Research Article

Omega-3 fatty acid supplementation candidates can be selected using fatty acid profiling

Jan Galuszka[1], Jitka Vostalova[2], Blanka Cervena[3], Dana Galuszkova[4], Vilim Simanek[2], Michal Holcapek[3], Eva Cifkova[3], Miroslav Lisa[3], Jana Vrbkova[5], Martina Bancirova[2], Milos Taborsky[1] and Dalibor Novotny[6]

[1] Department of Internal Medicine I—Cardiology, University Hospital Olomouc, Olomouc, Czech Republic
[2] Department of Medical Chemistry and Biochemistry, Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic
[3] Department of Analytical Chemistry, University of Pardubice, Pardubice, Czech Republic
[4] Department of Transfusion Medicine, University Hospital Olomouc, Olomouc, Czech Republic
[5] Institute of Molecular and Translational Medicine Department, Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic
[6] Department of Clinical Biochemistry, University Hospital Olomouc, Olomouc, Czech Republic

The aim of this study was to evaluate the fatty acid (FA) profile in the plasma and erythrocyte membranes of apparently healthy men compared to male patients with various cardiovascular diseases (CVD). The plasma FA profiles were analyzed in five groups of subjects aged 37–57 years: groups 1 and 2 were male voluntary blood donors with a body mass index (BMI) 25.1 ± 1.6, (Group 1, $N = 12$) and a BMI 32.9 ± 4.5, (Group 2, $N = 11$) respectively, male patients with chronic heart failure due to dilated cardiomyopathy (Group 3, $N = 10$), chronic atrial fibrillation without heart failure or coronary heart disease (Group 4, $N = 10$) and patients after myocardial infarction (Group 5, $N = 11$). The FA profile in plasma and erythrocytes was measured by gas chromatography (GC) with flame ionization detection and basic plasma lipid parameters were then estimated. Deficiencies of eicosapentaenoic acid and docosahexaenoic acid were found in all the groups of CVD patients and in Group 2 (BMI 32.9 ± 4.5) of this Central European population. Unfavorable alterations in FA composition and clinical biochemistry parameters were found in patients with dilated cardiomyopathy (Group 3). Further, 75% of Group 1 (blood donors) had an average plasma PUFA level in the worst quartile for relative risk of cardiovascular events. Tailored n-3 PUFA supplementation should be recommended not only for secondary prevention in patients suffering from CVD but also for primary prevention in overweight and obese persons with a proven deficiency.

Practical applications: Plasma and erythrocyte membrane n-3 polyunsaturated fatty acid (PUFA) levels are an important parameter of diet composition as well as a marker of cardiovascular risk. Regular sea fish intake or alternative supplementation with the n-3 PUFA recommendation is in all probability insufficiently realized, particularly in inland countries. In addition to contemporary guidelines and practice, research is providing data on the use of FA analysis for revealing people with an n-3 PUFA deficiency. This approach could benefit people suffering from CVD as well as for overweight and obese populations with demonstrated deficiency.

Keywords: Cardiovascular diseases / Lipidomics / n-3 PUFA / n-6 PUFA / Obesity

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Correspondence: Dr. Jan Galuszka, Department of Internal Medicine I—Cardiology, University Hospital, I. P. Pavlova 6, Olomouc, 775 20, Czech Republic
E-mail: jan.galuszka@fnol.cz
Fax: +420 588442500

Abbreviations: AIP, atherogenic index of plasma; **ApoA1,** apolipoprotein A1; **ApoB,** apolipoprotein B; **BMI,** body mass index; **CRP,** C-reactive protein; **CVD,** cardiovascular diseases; **DHA,** docosahexaenoic acid; **DPA,** docosapentaenoic acid; **EPA,** eicosapentaenoic acid; **FA,** fatty acids; **FAME,** fatty acid methyl esters; **GC,** gas chromatography; **HDL,** high-density lipoprotein; **LDL,** low-density lipoprotein; **Lp(a),** lipoprotein(a); **PUFA,** polyunsaturated FA; **TAG,** triacylglycerols

1 Introduction

Lipid metabolism research is closely linked to the medical condition of arterial atherogenesis. Atherosclerosis is a chronic disease involving the vascular wall, causing target organ damage in advanced stages, and atherosclerotic CVD remains the leading cause of premature death worldwide [1].

