REVIEW
Polyunsaturated fatty acids in health and disease

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Abstract: Polyunsaturated fatty acids (PUFAs) are necessary for overall health. Two PUFAs families, n-6 and n-3 fatty acids, are physiologically and metabolically distinct. The proportion of PUFAs in serum and erythrocyte phospholipids, which depends on endogenous metabolism controlled by genetic polymorphisms and dietary intake, is an important determinant of both health and disease. Both n-3 and n-6 PUFAs are processed to powerful promoters of eicosanoids synthesis at the cyclooxygenase and lipoxygenase level. Evidence from observational and intervention studies suggest that n-3 PUFAs are cardioprotective, perhaps through their anti-inflammatory, anti-arrhythmic, lipid-lowering and antihypertensive effects. In contrast, dietary n-6 PUFAs have pro-inflammatory effects. Low n-3 and elevated n-6 PUFAs levels were found in patients with cancer on different sites. The present review focuses on current knowledge related to PUFAs intake and status in health and disease, with reference to the Serbian population.

Keywords: n-3; n-6; PUFA; inflammation; cardiovascular disease; chronic diseases.

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1. INTRODUCTION

Polyunsaturated fatty acids (PUFAs) play important roles in maintaining normal physiological conditions and, consequently, in human health. Two PUFAs families, n-6 and n-3 fatty acids (FA), are physiologically and metabolically distinct. Their precursors, linoleic acid (18:2n-6; LA) and α-linolenic acid (18:3n-3; ALA) are essential fatty acids (EFA), meaning that they cannot be synthesized in the human body and must be obtained from the diet. Thus, LA can be converted *via* γ-linolenic acid (18:3n-6) and dihomo-γ-linolenic acid (20:3n-6; DGLA) to arachidonic acid (20:4n-6; AA) (Fig. 1). Arachidonic acid plays important biological roles. It is released from phospholipids by phospholipase A2 and is the precursor of pro-inflammatory eicosanoids, which include prostaglandins of the two series (PGE2, PGD2), leukotrienes of the four series (LTA4, LTB4, LTC4, LTD4 and LTE4) and lipoxines.[1] Their production is catalyzed by cyclo-oxygenase, lipoxygenase and epoxygenase enzymes, respectively. By an analogous set of reactions catalyzed by the same enzymes, precursor of n-3 PUFAs, ALA, can be converted into eicosapentaenoic acid (20:5n-3; EPA), and further to docosapentaenoic acid (22:5n-3; DPAn-3) and docosahexaenoic acid (22:6n-3; DHA). This is achieved by insertion of additional double bonds into the acyl chain (*i.e.*, unsaturation) and by elongation of the acyl chain. EPA is a precursor of the other classes of eicosanoids, namely the three series of prostaglandins and the five series of leukotrienes. Eicosanoids derived from AA have opposing properties from those originating from EPA. Therefore, an increase in the dietary intake of LA changes the physiological state to a prothrombotic, proconstrictive, and pro-inflammatory one. Many of the chronic conditions, cardiovascular disease, diabetes, cancer, obesity, auto-immune diseases, rheumatoid arthritis, asthma and depression are associated with an increased production of thromboxane A2, leukotriene B4, IL-1, IL-6, tumour necrosis factor (TNF), and C-reactive protein.[2,3] All these factors are increased by increased n-6 PUFAs intake and decreased by increased n-3 PUFAs intake, either ALA or EPA and DHA. However, there is one exception. DGLA of the n-6 family can be further converted by inflammatory cells to 15-(*S*)-hydroxy-8,11,13-eicosatrienoic acid and PGE1. This is interesting because these compounds possess anti-inflammatory and anti-proliferative properties. PGE1 could also induce growth inhibition and

