempseed, and soy oils. Once it is ingested, ALA can be elongated and desaturated by several enzymes to EPA, DPA, and DHA. A representation of the n-3 PUFAs metabolism is presented in Fig. 1. Seaweeds are also a source of long-chain n-3 PUFAs such as EPA, DPA, and DHA. Since seaweeds constitute the first element of the marine food chain, EPA, DPA, and DHA are also found in sea animals such as fish and shellfish. Long-chain n-3 PUFAs can thus be ingested by humans directly from marine animals. As soon as 1993, Yang et al. [24] showed that the cardioprotective effect of fish oil in ischemia-reperfusion was abolished by pre-treatment with the cyclooxygenase inhibitor indomethacin. This considerably restricted the number of fatty acids potentially involved: only arachidonic acid (ArA) and EPA are the substrates of this enzyme which lead to the formation of the 2-

and 3-series prostaglandins, respectively. However, the estimation of the fatty acid involved is more difficult than it seems. Indeed, prostaglandins are synthesized from PUFAs released from membrane phospholipids by the phospholipase A2. Yet, feeding animals with fish oil is known to increase EPA, DPA, and DHA in cardiac phospholipids and to reduce ArA [19]. So, it is difficult to know whether the increase in membrane EPA or DPA or DHA or the decrease in ArA is responsible for the cardioprotective effect. This question has however been partially answered by feeding rodents or incubating cardiomyocytes with individual n-3 PUFAs (EPA or DHA). EPA seems to be the effective agent compared with DHA [31, 35–37], since this fatty acid was cardioprotective and DHA had no beneficial effect. Both dietary EPA and DHA reduced the ArA proportion of cardiac membranes [31], which excludes the decrease in ArA as the vector of the n-3 PUFAs–related cardioprotective effect. This ascertainment must however be modulated by the work of Ip et al. [38] which showed that the replacement of saturated fat in the diet by n-6 PUFAs was detrimental for the heart. The pro-inflammatory properties of n-6 PUFAs [39] thus also could partially intervene. ALA is also cardioprotective [18, 21, 40] since it can be transformed in the organism and incorporated as EPA in cardiac phospholipids. However, it must be ingested in a threefold larger quantity than fish oil to allow the similar EPA incorporation in myocardial membranes [41].

The mechanism of EPA-related cardioprotection obviously involves prostanoid synthesis [21, 42]. At the cardiomyocyte level, it was shown to imply the mitochondrial function [19, 20]. The fatty acid prevented ischemia-reperfusion-induced decrease in oxidative phosphorylation. This was explained by a lower matrix calcium accumulation during the pathology [43]. Calcium, a known effector of the mitochondrial permeability transition pore, favors the leak of matrix hydro-soluble elements, reduces oxidative phosphorylation, and triggers apoptosis. Nitric oxide production and calcium-activated potassium channel opening are involved in the cardioprotection [15]. However, the prevention of oxidative stress seems to be an important element in the EPA beneficial effect: n-3 PUFAs were reported to increase the enzymatic antioxidant defenses (superoxide dismutase, catalase, and glutathione peroxidase) [13, 14], preserve the reduced glutathione to oxidized glutathione ratio [13], and decrease oxidative stress [13, 14, 20]. The protective action might be related to an activation of the nuclear factor Nrf2 known to favor the formation of enzymatic antioxidant defenses and inhibition of the pro-apoptotic NF-kB [14]. It might also result from the activation of PPAR-delta [23]. Importantly, n-3 PUFAs not only act when they are administrated before ischemia but also have a beneficial influence as post-conditioning agents [17].