Over time, numerous different risk factors for CVD have been defined and recently summarized in the guidelines of the European Society of Cardiology. Besides such factors as smoking, sedentary lifestyle, lipid and glucose metabolism disturbance, blood pressure, increased BMI and nutrition are also mentioned [1]. The latter is an increasingly acknowledged factor in chronic non-infectious disease development and atherogenesis in particular.

In this regard observations of great importance were the link between diet and blood lipid composition. The relationship between eating habits and serum lipids in Danes and Greenland Inuits has been studied among the first. A hypotriglyceridemic effect of n-3 PUFA eicosapentaenoic (EPA, 20:5) and docosahexaenoic (DHA, 22:6) acids were revealed, DHA and EPA blood levels are now suggested biomarkers of fish consumption as well as a marker of cardioprotection [2]. Advances in diagnostic technologies in the last two decades, specifically mass spectrometry based-analysis, have enabled the development of a new discipline—lipidomics, the systematic study of the quantity, structure, and function of lipids [3]. Tissue and blood FA composition is related to dietary habits as well as the quality and quantity of dietary fat. FA status in blood and tissues is now an important aspect of a complete strategy for prevention/modulation of CVD development. In summary, n-3 PUFAs participate in varying measure in the following: decreasing the synthesis of mitogens and cytokines, whole blood viscosity, platelet aggregation, postprandial lipemia, albumin leakage, reduction of intimal hyperplasia, reduction of vasospastic response to vasoconstriction, blood pressure, and cardiac arrhythmias. Further in contrast, n-3 PUFA also stimulate endothelial production of nitric oxide, increase platelet survival, bleeding time, arterial compliance, cardiac beta-adrenergic receptor function, and post-ischemic coronary blood flow [4, 5]. The pleiotropic biological effects of n-3 PUFA has interdisciplinary overlap with CVD, from blood homeostatis, in prenatal medicine, psychiatry, neurology, rheumatology, and oncology to metabolic disturbances (e.g., metabolic syndrome) [4, 6]. We now have data on the links between dyslipidemia, life-style and CVD from epidemiological studies [7–9], interventional studies on diet [10], and various sorts of hypolipidemics in primary [11] and secondary prevention [12]. Moreover, from the pathophysiological viewpoint, n-3 PUFA act in synergy with various cardiovascular medications, e.g., antihypertensive drugs and antiplatelet agents [4, 5]. The current state of knowledge on n-3 PUFA benefits is expressed in the conclusions of the European Society of Cardiology guidelines for the management of ventricular arrhythmias and sudden cardiac death, atrial fibrillation, dyslipidemias, and CVD prevention. [1, 13–15]. Lipidomic technology offers new possibilities for understanding, lipid participation in CVD development [3].

The aim of this study was to assess the relationship between plasma lipid parameters, plasma and erythrocyte FA composition in men suffering from different types of CVD (dilated cardiomyopathy, atrial fibrillation/flutter, coronary heart disease), asymptomatic but overweight to obese persons (BMI 28.4–37.4), and a reference group of asymptomatic men with normal weight to overweight (BMI 23.5–26.7).

