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Mini review

$n-3$ PUFAs—From dietary supplements to medicines

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Abstract

Although there has been a great progress in the prevention of cardiovascular diseases, the mortality of patients with acute myocardial infarction (AMI) still remains high. One of the most important underlying causes explaining this phenomenon is the sudden cardiac death. Nearly half of all cardiovascular deaths in the USA each year is attributed to this unpredictable and unexpected complication of AMI. Hence, there is an urgent medical need for a targeted therapy to reduce the incidence of sudden cardiac death.

Since 1980 there have been several epidemiological and other studies concerning the benefits of $n-3$ polyunsaturated fatty acids ($n-3$ PUFAs) in cardiovascular health and prevention. Results from one of the largest studies, GISSI Prevenzione Trial show that adding the $n-3$ PUFAs to standard therapy of patients who survived AMI reduces sudden cardiac death (44% risk reduction, $p=0.0006$). In addition, significant decline in all-cause cardiovascular mortality (21% risk reduction, $p=0.0064$) further emphasizes the role of $n-3$ PUFA in cardiovascular prevention. To date, beneficial effects of $n-3$ PUFA are attributed to their antiarrhythmic, lipid lowering, antithrombotic and anti-inflammatory properties.

To conclude, EPA and DHA improve the prognosis of cardiovascular patients in the secondary prevention of sudden cardiac death without any documented side effects.

Keywords: $n-3$ (omega-3) polyunsaturated fatty acids ($n-3$ PUFA); EPA; DHA; Sudden cardiac death; Cardiovascular risks

1. Introduction

Over the recent decades there has been a significant progress in the management of AMI patients. Building of coronary intensive care units and fibrinolytic and primary angioplastic therapies have markedly improved their prognosis. Pharmacotherapy of patients with AMI has also shown a great progress with respect to their survival—particularly statins, beta-blockers, angiotensin converting enzyme inhibitors and anti-aggregating agents improve the AMI patients survival, but the mortality still remains high. Hence, the biggest issue in cardiovascular medicine is still the primary prevention of coronary artery disease. The role of $n-3$ PUFA in that issue is frequently discussed.

The first reference to the cardioprotective effects of fish oil (rich in $n-3$ PUFA) comes from Greenland Eskimo population and dates back to 1976—the year when also first statin was invented [1,2]. Since then statins have raised particular interest in internal medicine supported by studies and their prescription has become very popular [3]. Furthermore, fish oil as a nutritional supplement has been a focus of many randomized clinical trials and to date it is already available for prescription in many countries of the European Union (registered as Omacor® for additional treatment of AMI patients). Beside statins and life style changes, the $n-3$ PUFAs, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), could play an important role in cardiovascular disease prevention (particularly in patients with hearth failure) [4–6].

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Similar to most other developed countries, also in Slovakia the high consumption of saturated fat still remains a big problem causing dyslipoproteinemias—one of the main risk factor of cardiovascular diseases [7].

The most important PUFAs are omega-3 and omega-6 fatty acids The most important representatives of $n-6$ PUFAs are linoleic and arachidonic acids. In $n-3$ PUFA group eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (occurring mostly in fish oil), and α-linolenic acid (mostly in vegetable oils) are of highest importance [7].

It seems that not only the high consumption of saturated fats is an important risk factor of atherosclerosis, but also the ratio of $n-3$ to $n-6$ in the nutrition is of importance one typical example is the India, where the population is mostly vegetarian with low consumption of saturated fatty acids. Hindus have, however, a very low $n-3$ PUFA but high $n-6$ PUFA intake with $n-6$ to $n-3$ ratio 30–50:1. CAD mortality is more than 30% higher than expected in this population despite the non-existence of other traditional atherosclerosis risk factors. Another example is the population of Greenland Eskimos, where the ratio of $n-6$ to $n-3$ is 1:1. The CAD mortality in Eskimos is about 7%. It is well established that their consumption of saturated fats is relatively high. The so-called South-Asian paradox supports the nutritional protective role of omega-3 fatty acids [7].