differentiation of cancer cells.[4] The mechanism of DGLA action has not yet been elucidated.
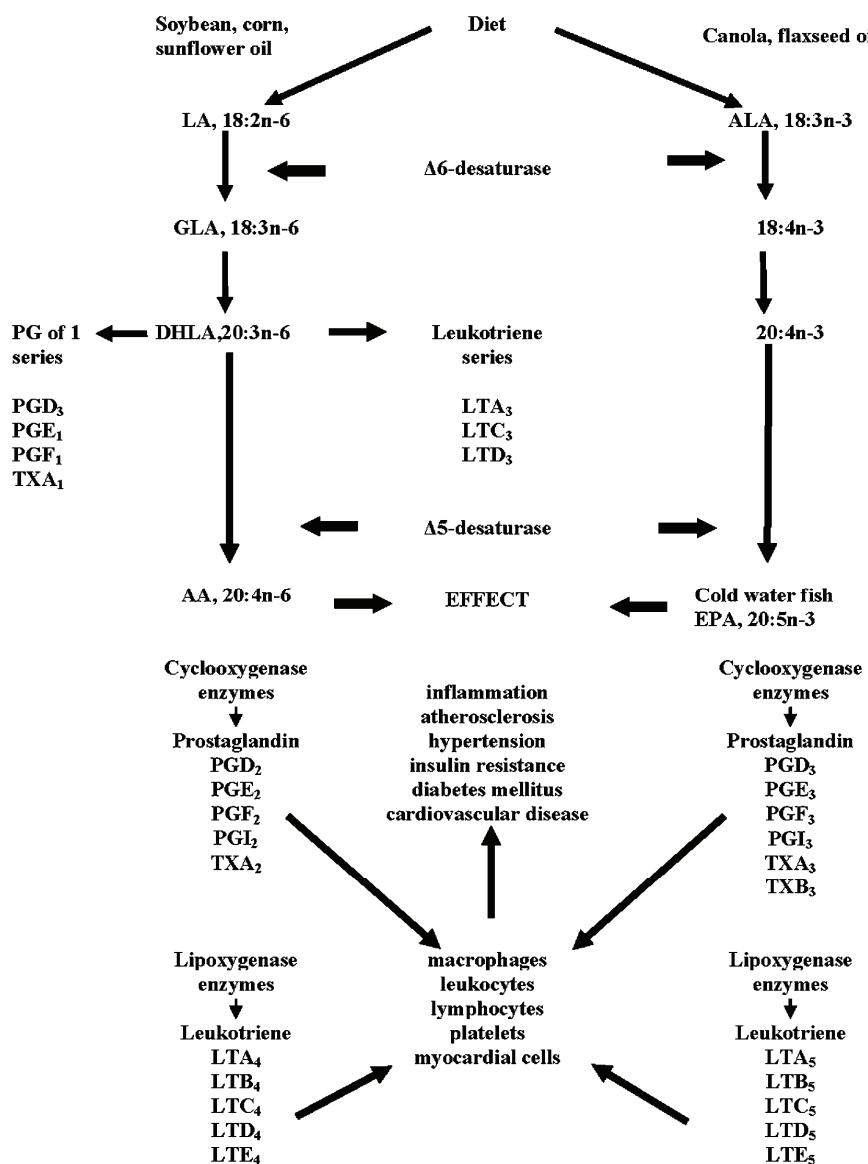
Fig. 1. Dietary sources, metabolism of n-3 and n-6 PUFAs and clinical outcomes.

It is well known that PUFAs favourably affect the blood lipid profile (Table I). LA is associated with a lower risk of atherosclerosis, cardiovascular heart disease (CHD) and type 2 diabetes.[5–8] Consumption of ALA has also been sug-

TABLE I. Potential beneficial effects of PUFA on physiological parameters[6,7,9,11]

Total PUFA	Diseases	Physiological parameters
ALA	Cancer/coronary heart disease	↓Serum total cholesterol
	Cardiovascular disease Myocardial infraction Cardiac arrhythmias Colon cancer Deficiency symptoms	↓Serum total cholesterol ↓Platelet aggregation, adhesion of monocytes to vessel walls, vascular dilatation, blood pressure, inflammatory processes, and immune reaction ↑Leukocyte function ↑Neural integrity n-3 deficiency in pre- and postnatal nutrition of infants affects: neural integrity, learning and visual abilities and depressed development of retinal function and visual acuity
EPA and DHA	CHD Fatal myocardial infraction Inflammatory diseases Bipolar disorder, cognitive decline, aggression, hostility, Anti-social behaviour Age-related maculopathy	↓Production of PGE2 metabolites ↓Thromboxane A2, a potent platelet aggregator and vasoconstrictor ↓Leukotriene B4 formation, an inducer of inflammation, and a powerful inducer of leukocyte chemotaxis and adherence ↑Thromboxane A3, a weak platelet aggregator and weak vasoconstrictor ↑Prostacyclin PGI3, leading to an overall ↑ in total prostacyclin by ↑ PGI3 without a ↓ PGI2, both PGI2 and PGI3 are active vasodilators and inhibitors of platelet aggregation ↑Leukotriene B5, a weak inducer of inflammation and a weak chemotactic agent ↓Serum triglycerides, VLDL-C ↓Platelet aggregation, adhesion of monocytes to vessel walls, vascular dilatation, blood pressure, inflammatory processes, and immune reaction ↑Rod photoreceptor, visual acuity, neural function (infants)
LA	CVD mortality Deficiency disease	↓Serum total cholesterol, LDL-C, HDL-C ↑Platelet aggregation, adhesion of monocytes to vessel walls, vascular dilatation, blood pressure, inflammatory processes, and immune reaction

gested to reduce the risk of CHD events.[9–12] Nevertheless, clinical benefits have not been confirmed in all studies, and further research on the association between ALA consumption and the incidence of CHD are required. The long chain n-3 PUFAs, EPA and DHA, consumption have demonstrated physiological benefits on blood pressure, heart rate, triglycerides, likely inflammation, endothelial function, and cardiac diastolic function.[13–16] Furthermore, consistent evidence for a decreased risk of fatal CHD and sudden cardiac death at consumption of > 250 mg day^{-1} of EPA plus DHA were also reported.[17] For primary prevention of car-

diac arrest, minimum intakes of 250 mg day^{-1} of marine EPA and DHA have been suggested.[14]

DHA also plays a major role in cognitive functions. Therefore, its intake is very important during pregnancy, in young children but also in the elderly. DHA is involved in normal development of the brain and retina during foetal development and the first 2 years of life.[18–20] In healthy children a positive associations between DHA levels in blood and improvements on tests of cognitive and visual function was found.[21] A study in human adults using positron emission tomography showed that a normal human brain consumes around 17.8 and 4.6 mg day^{-1} of AA and DHA, respectively, and that brain AA consumption is increased in Alzheimer disease patients.[22] In addition, some clinical evidence suggests that an AA/DHA ratio greater than 1/1 is associated with improved cognitive outcomes.[23] These findings suggest that recommendations for adequate intakes of DHA and other PUFAs in pregnant women, young children and elderly are urgently needed. The present review focuses on the current knowledge related to PUFAs intake and status in health and disease, with reference to the Serbian population.