2 Materials and methods

It was conducted according to the guidelines of the Declaration of Helsinki and all the procedures involving human subjects were approved by the Ethics Committee of the University Hospital and Faculty of Medicine and Dentistry, Palacky University in Olomouc, Czech Republic. The participants signed an informed consent before the start of the study. Recruitment took place from November 2011 to February 2012 at the Department of Internal Medicine I—Cardiology and the Department of Transfusion Medicine, University Hospital in Olomouc. A total of 54 non-alcohol dependent men aged 37–57 years were invited to participate in the study. Voluntary blood donors represented apparently healthy persons and were divided into two subgroups according to their BMI: 23.5–26.7 (Group 1, $N = 12$) and 28.4–37.4 (Group 2, $N = 11$). The original intention was to put together a reference group of voluntary blood donors with a BMI below 25, but there were small weight changes from the time of selection to randomization in Group 1 and hence the BMI ranged from normal to a little overweight. Asymptomatic persons with markedly increased BMI (Group 2) represent a transition between health and disease because increased BMI is a recognized risk factor for CVD [2]. Men suffering from CVD were divided according to diagnosis: into patients with chronic heart failure caused by dilated cardiomyopathy with a left ventricle ejection fraction $<35\%$ (Group 3, $N = 10$); patients suffering from a chronic form of atrial fibrillation/flutter (arrhythmias) without chronic heart failure or coronary heart disease with a left ventricle ejection fraction $>35\%$ (Group 4, $N = 10$) and patients with coronary heart disease after myocardial infarction with a left ventricle ejection fraction $>35\%$ (Group 5, $N = 11$). The patients groups represent different types of CVD involving lipid participation in problems associated with cardiolipotoxicity (Group 3), arrhythmogenesis (Group 4), and atherogenesis (Group 5). Exclusion criteria included diabetes mellitus types I and II, organ transplantation, cardiologic surgical events, chronic inflammatory diseases or chronic inflammatory status, immunosuppressive therapy, significant impairment in renal or hepatic function

Table 1. Baseline characteristics of subjects

Parameters	Units	Group 1 ($n=12$)	Group 2 ($n=11$)	Group 3 ($n=10$)	Group 4 ($n=10$)	Group 5 ($n=11$)
Age[a]	year	44.8/46.5/49.3	43.0/44.0/48.5	51.0/51.5/52.8*	48.8/52.5/53.0	49.5/53.0/53.5*
BMI[a]	kg/m^2	24.4/25.1/25.9	31.1/32.3/34.0*	24.4/26.3/32.1	26.2/27.8/30.8*	29.1/30.3/33.1*
Smoking	%	25	18	30	10	36
Hypertension	%	0	0	50	70	81
Arrhythmias	%	0	0	70	100	9
Lipid lowering therapy	%	0	0	20	20	100

[a]Data are expressed as 1st quartile/median/3rd quartile.
*$p < 0.05$.

(creatinine above 150 μmol/L, transaminases twice the upper normal limit), neurological or active musculoskeletal disorders, positive serologic test for infections (hepatitis types B and C, human immunodeficiency virus, syphilis), use of psychotropic medication, and use of fish oil dietary supplements. Lipid lowering medication (statins) was used by 20% of the participants in Groups 3 and 4 and in 100% in Group 5 based on the recommendations of the guidelines [15].

2.1 Health investigation and blood sampling

The following were routinely assessed during the health examination: (i) detailed medical history; (ii) assessment of all concurrent medication, treatments, and dietary habits; (iii) anthropometric measures (weight, height, BMI); (iv) physical, electrocardiographic, and echocardiography examinations for assessment of heart compensation; (vii) vital signs including pulse rate, systolic, and diastolic blood pressure; and (vii) blood collection.

2.2 Clinical biochemistry

Basic biochemical parameters were determined in all samples immediately after sampling: total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triacylglycerols (TAG), apolipoprotein A1 (ApoA1), apolipoprotein B (ApoB), lipoprotein(a) (Lp (a)) and C reactive protein (CRP, assessed by more sensitive assay) using a HITACHI Modular Evo P analyzer (Hitachi, Japan).

2.3 Lipid analysis

Preparation of fatty acid methyl esters (FAME) for gas chromatography (GC). Heparin-lithium blood was centrifuged in order to isolate plasma and erythrocytes. Erythrocytes were washed three times with phosphate buffered saline (pH = 7.4). For preparation of FAMEs, the plasma or erythrocytes (100 μL) were mixed with sodium methoxide (0.25 M) in methanol (1600 μL) and the samples were kept in a water bath (65 °C, 10 min). Hexane (1 mL) and NaCl (1 M; 1 mL) were then added. The FAMEs extracted from the mixture using hexane were used for GC analysis.