Effects of n-3 PUFAs on arrhythmia severity

N-3 PUFAs act on arrhythmogenesis directly as circulating fatty acids or indirectly after their incorporation into membrane phospholipids. Numerous studies have shown the anti-arrhythmic action of acute n-3 PUFAs administration. These beneficial properties are mainly based on electrophysiological studies on cultured neonatal or adult isolated ventricular myocytes. The first work suggesting an impact of n-3 PUFAs on cardiac electrical activity reported an EPA- and DHA-induced decrease in contraction frequency of cultured neonatal rat cardiomyocytes [44]. Thereafter, Kang and Leaf [45] showed that EPA and DHA reduced cardiomyocyte arrhythmias induced by lysophosphatidylcholine and palmitoylcarnitine. Numerous investigations were then conducted to understand the mechanism of this effect. They concluded that EPA and DHA stabilized the cardiomyocyte electrical activity via the inhibition of ionic channels. In contrast with saturated and monounsaturated fatty acids, EPA and DHA inhibit sodium [46], L-type calcium [47], and outward potassium currents [48]. A higher electrical activity is thus necessary to trigger an action potential, the refractory period is prolonged [49], and acute n-3 PUFAs administration displays anti-arrhythmic properties. If the effect of n-3 PUFAs on the sodium current is important to determine the cardiomyocyte excitability, modulating the L-type calcium current is also of utmost importance in the anti-arrhythmic action of these molecules: they were shown to suppress calcium sparks triggered by cardiomyocyte electrical stimulation [47, 50] maybe through the reduction of calcium availability for sarcoplasmic reticulum calcium uptake and a decrease of calcium release via ryanodine receptors [51]. However, other mechanisms can explain a lower calcium uptake by the cardiomyocytes, notably n-3 PUFAs–induced inhibition of the sodium/proton [52] and sodium/calcium [53] exchanges. The anti-arrhythmic properties of circulating n-3 PUFAs were ascertained during ischemia and/or reperfusion in the in vivo situation in animal models and even in humans. In the running dog, n-3 PUFAs infused before ischemia decreased the occurrence of arrhythmias developing during the ischemic episode [54]. In the pig subjected to cardiac ischemia and reperfusion, pericardial delivery of DHA from the 40th min before ischemia to the 5th min of reperfusion reduced the infarct size and the incidence of arrhythmias, resulting in no animal death [55]. In a model of long QT interval of isolated rabbit heart, EPA and DHA decreased the incidence of afterdepolarizations and torsades de pointe [56]. Finally, in humans, recent n-3 PUFAs ingestion reduced the risk of sudden cardiac death [57]. The beneficial effect of circulating n-3 PUFAs on the incidence of malignant arrhythmias compared with saturated and monounsaturated fatty acids is thus obvious, but there is no clear evidence showing that n-6 PUFAs exert less anti-arrhythmic activity. This might explain the results of the meta-analyses showing

the n-3 PUFAs' inefficiency in the reduction of fatal cardiac arrhythmias. However, other animal studies evaluating the effects of n-3 PUFAs incorporated in cardiac phospholipids clearly show a strong influence of these fatty acids on cardiac arrhythmogenicity.

Long-term dietary treatment with n-3 PUFAs leads to an incorporation of these fatty acids in cardiac phospholipids at the detriment of n-6 PUFAs. The effects of these changes in membrane composition on the severity of arrhythmias have been studied for a long time. The first studies mentioning a protective effect of n-3 PUFAs on arrhythmogenicity have been performed in the marmoset monkey [58, 59]. They showed that dietary PUFAs, particularly those of the n-3 series, increased the ventricular fibrillation threshold compared with dietary saturated fatty acids in paced animal and during ischemia. The anti-arrhythmic properties of n-3 PUFAs have been confirmed in several other animal models including in vivo and ex vivo preparations [12, 60–62]. These anti-arrhythmic effects have been observed during ischemia [12, 58, 59, 61, 62], although several studies did not observe any beneficial effect during coronary occlusion [15, 63, 64].