2. PUFAs INTAKE AND STATUS

2.1. Dietary sources

LA is present in significant amounts in many vegetable oils, including corn, sunflower, grape seed and soybean oils, and in products made from these oils, such as margarines. ALA is found in green plant tissues, in some common vegetable oils, including soybean and rapeseed oils, in some nuts and in particular in linseeds and linseed oil.[24] Arachidonic acid is mostly present in meats and its intake is estimated at 50 to 500 mg day^{-1}. The richest sources of EPA, DPA and DHA are oily fish (tuna, salmon, mackerel, herring, and sardine). One oily fish meal can provide between 1.5 and 3.5 g EPA+DHA.[25] Consumption of 1 g fish oil capsule per day can provide about 300 mg of these fatty acids. In the absence of oily fish or fish oil consumption, the intake of n-3 PUFAs is likely to be 100 mg day^{-1}.[26] According to habitual dietary information in Serbia, low fat consumers have an intake of 5.4 % of daily energy intake (%E) and high fat consumers around 5.9 % E from PUFAs.[27]

2.2. n-6 to n-3 PUFAs ratio

The intake of LA in western countries has increased greatly in the last few decades, due to the introduction and marketing of cooking oils and margarines.[28] Typical intakes of both EFA exceed requirements. However, replacing lard with sunflower oil in the diet has resulted in a marked increase in the ratio of n-6 to n-3 PUFAs. This ratio is typically between 5 and 20 in most Western populations.[29] A lower n-6 to n-3 PUFAs ratio consumption has been recommended in order to

reduce the formation of pro-inflammatory eicosanoids from n-6 PUFAs and to increase the production of anti-inflammatory mediators from n-3 FA.[2,30] Additionally, it was suggested that lowering the n–6 FA intake would have the same health effects as increasing n-3 FA intake. Wall *et al.*[31] recently reviewed that reductions in the n-6 to n-3 FA ratio in the diet may lower the incidence of many chronic diseases that involve inflammatory processes; these include cardiovascular diseases, inflammatory bowel disease, cancer, rheumatoid arthritis and psychiatric and neurodegenerative illnesses. Thus, the specific n-6/n-3 ratio in the diet is of particular interest for maintaining overall health.

2.3. Recommendations for intake of PUFAs

In the light of new evidence for associations between low intakes of some PUFAs and increased risk of chronic disease that was mentioned above, optimal criteria for dietary recommendation aim to achieve optimal health and to reduce risk of developing chronic disease.[32] A World Health Organization report from 1994[33] did not suggest nutrient intake values for total PUFAs, but focused on the ratio of LA/ALA in the diet. Recent reports indicated that in healthy adults, the minimum intake levels for EFA should be 2.5 % LA plus 0.5 % ALA of daily energy intake to prevent deficiency symptoms.[32] Recommendations on the intake of PUFAs in healthy adults from Nutrition bodies and International Dietary Recommendations[32–40] are listed in Table II. An effective intake for the prevention of chronic diseases is higher, 6–11 % *E*, which is considered as the optimal range for the total intake of PUFAs.[2,32,40] Currently, there is no upper n-6 PUFAs value in the Eurodiet core report.[39] These different positions reflect the current worldwide debate on the relevance of an upper limit in dietary n-6 PUFA intake and highlight the need for further *in vivo* investigations. At the moment, the nutrition body supports the recommendation for n-6 PUFA intake above 5 %, and ideally about 10 % of total energy. However, balance in the n-6/n-3 ratio issue was debated in detail by Stanley *et al.*[41] and Harris,[42] who concluded that this ratio is not relevant for setting up recommendations. Based on both evidence and conceptual limitations, there is no compelling scientific rationale for the continued recommendation of a specific ratio of n-6 to n-3 PUFAs or LA to ALA.

2.4. PUFAs intake in relation to status biomarker

The proportion of PUFAs in serum and erythrocyte phospholipids, an important determinant of both health and disease, depends on the dietary intake and endogenous metabolism controlled by genetic polymorphisms. The FA composition of serum phospholipids is genetically controlled by the FADS1 and FADS2 gene cluster. Based on this genetic variation, individuals may require different amounts of dietary PUFAs to achieve comparable biological effects.[43] Nevertheless, the FA composition in serum lipids can be used not only as a biomarker of fat quality intake, but also as an indicator of disease risk.