GC conditions: For the FAME separation, the GC with flame ionization detection Agilent 7890 (Agilent Technologies) and TR-FAME column (60 m × 0.25 mm × 0.25 μm, Thermo Scientific) was used. The flow rate of

Table 2. Selected biochemical markers of participants

Parameters	Units	Group 1 ($n=12$)	Group 2 ($n=11$)	Group 3 ($n=10$)	Group 4 ($n=10$)	Group 5 ($n=11$)
IL-6	[μg/L]	1.50/1.50/1.53	1.50/1.70/2.50	1.78/3.45/4.08*	1.50/1.60/2.18	1.50/1.60/1.90
CRP	[mg/L]	0.40/0.65/1.28	1.03/1.55/2.05	0.70/1.20/3.15	0.98/1.60/4.08	0.90/1.30/2.10
Chol	[mmol/L]	4.75/5.36/5.63	4.91/5.28/6.01	4.74/5.19/5.71	5.00/5.60/7.23	3.80/4.15/4.37*
TAG	[mmol/L]	0.86/0.95/1.28	1.02/1.28/1.97	1.42/1.87/2.74*	0.92/1.25/2.52	0.97/1.32/1.93
HDL	[mmol/L]	1.40/1.52/1.70	1.12/1.29/1.44*	1.05/1.35/1.49	1.19/1.29/1.64	1.13/1.15/1.21*
LDL	[mmol/L]	2.58/3.21/3.66	2.95/3.46/3.81	2.38/2.87/3.14	3.32/3.51/4.65	2.15/2.34/2.49*
AIP		−0.21/−0.18/−0.08	−0.08/0.08/0.23*	0.00/0.16/0.46*	−0.23/−0.06/0.31	−0.11/0.09/0.27*
ApoA1	[g/L]	1.57/1.73/1.85	1.38/1.60/1.69	1.56/1.60/1.65	1.46/1.57/1.67	1.43/1.54/1.62*
ApoB	[g/L]	0.82/0.96/1.06	0.94/1.07/1.14	0.86/0.96/1.21	1.00/1.05/1.54	0.77/0.84/0.92
Lp(a)	[g/L]	0.09/0.23/0.43	0.14/0.25/0.53	0.09/0.35/0.37	0.16/0.38/0.82	0.27/0.43/0.96

Data are expressed as 1st quartile/median/3rd quartile.
*$p < 0.05$.