Severe ventricular arrhythmias potentially leading to cardiac death are observed during ischemia, but their incidence and duration are low compared with those occurring during reperfusion. Yet, reperfusion is currently performed in cardiology units to keep the cardiac tissue from an inevitable necrosis. Reperfusion arrhythmias explode soon after the ischemic event during a critical period whose duration depends on the species studied. In man, this period can last approximately 10 days. Since these arrhythmias are lethal, it is important to determine the influence of n-3 PUFAs on these electrical abnormalities after the re-establishment of the coronary flow in the ischemic area. Opinions are divided as to whether n-3 PUFAs are anti-arrhythmic after an infarction. Performing a coronary artery ligation in the living rat, McLennan [62] observed a reduced mortality in n-3 PUFAs–fed rats compared with their counterparts fed a n-6 PUFAs–rich diet. This was however not explained by the arrhythmia score which was similar in both groups. In contrast, Isensee et al. [61] noted an anti-arrhythmic effect of n-3 PUFAs compared with PUFAs of the n-6 series. Abdukeyum et al. [12] observed the same effect. In contrast, other investigators experimenting the living rabbit or isolated rat heart did not note any effect of n-3 PUFAs on severe reperfusion arrhythmias [15] or even observed a tendency toward a pro-arrhythmic effect [63]. In an unpublished work performed in our laboratory in the isolated working rat heart, we even observed a pro-arrhythmic effect of dietary n-3 PUFAs after coronary artery ligation: the results, presented in Fig. 2, clearly demonstrate the deleterious effect of dietary n-3 PUFAs compared with n-6 PUFAs. Patch-clamp studies in isolated ventricle myocytes of animals fed a n-3 PUFAs–rich diet are also controversial. When the cardiomyocytes are isolated from healthy pig ventricles, membrane n-3 PUFAs decrease action potential duration, inhibit I(Ca,L) and the Na/Ca exchange, and activate I(K1) et I(Ks), rendering the cell less prone to eventual arrhythmic events [65]. This was confirmed by Den Ruijter et al. [66] who showed that E4031-induced early post-depolarizations in myocytes extracted from healthy pig ventricles were reduced by membrane n-3 PUFAs. This might be due to a reduced ability of n-3 PUFAs–rich cardiomyocytes to produce triphosphate inositol after adrenergic stimulation [67] and consequently to increase calcium sparks. However, researchers patch-clamping myocytes isolated from a post-ischemic ventricle of dogs fed a n-3 PUFAs–rich diet do not share the same opinion. They discriminated myocytes resistant to ventricular fibrillation (VF−) from those that were susceptible to this arrhythmic abnormality (VF+) and found that membrane n-3 PUFAs did not reduce calcium sparks in VF+ and increased them in VF− [68]. They concluded that n-3 PUFAs were pro-arrhythmic in post-ischemic myocardium. This was confirmed by another more recent study using the same model which showed that membrane n-3 PUFAs increased the action potential duration without improving the repolarization phase in myocytes extracted from post-AMI ventricles [69]. Finally, Billman reviewed carefully the effects of n-3 PUFAs [70] and concluded that "Despite initial encouraging results, more recent clinical prevention and animal studies have not only failed to reduce sudden cardiac death but actually increased mortality in angina patients and increased rather than decreased malignant arrhythmias in animal models of regional ischemia."

Harmonization of the opinions about n-3 PUFAs and reperfusion arrhythmias

Rather than exacerbating tensions between those who believe that membrane n-3 PUFAs increase lethal reperfusion arrhythmias and those who think that these fatty acids are anti-arrhythmic, we feel that both opinions are right. To reach this hypothesis, we have started from the two postulates presented earlier in this manuscript: (i) n-3 PUFAs incorporated in membrane phospholipids, namely the eicosapentaenoic acid, reduce ischemia-reperfusion-induced cellular damage; (ii) these membrane fatty acids are either pro- or anti-arrhythmic during post-ischemic reperfusion.

The relation between the duration of ischemia (thus the intensity of cellular damage induced by ischemia-reperfusion) and the severity of reperfusion arrhythmias is well known: it is a bell-shaped curve [71]. Since membrane n-3 PUFAs reduce ischemia-reperfusion-induced cellular damage compared with n-6 PUFAs, these fatty acids help shift the curve toward the right (Fig. 3). The shape of the curve is easily explained by the electrical conductivity of the reperfused myocardium: when cellular damage is low, the