TABLE II. Dietary recommendations for PUFAs intake; AI = adequate intake; E% = energy of fat; IOM = Dietary reference intakes of the American Institute of the Medicine's Food and Nutrition Board; AHA = American Health Association Science Advisory, Australia/NZ = Nutrient reference values for Australia and New Zealand; BNF = British nutrition foundation; FS = French food safety agency report; EDC = Eurodiet core report; LA = linoleic acid, LNA = α-linolenic acid, AA = arachidonic acid, DHA = docosahexaenoic acid, EPA = eicosapentaenoic acid; m = male, f = female

PUFA	FAO/WHO E%[33,34]	IOM[35]	AHA E%[7,37]	Australia/ NZ E%[38]	BNF E%[54]	EDC E%[39]	AI for adults g day⁻¹, E%[39]	AI for infants formula/diet of FA[39]	Recent report[32,40] E%
n-6 PUFA	4–10 5–8	5–10 E%	At least 5–10	4–10	–	4–8	–	–	Ranging 2–10
LA	2.5	m 17 g f 12 g	–	8–13 g	Min. 1	–	2; upper limit 3 4.44 g; 6.67 %	10	–
AA	0.5	–	–	–	–	–	–	0.5	–
n-3 PUFA	1–2	–	–	–	–	–	–	–	–
LNA	None/min <0.05	m 1.6 g f 1.1 g	–	0.8–1.3 g	0.6 g; 1.2 %	2 g	2.2 g; 1 %	1.5	Ranging 0.2–2
EPA	None %	–	Patient with CHD: 1 g/fish oil/d Without CHD: 500 mg/d TG↑ 3–4 g	–	–	200 mg	0.22 g, at least 0.1	upper limit < 0.1	90–650 mg/d
DHA	None %	–		–	–	–	0.22 g, at least 0.1	0.35	40–3000 mg/d
EPA +DHA	None %, / 0.25 g/d	–		–	–	–	0.65 g, 0.3%	–	for different age groups
Total PUFA (n-3 + n-6)	6–10	6–12	–	–	6–10	–	–	–	10–12 us upper level

PUFAs are important constituents of the phospholipids of all cell membranes. LA, ALA and their metabolic products, AA, EPA and DHA are crucial structural and functional components of cellular and intracellular membranes in the human body, but especially in brain, heart, retina, and testes. Phospholipids play an essential role in membrane structure and function. PUFAs of both the n-6 and n-3 series are incorporated into membrane phospholipids, and the AA/EPA ratio ranges between 1:1 and 5–10:1. The higher ratio stimulates the incorporation of EPA, because of the greater affinity of enzymes for EPA.[44] The length and degree of unsaturated FAs in membrane phospholipids are the main determinants of fluidity, transport systems, activity of membrane-bound enzymes, and susceptibility to lipid peroxidation.[45–47] In this context, an altered FA composition with reduced levels of PUFAs and increased contents of saturated FA (SFA), that consequently decrease the PUFA/SFA ratio in erythrocyte membranes, may be associated with lower membrane fluidity in patients with chronic diseases.[48,49] This is often found in elite athletes.[50–52]

It was previously established that erythrocytes reflect the general FA metabolism in other organs and tissues.[53,54] A poor n-3 PUFAs status is often related to a low consumption of cold-water fish, as the primary source of EPA and DHA, and then to income status, national and social eating habits. However, an inadequate EFA intake is not the only cause for a disturbance in the FA profile. EFA deficiency is also present in chronic inflammatory conditions,[55] increased oxidative stress related to PUFAs oxidation[56] and in elevated intracellular calcium concentration.[57] A number of studies showed that different pathologies, such as cancer, diabetes, coronary heart disease, pancreatitis, *etc*., could be associated to altered FA profiles of plasma or serum phospholipids.[58–61] Patients with renal failure, liver cirrhosis or diabetes mellitus often have plasma FA profiles similar to those with nutritional deficiency of EFA.[62–65] Considering limited storage of n-3 FA in adipose tissue in both patients and healthy people, a continued dietary supply with the optimal n-6/n-3 ratio of PUFAs has been suggested. A diet supplemented with n-3 PUFAs partially replace n-6 PUFAs in the majority of the membranes of cells (*e.g*., erythrocytes, platelets, monocytes, lymphocytes and granulocytes, and endothelial neuronal, colon, and hepatic cells), suggesting that in spite of pathologies, diet could markedly change the FA profiles in patients.

In the Serbian population with type 2 diabetes[59] who also had abnormal lipid levels, the total n-3 PUFAs in plasma were lower, while the n-6/n-3 ratio was higher when compared to healthy subjects (Table III). EPA, DHA and total n-3 PUFAs in the erythrocyte phospholipids in these patients[59] were also low (Table IV). Patients with hyperlipidemia[60] had a significantly lower proportion of EPA and DHA than healthy subjects. Suboptimal levels of n-3 FA in erythrocytes have been found in obese subjects,[67] as well as a lower proportion of EPA, DHA and total n-3 FA, and a significantly higher n-6/n-3 ratio in insulin-resistant

obese women when compared to obese women with normal glucose tolerance (Table IV). A similar n-3 FA status in serum phospholipids and red blood cells was reported in well-nourished patients[62] undergoing haemodialysis. Proportions of DHA and n-3 PUFAs in serum phospholipids of patients with non-Hodgkin lymphoma,[61] as well as in patients with obstructive jaundice[66] were extremely low, which led to very high n-6/n-3 ratios of around 15 in both groups of patients, demonstrating a complete imbalance of FA in these patients. However, the n-6/n-3 ratio in healthy subjects in the Serbian population was also very high (11–12), suggesting the importance of changing dietary habits in Serbia.[50–52,61,62]

TABLE III. Plasma phospholipids fatty acids composition (mol %) with reference to the Serbian population; HLP, hyperlipidemic patients; DM, diabetes mellitus; HD, haemodialyses patients; NHL, patients with non-Hodgkin lymphoma