Human body is unable to synthesize $n-3$ PUFAs due to missing enzymes catalyzing the double bond synthesis behind carbon 9 of the acid chain. The most important source of $n-3$ PUFAs is the fish oil. The structures of EPA and DHA are described in Fig. 1 [8].

The amount of $n-3$ PUFA varies in different fish species (Fig. 2). Unfortunately it depends also on the processing method (e.g. canned tuna compared to fresh one contains only 10% of EPA and DHA). For example the use of emulgated fats for frying may result in absorption of mostly $n-6$

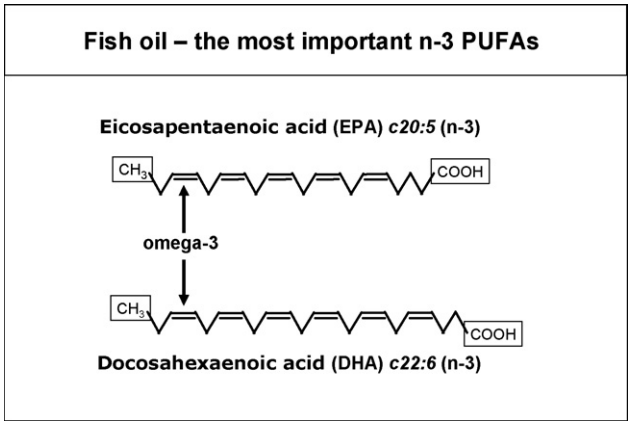

Fig. 1. The structures of the most important $n-3$ PUFAs in fish oil, EPA and DHA.

and to the lesser extent also of $n-3$ PUFA [8]. It is also well known that the same species of fish living in their natural environment contains different levels of $n-3$ PUFA compared to the same species bred in farms [9].

Another issue is the simultaneous intake of methylmercury, polychlorinated biphenyls and other organic substances obtained from fish living in polluted waters [9]. Therefore the Food and Drug Administration in USA does not recommend to pregnant women and children to consume of certain fish species (e.g. shark, swordfish and mackerel). With respect to contamination the more suitable species are tuna, salmon, codfish and barbell fish, and again the place of origin is very important depending on the water pollution [10]. Potential exposure to pollutants can be partly eliminated by removing the fish skin, containing highest concentrations of pollutants.

α-Linolenic acid can be found in high amounts in linseed, walnuts, rapeseed oil and in lesser amounts in hazelnuts

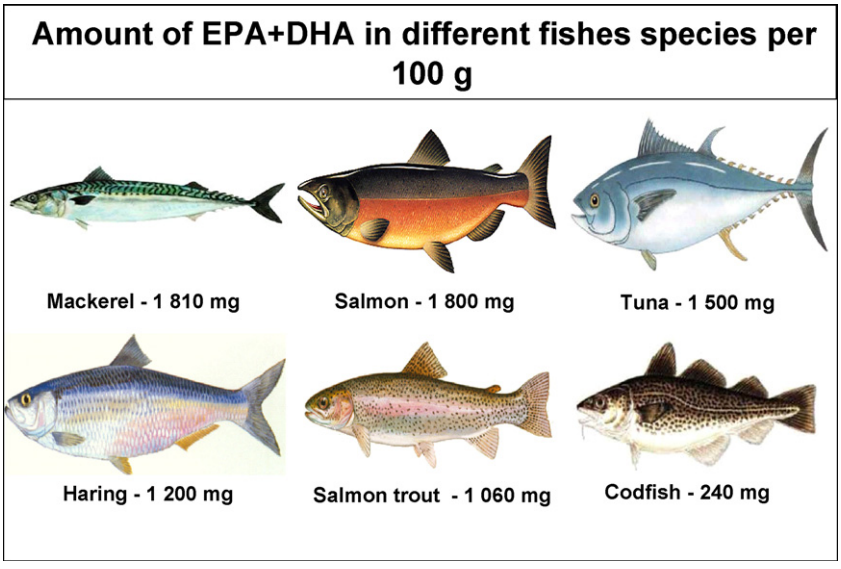

Fig. 2. Amounts of EPA + DHA in different fish species per 100 g.

and almonds. α-Linolenic acid is metabolized after absorption to EPA and in the next step to DHA by desaturase and elongenase enzymes, respectively. EPA is the source of eicosanoids and autacoids, such as prostacycline, thromboxane, leucotriens and many more.