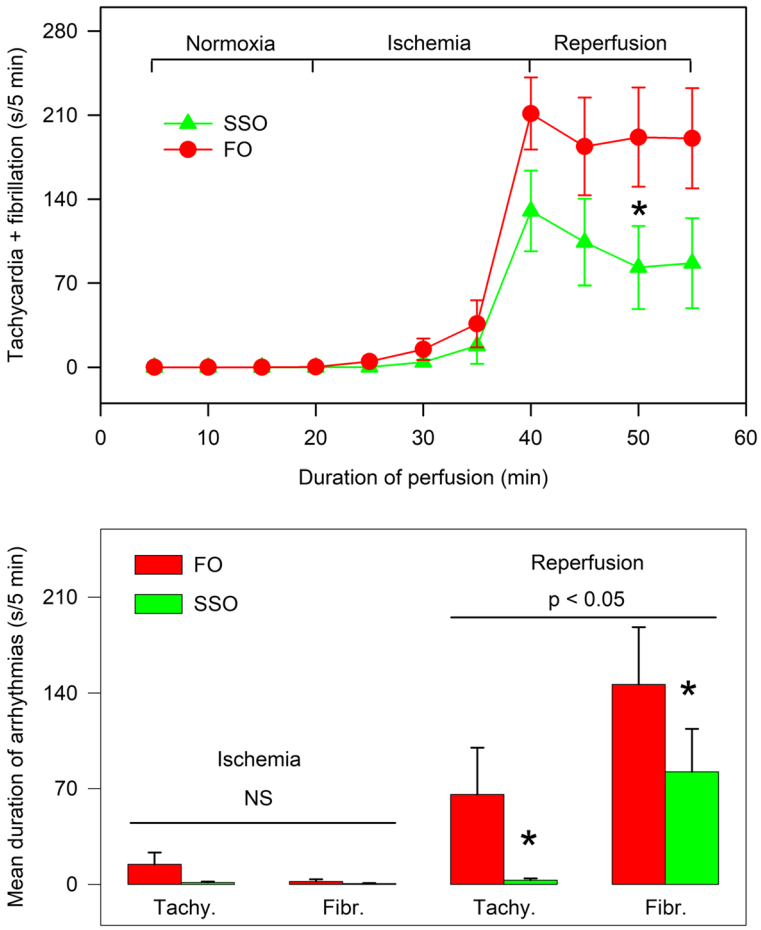

Fig. 2 Influence of dietary sunflower seed and fish oils on the severity of ischemic and reperfusion arrhythmias. After a 6-week feeding, rat hearts were perfused according to the working mode with a Krebs-Heinselett medium which was rich in calcium (2.5 mM), low in magnesium (1.2 mM), and low in potassium (3 mM). After 30 min of equilibration, a local ischemia was performed by left anterior descending artery ligation for 20 min. The ligation was then released to reperfuse the heart for 20 min. Upper panel: time-evolution of tachycardia and fibrillation during normoxia, ischemia, and reperfusion. Lower panel: cumulative duration of tachycardia (Tachy.) and fibrillation (Fibr.) during the ischemic episode and reperfusion. SSO: rats fed with 10% of n-6 PUFA–rich sunflower seed oil; FO: rats fed with 5% of n-3 PUFA–rich fish oil + 5% of sunflower seed oil. The number of experiments was 12 per group. *Significantly different by one-way analysis of variance

conductivity of the tissue is still good, but the electrical stimulus is slowed in some zones and reaches the non-ischemic healthy myocardium with a delay compared with the normal stimulus. This triggers a post-depolarization phenomenon also called re-entry loop. The more damaged the myocardium, the more re-entry loops and the more arrhythmic the heart is. This thus leads to tachycardia with periodic re-entry loops and fibrillation when these loops are irregular in their frequency. In the first part of the curve, arrhythmia severity thus increases proportionally with the intensity of cellular damage. However, the death of cardiomyocytes switches off the electrical activity in the unhealthy zone, progressively reducing the number of