Serum	Healthy controls (n = 27)[62]	HLP (n = 41)[60]	DM 2, HLP (n = 29)[59]	Obstructive jaundice (n = 13)[66]	HD (n = 29)[62]	NHL (n = 47)[61]
LA	26.5±2.8	23.1±1.4	22.6±2.4	24.8±3.4	25.5±2.9	20.2±2.4
DGLA	2.4±0.7	3.0±0.4	3.0±0.8	2.6±1.0	2.0±0.5	3.9±1.0
AA	11.6±2.3	11.4±1.0	13.2±2.9	8.8±1.50	11.1±2.2	14.3±1.5
22:4 n-6	0.4±0.2	0.4±0.1	0.6±0.3	0.2±0.1	0.4±0.1	0.6±0.2
Σn-6	40.8±2.9	38.0±1.7	39.5±3.4	35.6±3.2	39.0±3.3	38.8±2.6
ALA	0.11±0.01[52]	0.10±0.02	–	–	–	–
EPA	0.4±0.1	0.3±0.05	0.4±0.3	0.35±0.07	0.3±0.1	0.2±0.1
DPAn-3	0.6±0.1	0.5±0.1	0.6±0.2	0.35±0.10	0.5±0.1	0.4±0.2
DHA	3.6±1.1	3.1±0.3	2.5±0.7	2.2±0.7	3.0±0.9	2.1±0.7
Σn-3	4.7±1.4	4.0±0.3	3.5±0.8	2.6±0.8	3.8±1.2	2.7±0.7
n-6/n-3	8.8±1.6	9.4±0.9	11.2±2.7	14.2±3.5	9.6±1.3	15.4±4.6

3. BIOLOGICAL EFFECTS AND METABOLIC FUNCTIONS OF n-6 AND n-3 PUFAs

An increasing body of evidence suggests that n–3 PUFAs supplementation may improve defects in insulin signaling and prevent alterations in glucose homeostasis and further development of diabetes type 2.[63,68] These effects are possibly mediated through the peroxisome proliferator-activated receptors (PPARs), which are up-regulated by long-chain PUFA and in turn are related to the gene expression involved in lipid oxidation and synthesis.[69] Other pleiotrophic effects of n–3 PUFAs may contribute to decreased condition of the metabolic syndrome, such as modulation of inflammation, platelet activation, endothelial function and blood pressure.[70]

In addition, a high proportion of n-3 PUFAs in red blood cell membranes is associated with a reduced risk of primary cardiac arrest. The American Heart Association recommended that individuals at high cardiovascular risk should consume 1 g daily of fish oil.[7,71] It was shown that n-3 PUFAs oral supplementation quickly and effectively raised the blood n-3 PUFAs levels.[72] However, some new data and meta analyses showed no effect of n-3 supplementation and

TABLE IV. Erythrocyte phospholipids fatty acids composition (mol %) with reference to the Serbian population; IILP, hyperlipidemic patients; DM, diabetes mellitus: NGT, normal glucose tolerance; IR, insulin resistance; HD, haemodialyses patients; NHL, patients with non-Hodgkin lymphoma; Er, erythrocyte

Er	Healthy controls (n = 27)[62]	HLP (n = 41)[60]	DM 2, HLP (n = 29)[59]	Obesity, HLP (n = 30)[66]	Obesity, HLP, NGT (n = 12)[66]	Obesity, HLP, IR (n = 18)[66]	HD (n = 29)[62]
LA	15.5±1.8	13.5±1.1	13.0±3.5	12.6±1.7	12.2±1.6	13.0±1.7	14.8±2.0
DGLA	1.2±0.4	1.5±0.2	1.7±0.6	1.8±0.4	1.7± 0.3	2.0±0.4	1.3±0.4
ΛΛ	16.2±2.0	16.5±1.5	11.3±3.8	16.7±1.9	17.3±1.7	16.3±2.0	15.3±1.8
22:4 n-6	3.5±1.2	3.6±0.5	2.4±1.1	3.8±0.7	3.8±0.7	3.8±0.7	3.5±0.9
Σn-6	36.5±2.4	35.2±2.2	28.4±6.5	35.1±3.0	35.0±2.4	35.0±3.4	34.9±3.5
ALA	0.11±0.09[52]	0.10±0.02	–	–	–	–	–
EPA	0.5±0.1	0.3±0.1	0.3±0.2	0.4±0.2	0.5±0.2	0.4±0.2	0.2±0.1
DPAn-3	1.3±0.5	1.6±0.3	0.9±0.5	1.7±0.4	1.8±0.4	1.5±0.3	1.1±0.4
DHΛ	5.3±1.1	3.8±0.6	2.2±1.1	4.3 ±1.2	5.2±1.3	3.8±1.9	4.3±0.8
Σn-3	6.9±1.3	5.9±0.6	3.3±1.6	6.5±1.7	7.6±1.7	5.7±1.2	6.0±0.9
n-6/n-3	5.4±1.1	6.0±0.7	8.6±4.8	5.7±0.7	4.9±1.4	6.4 ±1.4	5.9±0.9

health benefits. Considering these facts, further work is required to confirm the association between plasma PUFAs levels and clinical outcomes.