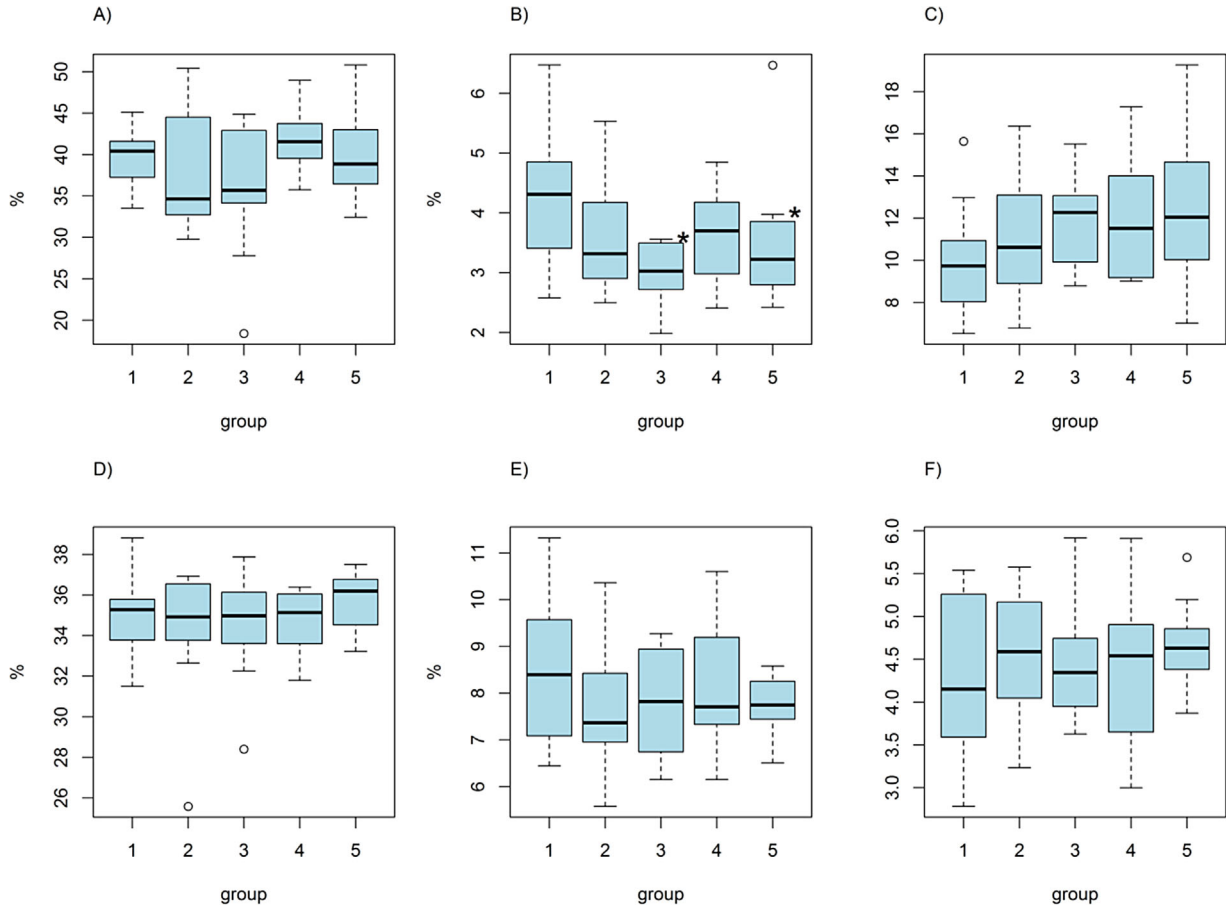

Figure 1. Distribution n-6 PUFA (A), n-3 PUFA (B), and n- 6/ n-3 PUFA ratio (C) in plasma and n-6 PUFA (D), n-3 PUFA (E), and n-6/ n-3 PUFA ratio (F) in erythrocyte in reference (Group 1) and obese (Group 2) participants and patients suffering CVD (Groups 3–5). n-6 PUFA include linoleic, gamma-linolenic, eicosadienoic, dihomo-gamma-linolenic, arachidonic, adrenic, and 4,7,10,13,16-docosapentaenoic acids. n-3 PUFA include alpha-linolenic, eicosapentaenoic, 7,10,13,16,19-docosapentaenoic and docosahexaenoic acids. Box plots were used as graph illustrations of the significant differences between the groups. *p-value < 0.05 (Wilcoxon Exact Rank Test).

helium as a carrier gas was 1.025 mL/min. The temperature program was as follows: the initial temperature was 160 °C, ramped up to 210 °C at 2 °C/min and then ramped up to 235 °C at 22 °C/min. The injection volume was 3 μL and the split ratio was 1:15. The injector and detector temperature were 250 °C and 280 °C, respectively. Individual FAME were identified based on retention time of identical standards.

Table 3. Baseline of plasma n-3 PUFA levels in relation to risk of sudden death from cardiac causes

Relative risk quartile	1	2	3	4
n-3 PUFA[a] (%)	2.12–4.32*	4.33–5.19*	5.20–6.07*	6.08–10.20*
Distribution		(%)		
Group 1 ($n = 12$)	75	8	16	0
Group 2 ($n = 11$)	91	0	9	0
Group 3 ($n = 10$)	100	0	0	0
Group 4 ($n = 10$)	80	20	0	0
Group 5 ($n = 11$)	91	0	9	0

EPA, eicosapentaenoic acid; DHA, docosahaxaenoic acid; DPA, docosapentaenoic acid.
[a]n-3 PUFA (sum of EPA, DHA, DPA).
*Albert, C.M. et al. [23].

2.4 Statistical analysis

The data were analyzed using the two-sample Exact Wilcoxon test to evaluate the compliances in the distribution of the parameters between Groups 2–5 and Group 1. The level of significance was set at 5%. The values are presented as the 1st quartile/median/3rd quartile. Box plots were used as a graphic illustration of significant differences between the groups.