2. Cardioprotective properties of $n-3$ PUFAs

Cardioprotective properties of $n-3$ PUFA are derived from experimental, epidemiological and clinical studies. Especially based on the results of the GISSI Prevenzione and DART studies, several effects of $n-3$ PUFA have been demonstrated, e.g. antiarrhythmic, anti-inflammatory, antithrombotic and lipid lowering [4,5].

2.1. Antiarrhytmic properties

Several studies on healthy volunteers and on CAD patients have focused on the monitoring of the relationship between the administration of $n-3$ PUFAs and heart rate variability (HRV). Low HRV is linked to higher cardiovascular morbidity and mortality and is related to higher risk of sudden death mostly in AMI patients [11].

In a study of 55 patients after AMI significant positive correlation was found between the levels of DHA and EPA in platelets and HRV [12]. With increased level of $n-3$ PUFA in platelets a progressive lowering of arachidonic acid with accompanied increase of HRV occurred ($p<0.00001$). Another study has demonstrated a statistically significant decrease of heart rate ($p<0.0001$), faster heart rate lowering after physical activity ($p<0.01$) and increase of HRV in patients after AMI with reduced ejection fraction under 40% treated with $n-3$ PUFAs (585 mg DHA and 225 mg EPA) when compared to placebo group [13].

The antiarrhythmic properties of $n-3$ PUFA are related to electric stabilization of cells via their effects on ion channels (mostly sodium, calcium and potassium) and the interactions of $n-3$ PUFA with the phospholipid bi-layer of membranes. Interestingly the enrichment of phospholipid membranes with $n-3$ PUFAa is not linked with any antiarrhythmical effect [14]. Redundant removal of $n-3$ PUFA from these membranes using bovine albumin causes no improvement in arrythmogenic capability [15]. Infusion of free $n-3$ PUFA into the blood is accompanied by significant antiarrhythmic effect, hence not the esterified, but free $n-3$ PUFAs are responsible for the antiarrhythmic effect. 3-PUFAs are released from the SN-2 position of membrane phospholipids under ischemic influence or ventricular tachycardia and may have immediate cardioprotective effect [16].

$n-3$ PUFAs bind to the proteins of sodium channels with effects similar to certain antiarrhythmic agents (e.g. mexiletin) [17]. Extended administration of mexiletine leads to up-regulation of sodium channels. On the other hand $n-3$ PUFAs do not lead to any negative side effects and show better safety profile compared to mexiletine. This is based on the fact that mexiletine increases the synthesis of mRNA coding α subunit of sodium channels, and $n-3$ PUFAs increase the hyper-polarization of ischemic cell membranes and extend the refractive period. Therefore a higher intensity of stimulus is needed to generate the action potential. $n-3$ PUFAs also have the ability to inhibit intracellular influx of calcium ions via L-channels, which is of great importance in ischemia induced calcium overload. In comparison to calcium channel blockers $n-3$ PUFAs have no negative effect on myocardial contractility [18].

2.2. Antithrombotic properties

Multiple effects of $n-3$ PUFAs on function of thrombocytes, coagulation including lowering of thrombocytes and increase of fibrinolytic activity have been documented in experimental studies [19]. Detailed scheme of antithrombotic properties of $n-3$ PUFAs is shown in Table 1.

2.3. Anti-inflammatory properties

The complex of anti-inflammatory properties of $n-3$ PUFAs can be ascribed to the reduced synthesis of arachidonic acid and linked to the inhibition of cyclo-oxygenase and lipooxygenase pathways of the inflammatory cascade. Anti-inflammatory properties of $n-3$ PUFAs may play an important role in the modification of subclinical vascular inflammation, the role of which is generally accepted in the pathogenesis of atherosclerosis, diabetes mellitus, hypertension, metabolic syndrome and chronic cardiac failure. Interestingly, a study on the anti-inflammatory effects of $n-3$ PUFA in patients with dyslipoproteinemia has shown,