3.1. PUFAs and dyslipidemia

One of the most investigated health effect of n-3 PUFA is their capability to reduce serum triglyceride levels. Many different mechanisms seem to be involved in the hypotriglyceridemic effect of n-3 PUFAs in humans. First, it is assumed that the lipid-altering effects of n-3 PUFA could modify gene expression.[73,74] At the gene transcriptional level, they can act on liver X receptor, hepatocyte nuclear factor-4a, farnesol X receptor and PPAR-alpha and PPAR--gamma. They simultaneously down-regulate genes encoding proteins that stimulate lipid synthesis and up-regulate genes encoding proteins that stimulate fatty acid oxidation, both processes resulting in lower serum triglyceride levels.[75–77] EPA and/or DHA supplementation in animal studies reduced the substrate for triglyceride synthesis and increased peroxisomal, mitochondrial FA β-oxidation[78] and decreased concentrations of all blood lipids.[79] These FA activate expressions of genes involved in β-oxidation controlled by PPAR-α receptors. It was also reported that fish oil supplementation decreased the fractional catabolic rates of high density lipoprotein (HDL) and increased the ratio of HDL--2/HDL-3 cholesterol.[80] This was related to a decrease in the levels of plasma triglycerides, which stabilizes HDL particles as they become larger, retain more cholesterol and are less susceptible to catabolism by hepatic and renal clearance pathways.[81] At the level of low density lipoprotein (LDL) cholesterol and very low density lipoprotein (VLDL), peroxidation of PUFAs stimulates apoB degradation, reduces VLDL secretion, stimulates lipoprotein lipase activity mechanisms and increases postprandial clearance.[82,83]

The dosage of n-3 PUFA that lowers triglycerides differs among studies. Bays[84] found that 4 g of n-3 PUFAs per day, in the form of fish oil capsules, reduced serum triglyceride levels by 35 to 45 %. In a meta-analysis of 72 randomized control trials, Harris[78] reported a serum triglyceride reduction of 25––30 % at a dosage of 3–4 g day^{-1} of EPA + DHA. The effect of n-6 PUFAs enriched diets was also studied. A meta-analysis of 60 controlled trials reported that replacement of carbohydrates with PUFAs (largely n-6) had a beneficial effect on the total cholesterol/HDL-cholesterol ratio, and on the LDL concentration.[85] Replacing SFA by n-6 PUFAs also led to a substantial reduction in the total cholesterol and LDL-cholesterol, a reduction of the total cholesterol/HDL--cholesterol ratio and thus may reduce the risk of CHD.[86–88] A recently published paper[89] showed that the plasma cholesterol value was negatively correlated with serum levels of EPA.

3.2. PUFAs and obesity and diabetes

Although a favourable effect of n-3 PUFAs on development of diabetes mellitus was shown in several studies, the overall pooled data findings do not support any benefits of oily fish/seafood or EPA+DHA intake on diabetes and suggest that ALA may be associated with a modestly lower risk.[90] However, some evidence indicates that higher proportion of n-3 PUFAs in the diet may have anti-obesity effects and protection against metabolic syndrome through a number of metabolic effects.

It was proposed that n-6 PUFAs may be involved in the differentiation of preadipose cells to adipocytes.[91,92] To date, no firm conclusion could be drawn from available *in vitro* studies[93,94] on the role of AA in the differentiation of preadipose cells. Moreover, animal studies investigating the effect of a diet enriched in n-6 PUFAs on adipose tissue produced conflicting results.[91,94–96] More research is required to ascertain whether a balance of n-3 and n-6 in the diet contributes to excessive development of adipose tissue.

3.3. PUFAs and inflammation response

The possible mechanisms in PUFAs modulation of inflammatory response were investigated in a number of studies but the data were often inconsistent. Based on preclinical studies, the underlining mechanisms include transcriptional down-regulation of the production of pro-inflammatory cytokines,[97] cyclooxygenase-2 activity,[98] and vascular surface expression of endothelial leukocyte adhesion molecules.[99] These effects are a consequence of altered gene expression. Animal studies showed that n-3 PUFAs supplementation inhibited the production of pro-inflammatory cytokines IL-1 and TNF.[100] Similar observations were reported in studies in humans. In particular, studies of fish oil supplementation in patients with active inflammation diseases, such as rheumatoid arthritis and Crohn's disease,[100] supported a potentially beneficial anti-inflammatory effect of n-3 PUFAs. Dietary supplementation with n-3 PUFAs in healthy subjects was associated with reduced levels of IL-1, thromboxane 2 and prostaglandin E2,[101,102] but not of C-reactive protein.[103]

A potential protective effect of PUFAs supplementation on the progression of renal disease based on its action on inflammation in the renal fibrosis process, was suggested in studies on animal models.[104] The actions of PUFAs interfere directly with mesangial cell activation and proliferation and extra-cellular matrix protein synthesis, and they are involved in the regulation of pro-inflammatory cytokine production.[105,106] It is possible that PUFAs suppress the activity of the angiotensin-converting enzyme, reduce angiotensin II formation, enhance endothelium nitric oxide generation, and down-regulate the expression of the transforming growth factor-β (TGF-β).[107]

The anti-inflammatory effects of n-3 fatty acids from seafood may contribute to their protective actions towards atherosclerosis, plaque rupture and cardiovascular mortality. In inflammatory bowel diseases, some trials reported improved gut histology, duration of morning stiffness, global assessments of pain, decreased disease activity, use of corticosteroids and relapse.[108] However, the therapeutic dose of n-3 PUFA has not yet been established.[109] For instance, reduction of pro-inflammatory eicosanoids and cytokines could be achieved with an intake of 2–4 g day^{-1} of 84 % EPA+DHA ethyl esters.[110] In the inCHIANTI study,[111] the intake of 7 g day^{-1} PUFAs led to higher plasma levels of AA and n-3 PUFA (mainly DHA) and these FA profiles were independently associated with lower levels of serum pro-inflammatory markers. Thies *et al*.[112] reported that a dietary supplementation with moderate amounts of long-chain n-6 or n-3 PUFAs neither significantly affected inflammatory cell numbers nor neutrophil and monocyte responses.