3 Results and discussion

The baseline characteristics of participants divided into five groups according to health status are shown in Table 1. Group 1 was used as a reference control group. The subjects in Groups 3 and 5 were significantly older than those in Group 1. This merely reflects the reality that people are generally more diseased (heart failure, myocardial infarction) with increasing age. The significantly higher BMI indices of Groups 2, 4, 5 than Group 1 reflects the situation in the Czech Republic with a high proportion of the population overweight or obese (above 50%). A similar situation can be found in many developed parts of the European Union as well as in the USA [1]. Biochemical markers of participants are shown in Table 2. Total cholesterol was elevated in all groups with the exception of patients after myocardial infarction (Group 5) which was significantly decreased. This could be explained by statin treatment according to the guidelines [16]. There was a significant ApoA1 decrease in the myocardial infarction Group 5 compared with Group 1. This corresponds with the data on the inverse association of Apo A1 with cardiovascular risk. [17]. A significant decrease in antiatherogenic HDL levels in Groups 2 and 5 points to the manifestation of dyslipidemia and a higher risk of CVD. Significant decrease in LDL cholesterol in subjects after

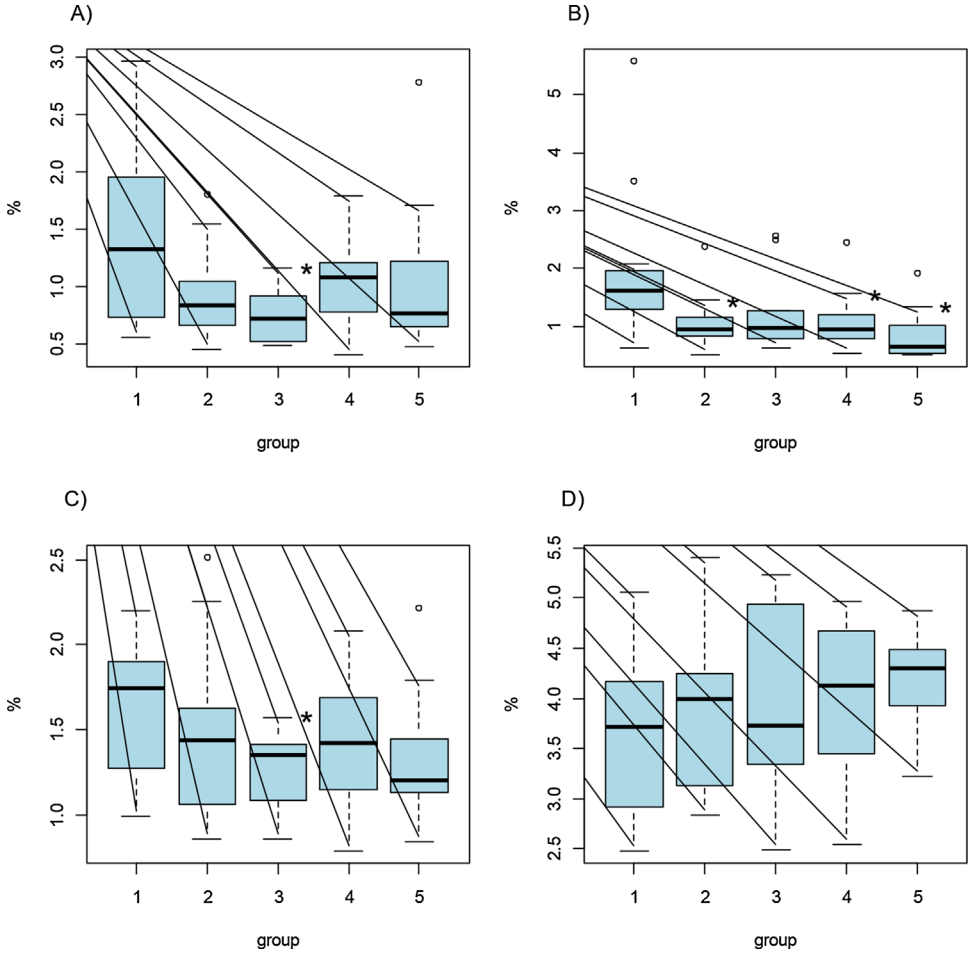

Figure 2. Content of EPA in plasma (A) and erythrocytes (B) and DHA in plasma (C) and in erythrocytes (D) in reference (Group 1) and obese (Group 2) participants and patients suffering CVD (Group 3–5). Box plots were used as graph illustrations of the significant differences between the groups. *p-value < 0.05 (Wilcoxon Exact Rank Test).