Table 1
Influence of $n-3$ PUFA on function of thrombocytes and blood coagulation [19]

Factor	Function in atherogenesis	Effect of $n-3$ PUFA
Arachidonic acid	Precursor of thromboxan and leukotriens	Lowering
Thromboxane A2	Thrombocytes aggregation, vasocontriction	Lowering
Fibrinogen	Blood coagulation increase	Lowering
Thrombocytes activation factor	Thrombocytes activation	Lowering
tPAI-1	Blood coagulation increase	Lowering
TDGF	Chemoattractant and mitogen of macrophages and smooth muscle cells	Lowering
TPA	Increase of fibrinolysis	Increase

tPAI-1: tissue plasminogen activator 1 inhibitor; TDGF: thrombocytes derivate growth factor; TPA: tissue plasminogen activator.

Table 2
Influence of $n-3$ and $n-6$ PUFA on systemic inflammatory markers [20]

Markers	Before treatment	After treatment	p
Group ALA ($n=50$)			
CRP (mg/l)	1.24 (0.72–3.70)	0.93 (0.56–1.80)	0.0008
SAA (mg/l)	3.24 (2.30–5.30)	2.39 (1.70–3.90)	0.0001
IL-6 (pg/ml)	2.18 (1.35–3.90)	1.70 (1.30–2.80)	0.01
Group LA ($n=26$)			
CRP (mg/l)	1.54 (0.62–3.10)	1.25 (0.64–1.70)	0.35
SAA (mg/l)	3.52 (2.10–4.90)	3.34 (2.15–4.40)	0.58
IL-6 (pg/ml)	1.77 (1.30–2.70)	2.20 (1.10–2.70)	0.69

ALA: α-linolenic acid ($n-3$ PUFA); LA: linoleic acid ($n-6$ PUFA); CRP: C-reactive protein; SAA: serum amyloid A; IL-6: interleukin 6.

that the supplementation with high doses of $n-3$ PUFA (8.1 g α-linolenic acid) leads to a significant lowering of C-reactive protein, serum amyloid A and interleukin 6, and it is linked with a change of $n-6$ PUFAs to $n-3$ PUFAs ratio from 13.2:1 to 1.3:1 at the same time (Table 2) [20]. In the placebo group without $n-3$ PUFA added to nutrition, no lowering effect on inflammatory markers takes place. Anti-inflammatory effect $n-3$ PUFAs may be responsible for the deceleration of atherogenesis and high ratio of $n-6$ PUFA to $n-3$ PUFA for its acceleration [21].

2.4. Lipid lowering properties

Influence of $n-3$ PUFAs on serum lipid levels, mainly their effect on triglycerides is mostly linked with elevation of HDL-cholesterol [22]. In one study, 27 patients received statin therapy for 3 months followed by added $n-3$ PUFA (3 g per day) for the next 3 months together with statin. Statistically significant influence on triglycerides and HDL-cholesterol levels was documented (Table 3). Positive lipid-lowering effect was significant only with doses 2–4 g/day of $n-3$ PUFAs. Interestingly, other positive effects of $n-3$ PUFAs were present already at lower doses (1 g/day).

3. $n-3$ PUFAs as drugs

Fish oil is well known from the history for prevention of the rickets and other illnesses. Today we see the renaissance of fish oil and its application in the prevention of cardiovascular disorders. In the last decade several analyses of the effects of $n-3$ PUFA from fish oil and its cardioprotective effects have been published. One of the latest comes from Harpers and Jacobs study (Table 4) [23].

Probably the most significant results come from the Italian GISSI Prevenzione study and British DART study [4,5].

In GISSI Prevenzione study lower doses of highly purified $n-3$ PUFAs (1 g/day) than in the DART study were used. There was a statistically significant decrease of total cardiovascular mortality (about 21%, $p=0.0064$). One of the most important outcomes of the study was the significant lowering of sudden cardiac deaths (44%, $p=0.0006$). With these results there is no doubt about classification of fish oil among the drugs with evidence-based cardioprotective properties. A comparison of $n-3$ PUHA Omacor (registered trade mark) to the other drugs used in prevention of sudden death can be found in Table 5 [24].