3.4. PUFAs and oxidative stress

Both n-3 and n-6 PUFAs are highly susceptible to oxidation because of their multiple double bonds. This lipid peroxidation, leading to pro-inflammatory oxidised LDL and HDL, is highly suspected of contributing to the pathogenesis of atherosclerosis. Several studies showed that dietary supplementation of n-6 PUFAs increased the extent of LDL oxidation *in vitro* compared with a diet enriched in mono-unsaturated FA.[113] In contrast, markers related to LDL oxidation *in vitro* or malondialdehyde derived from LDL showed no correlation with n-6 PUFA intake in a group of healthy volunteers.[114] Furthermore, a double-blind controlled intervention in a cohort of healthy men showed that fish oil consumption combined with a high LA intake (21 g day^{-1}) did not raise the plasma level of oxidised LDL compared with the same fish oil consumption but combined with a low level of LA.[115] Parameters of oxidative stress were significantly improved after fish oil supplementation in an animal study.[79] EPA and DHA have beneficial effects in glomerular disease, which are attributed to their effect on the pro-oxidant and antioxidant status and EFA metabolism, as reviewed by Das.[107] However, recent evidence does not support the idea that n-3 PUFAs up-regulate oxidative stress. Further investigations would enable more definitive conclusions to be made.

3.5. PUFAs and blood pressure and mortality

As highlighted by a review of cross-sectional studies, an increase in the dietary intake of n-6 PUFAs is often associated with a decrease in blood pressure.[116] It was also reported that plasma levels of LA were inversely associated with systolic and diastolic blood pressures.[117] Combining the results from different studies in a meta-analysis, Morris *et al*.[118] found that at supraphysiological

doses of 5.6 g n-3 PUFAs in hypertensive subjects, systolic pressure was reduced by 3.4 and diastolic pressure by 2.0 mmHg. Possible mechanisms include modulation of the biosynthesis of eicosanoids: hydroxyeicosatetraenoates or epoxyeicosatrienoates.[119] A more recently published meta-analysis also supported the antihypertensive effects of n-3 PUFAs.[120] Similar findings were obtained in animal models and cell culture studies, which indicate that n-3 PUFAs supplementation can lower blood pressure and proteinuria, potentially by the vasorelaxation action of n-3 PUFAs with increased endothelium-derived releasing factor[107] and by having effects on TGF-β, renin, fibronectin and nitric oxide synthesis.[104]

Another meta-analysis of 25 case–control studies was performed by Harris *et al.*[121] in order to assess the association between the tissue contents of n-3 and n-6 PUFAs and CHD events. They found that content of LA in tissue was significantly decreased in patients with CHD events. Similar results were found in a study by Block *et al.*[122] on the relation between acute coronary syndrome and the fatty acid content of whole-blood cell membranes. The renal, cardiovascular and reduced mortality benefits of n-3 fatty acids are still areas of active investigation. Kutner *et al.*[123] in a prospective cohort study showed that dialysis patients with a high intake of fish live longer, with an approximately 50 % lower rate of mortality over 3 years. Regarding the role of n-3 PUFAs and CVD, a randomized clinical trial by Svensson *et al.*[124] found that compared with placebo groups, patients receiving n-3 PUFAs supplementation (1.7 g day^{-1}) had a protective effect on the rate of myocardial infarctions but led to no improvement in the primary end point of total cardiovascular events and death, with a follow-up of 2 years. The same authors reported that there was no change in heart rate variability in haemodialyzed patients during 8 weeks of n-3 PUFA supplementation at a dosage of 1.7 g day^{-1}.[125] A recently published controlled study by Kirkegaard *et al.*[126] showed an inverse association between the presence of arterial fibrillation and plasma DHA. This is very important because high risk of sudden cardiac death is often caused by arrhythmias. Finally, a new meta-analysis of 20 clinical studies[127] looking at the effects of n-3 PUFAs in patients at high risk for cardiovascular events showed that the supplements had no effect on hard clinical outcomes, including all-cause mortality, cardiac death, sudden death, myocard infarction or stroke. In the future, better-powered studies would need to be conducted to resolve the relationship between n-3 PUFAs status and the mortality risk.