myocardial infarction (Group 5) is probably due to the beneficial dyslipidemic modulation of statins. The plasma levels of TAG were significantly increased in Group 3. Triglycerides are a biomarker of cardiovascular risk and parameter of metabolic health, greatly influenced by lifestyle, hormonal milieu, and genetic factors [18]. Elevated TAG are currently considered an additional causal risk factor for cardiovascular disease and all-cause mortality [19]. The highest TAG level in Group 3 may have been partly due to limited physical activity owing to heart failure as data reveal an up to 50% TAG level reduction can be achieved through lifestyle changes [18]. Overweight is frequently associated with increased TAG concentrations [16, 20]. The Food and Drug Administration recommends the use of n-3 PUFA as an adjunct to the diet if TAG exceeds 5.6 mmol/L [15]. There was no evidence of such high plasma TAG levels in any of the participants. The atherogenic index of plasma (AIP) is defined as the logarithm of the molar ratio of TAG to high-density lipoprotein cholesterol and predicts the degree of cardiovascular risk [21]. There were significant increase in AIP in Groups 2, 3, and 5 (Table 2) while only Group 3 reached the criterion of moderate cardiovascular risk. However, based only on a triglyceride analysis, the criterion for n-3 PUFA administration was not fulfilled despite the AIP being increased [15]. The level of pro-inflammatory cytokine IL-6, which promotes the clinical evolution of CVD was only significantly increased in patients suffering from heart failure (Group 3). The significant increase in IL-6 in Group 3 with dilated non-ischemic cardiomyopathy may be caused by heart failure progression and the systemic neurohumoral response [22].

No significant differences in the level of n-6 PUFA in plasma or erythrocytes were found in any group. However, compared to Group 1, plasma n-3 PUFA levels were significantly lower only in subjects with heart failure due to dilated cardiomyopathy (Group 3) and in the myocardial infarction group (Group 5). Both groups represent structural heart diseases. The n-6/n-3 PUFA ratios were non-significantly higher in the plasma as well as in the erythrocytes in Groups 2–5 compared with Group 1 (Fig. 1). The low n-3 PUFA levels signalize increased cardiovascular risk in all groups and particularly among patients with structural heart disease (Groups 3, 5) which correspond with earlier published data on the inverse correlation between n-3 PUFA blood level and risk of sudden death [23]. Further, 75% of participants in control Group 1 had an average plasma PUFA level in the worst quartile for relative risk of cardiovascular events (Table 3). Moreover two participants (one from Group 3 and one from Group 5) had plasma n-3 PUFA levels below the lower limit of the quartile with the highest risk for cardiovascular events.

There was significantly decreased erythrocyte EPA in Groups 2, 4, and 5 and significantly lower plasma EPA in Group 3. Plasma DHA levels were decreased significantly in Group 3 (Fig. 2). Group 3 defined by a history of heart failure and significant depression of the left ventricle ejection fraction primarily represented those participants with the worst prognosis.

Our study revealed an accumulation of unfavorable risk markers: significantly increased IL-6, AIP, TAG, significantly decreased: plasma n-3 PUFA, plasma EPA, erythrocyte EPA. These preliminary results showed that the FA profile analysis together with analysis of inflammatory markers may be a useful approach for individualized therapy adjustment.

4 Conclusions

Deficiency in EPA and DHA was found in all groups of CVD patients as well as in obese persons, and 75% of Group 1 (apparently healthy blood donors) had an average plasma PUFA level in the worst quartile for relative risk of cardiovascular events. The research suggests that FA profiling along with routine cholesterol, TAG and lipoprotein, and inflammatory marker analysis would also be desirable in the higher risk population. This study contributes important information to complete risk assessment. It can be assumed that populations of inland countries have an EPA and DHA intake commonly under the recommended level for cardiovascular health [1]. Our preliminary data on limited groups of patients suffering from specific CVD confirm the importance of individual FA analysis in any proposed n-3 PUFA supplementation strategy.

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The authors declare no conflict of interest.

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