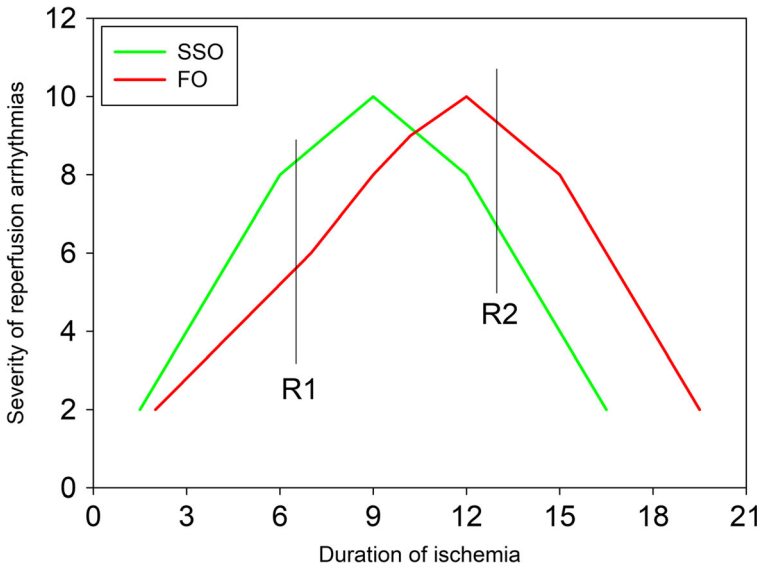

Fig. 3 Schematic representation of the influence of n-6 (SSO, sunflower seed oil) and n-3 (FO, fish oil) polyunsaturated fatty acids on the relationship between the severity of reperfusion arrhythmias and duration of ischemia. The longer the duration of ischemia, the more severe the cellular damage. R1: marker for low cellular damage; R2: marker for high cellular damage

re-entry loops and severity of reperfusion arrhythmias. When cellular damage increases, the occurrence and duration of reperfusion arrhythmias are thus progressively decreased: this is the second part of the curve. Since membrane n-3 PUFAs slow down the occurrence of cellular damage, they also reduce the severity of reperfusion arrhythmias in the first part of the curve (point R1 in Fig. 3). However, this is completely different in the second part of the curve (point R2 in Fig. 3): membrane n-3 PUFAs become pro-arrhythmic.

The severity of cellular damage during ischemia-reperfusion can be modulated by the duration of ischemia, but other factors as well: the temperature of the animals or perfusion fluid, calcium and magnesium concentrations of the liquid irrigating the heart or other environmental modifications altering intrinsic regulatory elements induced by the diet, long-term pharmacological treatments, or other factors. Notably, all the publications showing an anti-arrhythmic effect of membrane n-3 PUFAs during post-ischemic reperfusion were based on an ischemic event of low intensity. This is the case for the study by McLennan [62] in the living rat which showed that the mortality during reperfusion was decreased by membrane n-3 PUFAs after an ischemic episode of only 5 min. Interestingly, in the same study, the author also performed a 15-min ischemia in the same model and did not observe any difference in the severity of reperfusion arrhythmias after feeding saturated, n-6 or n-3 PUFAs. This clearly indicates that the effects of dietary PUFAs on the severity of reperfusion arrhythmias depend on the duration of ischemia and intensity of cellular damage. This is also the case for the work by Isensee and Jacob [61] who performed a short-duration (10 min) ischemia and observed an anti-arrhythmic activity of long-term dietary fish oil. The study published by Abdukeyum et al. [12] in the isolated rat heart describing the effect of 30-min ischemia with high-calcium (2.5 mM) but also high-magnesium concentrations (1.6 mM) in the perfusion medium agrees with this postulate. Magnesium is recognized as a performant anti-calcium compound, and its high concentration in the perfusion fluid probably contributed to reduce the severity of ischemia-reperfusion cellular damage drastically. The only exception to this rule is the study in the living rabbit by Ogita et al. [15] who performed a 30-min occlusion followed by a 3-h reperfusion. Despite the long-duration ischemia, they showed a tendency toward an anti-arrhythmic effect of membrane n-3 PUFAs based on the occurrence of ventricular premature contractions. These results can be related to the characteristics of the animal model. Rabbit calcemia is high (3.4 mM compared with approximately 1.5 mM in the rat) depending mainly on the dietary supply. Moreover, its cellular calcium metabolism may be different from that of rodents. Furthermore, the authors were not able to display the results about the duration of fibrillation probably because of animal death: they only stated that the occurrence of fibrillation during reperfusion did not vary among the groups. It thus cannot be said for sure that dietary n-3 PUFAs are truly anti-arrhythmic.