There is a growing evidence that $n-3$ PUFA in the form of fish oil or capsules containing the purified $n-3$ PUFA can have a place in the newest guidelines of AHA (American Heart Association) for ischemic coronary heart disease. The use of one gram of EPA + DHA a day (2–4 g in patients with hypertriglyceridemia) as a cardiovascular preventive dose seems reasonable [25].

4. Discussion

The studies in Eskimo communities indicate that fish eating is effective in the primary prevention of cardiovascular diseases [1]. Results of the secondary prevention in GISSI-Prevenzione trial has shown that dietary supplementation with $n-3$ PUFA led to a clinically important and statistically significant benefit [4]. There is conflicting evidence on the benefits of foods rich in vitamin E (alpha-tocopherol), $n-3$ polyunsaturated fatty acids (PUFA), and their pharmacological substitutes. The supplementation of vitamin E had no benefit and the effect of the combined treatment was similar to that for $n-3$ PUFA [4]. The $n-3$ PUFA could also be powerful as drugs.

There are lots of steps to get approval for a registered drug. It means lots of pre-clinical trials with cell cultures, animals, clinical trials with healthy volunteers to find out the tolerance of the candidate compound, then clinical trials for indicated cases. Also the side effects need clarification. Controlled clinical double-blinded trials are next and the postmarketing and postregistrated researches are the final steps. During this final period the drug is followed at least 5 years. They are not very

Table 3
Influence of additional therapy of $n-3$ PUFA added to statin therapy on lipids levels [22]

Parameter	Before treatment $n=27$	After 6 weeks with statins treatment	After 3 months treatment with statins and $n-3$ PUFA (3 g/deň)	p
Statins and $n-3$ PUFA—complementary hypolipidemic effect				
Total cholesterol	6.48 ± 1.34	5.12 ± 0.86	5.04 ± 0.85	NS
LDL cholesterol	4.21 ± 0.89	3.04 ± 0.72	3.08 ± 0.76	NS
HDL cholesterol	0.92 ± 0.22	1.01 ± 0.29	1.21 ± 0.20	<0.05
TG	2.96 ± 0.99	2.34 ± 1.01	1.64 ± 0.81	<0.01

Table 4
Randomized controlled trials of $n-3$ PUFA (EPA, DHA) published to date [23]

Study	$n-3$ dose	Controls/doses	n	Average period of monitoring /months	Total mortality (RR, 95% CI)
GISSI-I	EPA + DHA (1:2)1.85 g/d	Placebo or vit.E	11,234	42	0.79 (0.66–0.93)
Singh-India	EPA + DHA (1:1)1.8 g/d	100 mg Al. hydroxid	360	12	ND
Nilsen-N	EPA + DHA (1:2) 3,4 g/d	Corn oil 4 g/d	300	18	1.0 (0.45–2.2)
Von Schacky-D	EPA + DHA (3:2) 1.85 g/d	Vegetable oil 1.85 g/d	223	24	0.5 (0–5.5)
Leng-UK	EPA 0.27 g/d	Sunflower oil 3 g/d	120	24	1.0 (0.21–4.8)
Sacks-USA	EPA + DHA (3:2) 4.8 g/d	Olive oil 12 g/d	59	28	0.3 (0.01–7.1)
Burr-UK	EPA 2.4 g/week.	Diet without fish	2,033	24	0.73 (0.56–0.93)

easy steps and they take lots of time and cause expenses. These will increase the final price of the drugs and patients or their insurance must pay.

Already the first omega-3 PUFA medicine has been registered with trade mark Omacor® (www.omacorrx.com). It is the first and sofar the only FDA-approved $n-3$ fatty acid product to be prescriped. Its capsules contain 90% $n-3$ acids and small amount of vitamin E. The product is naturally derived. In Europe it is recommended for an additional treatment of patients after acute myocardial infarction.

In the primary prevention eating more fish or to use dietary supplements are recommended. There is, however, a big difference in amounts of EPA and DHA in different fish species. The ratio of EPA and DHA of $n-3$ PUFA in the supplements varies, too.