3.6. PUFAs and haematological parameters

The effect of fish oil supplementation and n-3 PUFAs on red blood cell deformability and aggregation has also been investigated.[128,129] Findings from these studies suggest that n-3 PUFAs have antithrombotic, antiproliferative and anti-aggregatory platelet effects.[130] These FAs can influence gene regulation by down-regulating gene expression of platelet-derived growth factors and suppress the platelet activating factor, a potent platelet aggregator and leukocyte acti-

vator.[81,131] n-3 DPA can be metabolised by lipoxygenase in platelets, to form 11-hydroxy-7,9,13,16,19- and 14-hydroxy-7,10,12,16,19-DPA. It was also reported that n-3 DPA is effective (more than EPA and DHA) in the inhibition of aggregation in platelets obtained from rabbit blood.[132] The results from human studies are not conclusive, and further investigations are required to clarify the role of n-6 PUFAs in susceptibility to thrombosis.

3.7. PUFAs and cancer

Data from epidemiological studies suggest that diets rich in n-6 PUFAs may be associated with cancer risk. Studies on patients with cancer at different sites have shown a poor n-3 FA status due to suboptimal intakes and possible metabolic disturbances. Low proportions of n-3 PUFAs in plasma and/or erythrocytes phospholipids were found in pancreatic, lung and prostate cancer, and non-Hodgkin lymphoma.[61,133,134] All n-3 PUFAs were shown to be particularly depleted in advanced cancer patients, during chemotherapy and in cancer patients close to death. Additionally, low plasma n-3 fatty acids were associated with loss of skeletal muscle in these patients.[135]

Considering all these findings, supplementation with EPA and DHA in patients suffering from cancer was the objective in many trials. Long chain n-3 PUFA have inhibitory effects in tumour formation, probably through alteration of prostaglandins synthesis and inhibition of cell proliferation in colon and breast cancer.[136] A beneficial effect of n-3 supplementation throughout antineoplastic therapy was confirmed through weight, lean body mass and treatment outcomes. In patients with pancreatic cancer, fish oil supplementation may prevent cachexia.[137] In contrast, n-6 PUFAs have been associated with a greater capacity to induce tumour formation.[136] As mentioned above, Western diet contains disproportionally high n-6/n-3 PUFA ratios, which is thought to contribute to cancer. In favour of this assumption is the proportion of n-6 PUFA in cancer patients, which was found to be very high in patients with non-Hodgkin lymphoma.

The nature of the anti-tumour effects of EPA are not clearly understood, but one of the mechanisms is competitive inhibition of the use of AA for the production of eicosanoids. Eicosanoids derived from AA have been associated with both tumour promotion and progression. EPA is also a potent angiogenesis inhibitor, which suppresses the production of crucial angiogenic mediators, namely: vascular endothelial growth factor, platelet-derived growth factor, cyclo--oxygenase 2, nuclear factor kappa beta and nitric oxide.[138]

4. CONCLUSIONS

In conclusion, PUFAs have important roles in a wide range of physiological and pathologic processes. However, more conclusive relationships between PUFAs and metabolic pathways of insulin resistance, obesity, pancreatic and liver function, diabetic nephropathy, asthma clinical outcomes, mental health and

PUFAs supplementation in cachexia should be established. Future supplementation studies in larger, randomized control trials are required to reveal the full potential of PUFAs in the prevention and therapy of chronic diseases.

LIST OF ABBREVIATIONS

AA   – arachidonic acid
ALA   – α-linolenic acid
CHD   – cardiovascular heart disease
DGLA – dihomo-γ-linolenic acid
DHA   – docosahexaenoic acid
DPA   – docosapentaenoic acid
EFA   – essential fatty acids
EPA   – eicosapentaenoic acid
FA   – fatty acid
HDL   – high density lipoprotein
LA   – linoleic acid
LDL   – low density lipoprotein
PPAR   – proliferator-activated receptors
PUFA   – polyunsaturated fatty acid
SFA   – saturated fatty acid
TGF   – transforming growth factor
TNF   – tumour necrosis factor
VLDL – very low density lipoprotein
E%   – percentage of energy

И З В О Д

ПОЛИНЕЗАСИЋЕНЕ МАСНЕ КИСЕЛИНЕ У ЗДРАВЉУ И БОЛЕСТИ

ДАНИЈЕЛА РИСТИЋ-МЕДИЋ, ВЕСНА ВУЧИЋ, МАРИЈА ТАКИЋ, ИВАНА КАРАЏИЋ и МАРИЈА ГЛИБЕТИЋ

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Полинезасићене масне киселине (ПМК) су неопходне за нормално функционисање организма. Две ПМК фамилије, n-6 и n-3 масне киселине се физиолошки и метаболички разликују. Удео ПМК у фосфолипидима серума и еритроцита је важан показетељ здравља и болести, и зависи од ендогеног метаболизма, који је контролисан генетским полиморфизмом, и уноса хране. И n-6 и n-3 ПМК су прекурсори за синтезу еикозаноида на циклооксигеназном и липооксигеназном нивоу. Опсервационе и интервентне студије указују да n-3 ПМК имају кардиопротективни ефекат, делујући анти-инфламаторно, анти-аритмогено, хиполипидемично и антихипертензивно. Насупрот томе, сматра се да n-6 ПМК имају про-инфламаторно дејство. Низак ниво n-3 и повишен удео n-6 ПМК је показан код пацијената са различитим типовима малигнитета. У оквиру овог рада дат је преглед најновијих сазнања о дијетарном уносу и биомаркерима статуса ПМК у промоцији здравља и превенцији болести, са посебним освртом на резултате у нашој популацији.

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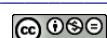
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