By contrast, studies performing more severe ischemia showed a pro-arrhythmic effect of dietary n-3 PUFAs compared with n-6 PUFAs. This is the case for the work by Demaison et al. [63] with an ischemia duration of 20 min in the presence of a perfusion fluid rich in calcium (2.5 mM) and poor in magnesium (1.2 mM). They noted that membrane n-3 PUFAs increased reperfusion arrhythmias (Fig. 4). However, reperfusion arrhythmias were almost totally suppressed in the n-3 PUFAs group with a treatment of the heart by lidocaine from the beginning of the perfusion. In contrast, reperfusion arrhythmias noted in the n-6 group were only marginally suppressed by the lidocaine treatment. This suggested that the myocardium of n-3 PUFAs–fed animals was less severely affected by ischemia-reperfusion than the heart of n-6 PUFAs rodents. This was ascertained by the evaluation of the mitochondrial function which was less altered in the n-3 PUFAs group (see [63] for details). However, the more serious arrhythmias in the n-3 PUFAs group indicate that the myocardium was located in the second part of the curve relating the intensity of cellular damage and the severity of reperfusion arrhythmias (point R2). The situation is more lethal in the whole animal compared with n-6 PUFAs–fed rats because of the severity of malignant arrhythmias, but the myocardium is less damaged.

Since n-3 PUFAs can either be pro- or anti-arrhythmic depending on the severity of ischemia-reperfusion-induced cellular damage, it seems logical that the meta-analyses in humans did not show any beneficial effect of these fatty acids on the severity of arrhythmias and coronary heart disease–related mortality: averaging pro-arrhythmic data plus anti-arrhythmic data for the n-3 PUFAs leads to a neutral mean which is comparable to that obtained with the n-6 PUFAs. This translates in an unchanged mortality rate whatever the dietary PUFAs supplied.

Strategies to enhance the beneficial effects of n-3 PUFAs

Patients eating a Western diet rich in n-6 PUFAs and poor in n-3 PUFAs are at a high risk for severe cellular damage during post-infarction revascularization. So, they need to be treated to increase cardioprotection. This can be done through pharmacological treatment with possible secondary effects or via a nutritional modification able to afford the protection. N-3 PUFAs, especially dietary EPA, are good candidates for that purpose. ALA can also act positively since it is elongated and desaturated in EPA in the organism, but this fatty acid must be supplied in greater quantities (approximately 3 times more) as compared with EPA.

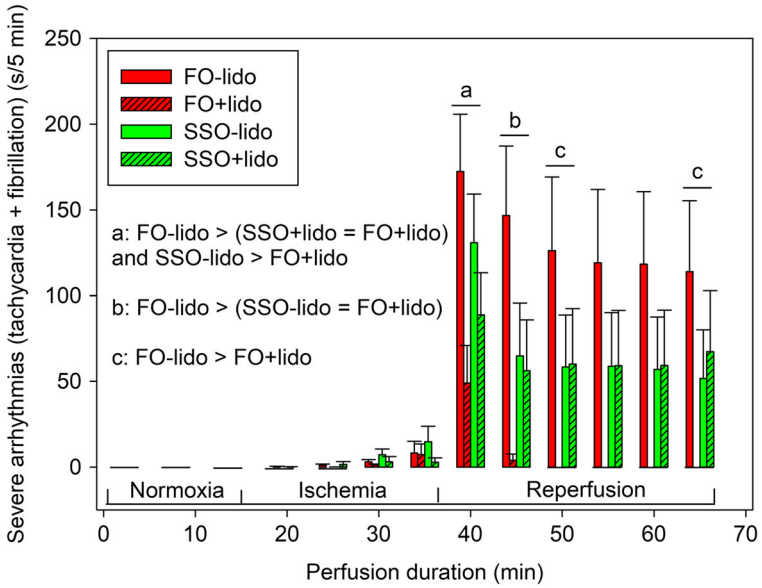

Fig. 4 Influence of the n-6/n-3 polyunsaturated fatty acid ratio and lidocaine treatment (5 μM) on the duration of severe arrhythmias during normoxia, ischemia, and reperfusion. After a 6-week feeding, rat hearts were perfused according to the working mode with a Krebs-Heinselett medium which was rich in calcium (2.5 mM), low in magnesium (1.2 mM), and low in potassium (3 mM). After 30 min of equilibration, a local ischemia was performed by left anterior descending artery ligation for 20 min. The ligation was then released to reperfuse the heart for 30 min. The lidocaine treatment was initiated as soon as the beginning of perfusion and maintained throughout the whole period of normoxia, ischemia, and reperfusion. The number of experiments was 12 per group. SSO: rats fed with 10% of n-6 PUFAs–rich sunflower seed oil; FO: rats fed with 5% of n-3 PUFAs–rich fish oil + 5% of sunflower seed oil; lido: lidocaine treatment; when SEM are not visible, the mean value is close to zero; a, b, c: significant differences as described on the figure. This figure is adapted from [63]