In the secondary prevention and treament especially in the elderly people the fish diets are probably not enough as the amounts of EPA can be reached only by rich meals which can be difficult for various reasons. Not all patients like fish and some are allergic. The supplements obtained in capsules avoid these difficulties. If one can consume fish oil, it should not be forgotten as it is cheap.

If we take a closer look on the $n-3$ PUFA preparations in every day use and calculate how much fish and how many days one is able to buy fish for the same price the capsuls look reasonable choices.

Little is known about the relation of the dietary intake of $n-3$ polyunsaturated fatty acids, i.e., docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) from fatty fish and alpha-linolenic acid (ALA) from vegetable oils in senior citizens with ischemic heart disease [26]. High combined dietary intake of EPA and DHA and possibly ALA, may lower the risk of coronary patients to get fatal event [26,27]. In patients the efficacy of lengthening of ALA to EPA and DHA has not been clarified. A high self-reported fish intake, however, associated with a slower progression of coronary atherosclerosis in postmenopausal women with coronary artery disease [28]. On other hand in healthy elderly subjects, ALA seems to affect concentrations of LDL-cholesterol and apoB more favorably than EPA/DHA, whereas EPA/DHA seems to affect tissue factor pathway inhibitor more beneficially [29].

Table 5
Comparison of different therapeutic modalities with respect to sudden cardiac death outcome [24]

Drug	Patient number	Relative risk of sudden death (95% CI)
Highly purified $n-3$ PUFA	11,323	0.56 (0.40–0.79)
ACE-I after MI	15,104	0.80 (0.70–0.92)
Aldosterone blockers	1,663	0.71 (0.54–0.95)
Flekainid, enkainid	1,455	3.60 (1.7–8.5)
Beta-blockers after MI	24,298	0.77 (0.70–0.84)
Amiodaron	6,500	0.71 (0.59–0.85)
D-Sotalol	3,121	1.77 (1.15–2.74)
Calcium blockers	20,342	1.04 (0.95–1.14)

A growing body of evidence shows that oxygen radicals and other products of free radical reactions are involved in aging and age-related degenerative diseases. Recent studies have suggested that fish oils have a potentially beneficial effect on age-associated diseases. Consumption of fish oil may increase the requirement for vitamin E, especially under conditions where oxidative stress is increased. Vitamin E requirement increases with increased intake of dietary polyunsaturated fatty acids. This relationship may be more important in elderly subjects. The studies have shown that plasma lipid peroxides are significantly higher in older than in young subjects. Thus, in conditions where the percentage of highly unsaturated fatty acid increases in the membranes, older subjects may be more susceptible to oxidative damage [30].

The older women receiving fish oil supplements for 3 months exhibited a greater increase in plasma $n-3$ PUFA than in the young subjects. Plasma vitamin E levels did not change significantly after supplementation. After 3 months supplementation in young women, the plasma vitamin E was, however, significantly lower than after 1 month supplementation. The vitamin E:TG-ratio significantly increased and vitamin E:(EPA + DHA) significantly decreased. All women showed a significant increase in plasma lipid peroxides after 2 months supplementation. The older women had significantly higher lipid peroxide levels than younger women. The lipid peroxide:TG-ratio, which declined in 3 months, was still significantly higher than baseline. These data indicate that although long-term fish oil supplementation may be beneficial in reducing plasma total TG, the susceptibility of plasma lipids to free radical attack may be potentiated [31].

By substituting membrane fatty acids with the potentially unstable ($n-3$) fatty acids of fish oil, older subjects have been found to be at a greater risk of oxidative stress than young sub-

jects. In addition, when exposed to eccentric exercise-induced oxidative stress, older men, receiving vitamin E supplements for 48 days, exhibited significantly lower levels of lipid peroxides in urine than controls. These data indicate that older subjects are more susceptible to oxidative stress and may benefit from the antioxidant protection provided by vitamin E [30].

To conclude, EPA and DHA improve the prognosis of cardiovascular patients in the secondary prevention of sudden cardiac death without any documented side effects. The first rather pure EPA has passed for these purposes the drug registration process. At present no such studies on primary prevention have been reported, although such studies should be done in young individuals at high atherosclerosis risk.

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