N-3 PUFAs can be administered at different stages of the pathology: before infarction, during ischemia, or at the beginning of the period following the revascularization intervention. The results obtained will be different depending on when the n-3 PUFAs are administrated, but also according to the severity of ischemia. Whenever n-3 PUFAs are administrated, it is of the utmost importance to maintain a steady treatment all the way through the critical post-revascularization period, i.e., during the 10 days following reperfusion. The GISSI prevention study performed this kind of treatment in secondary prevention and obtained excellent results on the survival rate [72]. Performing a regular administration during the critical period allows the optimal manifestation of the anti-arrhythmic effect of circulating n-3 PUFAs. It is thus important to maintain the level of plasma n-3 PUFAs as high as possible to reduce lethality at its lowest level. However, as it can be seen in the study by Demaison et al. [63], some reperfusion arrhythmias are resistant to anti-arrhythmic treatments: this is the case when ischemia-reperfusion-induced cellular damage is severe. In order to minimize these injuries, the best way consists in affording cardioprotection through a pharmacological or dietary treatment: n-3 PUFAs administered before or during the ischemic event can afford strong protection and help decrease cellular injuries. As it was explained in their last paragraph, the treatment will trigger either an anti- or a pro-arrhythmic effect during reperfusion, depending on the extent of the cellular damage. High circulating n-3 PUFAs (or a pharmacological anti-arrhythmic agent such as lidocaine) during the critical period of reperfusion will be able to suppress arrhythmias either partially or totally and increase patient survival. However, if cellular injuries are too serious, arrhythmias will become resistant to the anti-arrhythmic treatment: this can even occur with n-3 PUFAs' administration before reperfusion if the pathology is too serious, but the use of these fatty acids allows physicians to gain ground on the pathology by retarding cellular damage. The use of n-3 PUFAs before revascularization is thus of utmost importance to increase the survival rate, especially if the treatment is maintained throughout the critical reperfusion phase.

For the primary prevention of patients eating a Western diet, the problem is more complex, since n-3 PUFAs cannot be given before the ischemic event. Patients can be subjected to a sudden coronary artery occlusion by thrombus formation. They are then running a serious risk of fibrillation and sudden cardiac death. One of the main care then performed by the medical staff is a thrombolytic intervention. Alternatively, other patients can be subjected to atherosclerosis which slowly develops and allows the formation of new coronary vessels irrigating the unhealthy myocardium: they run fewer risks of malignant arrhythmias and sudden death, but they will have to undergo a revascularization intervention (a percutaneous coronary intervention or coronary artery bypass surgery) which can lead to abnormal electrical activity.

In the first patients with thrombus formation, n-3 PUFAs can be given as soon as the thrombolytic therapy is performed: an increase in circulating n-3 PUFAs may reduce malignant arrhythmias and somehow prevent sudden cardiac death provided the irrigation of the diseased myocardium is restored and if the cellular injuries are not too severe. The period of time between the infarction onset and medical care is thus crucial. For patients with no risk of sudden cardiac death (atherosclerotic individuals), the n-3 PUFAs treatment can be administered several days before the revascularization intervention: this allows the reduction of cellular injury and reperfusion arrhythmias, especially if a regular n-3 PUFAs treatment is performed during the critical period of reperfusion to maintain their level in the circulation as high as possible.

Conclusion

N-3 PUFAs in the circulation are anti-arrhythmic whereas those which are incorporated in membrane phospholipids are cardioprotective but can display either a pro- or an anti-arrhythmic effect during the re-establishment of the coronary flow. These two properties should allow an improvement of the survival rate after a coronary artery disease. However, they must be performed properly. Administrating n-3 PUFAs before revascularization is obviously beneficial as it decreases ischemia-reperfusion-induced cardiomyocyte injuries. Since such an intervention can trigger malignant reperfusion injuries, it is highly recommended to sustain the treatment throughout the critical period of reperfusion in order to maintain a high level of circulating n-3 PUFAs.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest with the contents of this article. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Agronomical Research.

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