N-3 polyunsaturated fatty acids in the treatment of atherogenic dyslipidemia

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Abstract

BACKGROUND: Atherogenic dyslipidemia contributes substantially to the residual cardiovascular risk. The aim of this study was to examine the effects of therapeutic doses of n-3 polyunsaturated fatty acids on the three major lipid abnormalities of atherogenic dyslipidemia, i.e. hypertriglyceridemia, low HDL cholesterol, and increased levels of small dense LDL particles, as well as on some new risk factors.

MATERIALS AND METHODS: A total of 60 hypertriglyceridemic patients were included in the study. Group S consisted of 36 patients who were already treated with statins, Group N of 24 patients not yet treated. Each patient was examined after six weeks on placebo and six weeks of treatment with n-3 PUFA (eicosapentaenoic and docosahexaenoic acid ethyl esters, 3.0 g/d).

RESULTS: Treatment with n-3 PUFA caused a decrease in plasma triacylglycerols (28%, p<0.001), and VLDL (–27%, p<0.001), an increase in HDL-C (+4%, p<0.01), and a decrease in sdLDL cholesterol (–16%, p<0.05). These changes were accompanied by a decrease in microalbuminuria (–30%, p<0.05), as well as in several parameters of oxidative stress. Analysis of the fatty acids composition of plasma phospholipids showed a significant increase in all n-3 PUFAs examined, accompanied by a decrease in n-6 PUFAs, as well as in monounsaturated acids. No significant differences in the effects of n-3 PUFA were found between the Groups S and N.

CONCLUSION: Our results support the opinion that hypertriglyceridemic patients benefit from the treatment with n-3 PUFA which improves several important metabolic factors of cardiovascular risk.

Abbreviations:

CD-LDL - conjugated dienes in LDL
CRP - C-reactive protein
DHA - docosahexaenoic acid (22:6n-3)
EPA - eicosapentaenoic acid (20:5n-3)
FA - fatty acids
GR - glutathione reductase
GPx - glutathione peroxidase
HbA1c - glycated hemoglobin
HDL - high density lipoproteins
LDL - low density lipoproteins
NEFA - nonesterified fatty acids
PON1 - paraoxonase-1
PUFA - polyunsaturated fatty acids
sdLDL - small dense LDL
TAG - triacylglycerols
TC - total cholesterol
thcy - total homocysteine
VLDL - very low density lipoproteins

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INTRODUCTION

Thanks to increasing experience in the preventive medicine, a close attention has recently been paid to the residual cardiovascular risk, i.e. the risk that persists after a successful intervention of the three classical risk factors – hypercholesterolemia (high LDL-C), arterial hypertension, and cigarette smoking. It is generally accepted that a prevailing role in the residual cardiovascular risk plays atherogenic dyslipidemia, a frequent component of the metabolic syndrome, characterized by hypertriglyceridemia, low plasma HDL-C, and increased proportion of small dense LDL particles (sdLDL). In the long-lasting discussion about the prognostic value of isolated hypertriglyceridemia, the view has now prevailed that even slightly elevated fasting and/or postprandial plasma triacylglycerols are associated with an increased risk of coronary heart disease (Yuan et al. 2007). Low concentrations of HDL-C were shown to be associated with a significant cardiovascular risk in several epidemiological studies (Zhang et al. 2008) and significant correlations were repeatedly found between plasma triacylglycerols, HDL-C, and sdLDL (Cromwell & Otvos 2004). At present, clinical and epidemiological research has also focused on some novel indicators of cardiovascular risk, such as lipoprotein Lp(a), total homocysteine (tHcy), C-reactive protein (CRP), and microalbuminuria (Romanens 2010).

Considerable evidence suggests that polyunsaturated fatty acids of the n-3 series (n-3 PUFA), especially eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) play an important role in the prevention and treatment of atherogenic dyslipidemia (Kremmyda 2011). Beneficial effects of n-3 PUFA on the lipid metabolism have been demonstrated fifty years ago, in connection with the original observations of a low incidence of cardiovascular disease in Inuits, who consumed high amounts of these fatty acids in their food. Clinical research has confirmed that n-3 PUFAs have positive influence on several components of the metabolic syndrome (Carpentier et al. 2006). The cardioprotective effectiveness of n-3 PUFAs added to statins was proved in several interventional studies, e.g. the GISSI-Prevenzione Trial (Marchioli et al. 2002) and the Japanese study JELIS (Yokoyama et al. 2007).

The aim of this study was to examine the effects of therapeutic doses of n-3 PUFA on both the classical and some new risk factors that operate in atherogenic dyslipidemia, as well as on the composition of fatty acids in plasma phospholipids. We have compared two groups of hypertriglyceridemic patients with the metabolic syndrome, those treated and not yet treated with statins.

MATERIALS, AND METHODS

A total of 60 participants were included in the study; they met the IDF criteria for the metabolic syndrome (Alberti et al. 2006) and their fasting plasma triacylglycerols exceeded 1.7 mmol/l. Group S consisted of 36 patients (24 males and 12 females) on statin therapy, Group N of 24 patients (15 males and 9 females) with no statins so far. The exclusion criteria were as follows: insulin dependent diabetes mellitus, age > 75 years, myocardial infarction or stroke in previous six months, chronic heart failure, renal or hepatic failure, obesity grade 2+ (BMI > 35 kg/m²), serious endocrinopathies, pregnancy and breastfeeding. The study was approved by the Joint Ethical Committee of the First Medical Faculty, Charles University, and General Faculty Hospital in Prague. All participants obtained detailed information on the research and signed their informed consent. Basal clinical and biochemical data of the patients are given in Table 1. At the beginning of the six week run-in stage, the participants were asked to follow the NCEP step II dietary recommendations. From the participants, 34 patients were treated for hypertension (19 with ACE inhibitors or sartans, 15 with the combination of ACE inhibitors and calcium channel blockers). The treatment did not change during the study. The study was designed as an add-on double blinded study, each subject served as his/her own control, so that individual responses to placebo and to n-3 PUFA (both administered per os for six weeks) can be compared. The placebo (blistered indistinguishably from active compound) contained oleic acid (75%), palmitic acid (10%) and linoleic acid (9%) w/w and was purchased from the same producer as the Maxicor®. In the experimental period, Maxicor® from SVUS Pharma, a.s., Czech Republic, with (EPA, 63%), (DHA, 23%) and stearidonic acid (18:4n-3, 14%) w/w was given in a daily dose of 3000 mg (1000 mg 3 times a day). The patients in the S group continued with their statin therapy (26 patients atorvastatin 20 mg, 6 patients fluvastatin 80 mg, 4 patients simvastatin 40 mg daily). Clinical and biochemical examinations of all participants were performed at the end of both periods.

Blood samples were obtained after 12 hours of fasting. Total cholesterol (TC), triacylglycerols (TAG), glucose and uric acid in plasma were assessed by enzymatic colorimetric methods, HDL-C in the supernatant after precipitation of apo B containing lipoproteins. VLDL and LDL were separated by sequential centrifugation in isopycnic gradient using the air-cooled ultracentrifuge Beckmann L-55 (Schumaker & Puppione 1986). Subfractions of LDL were analyzed by high-performance gel electrophoresis using polyacrylamide gel tubes (Lipoprint® LDL System, Quantimetric, U.S.). LDL were separated into 7 subfractions of LDL were analyzed by high-performance liquid chromatography (Araki & Sako 1987), nonesterified fatty acids (NEFA) by an
enzymatic colorimetric method (Randox Laboratories, UK). From the values of fasting glucose and insulin, the indexes HOMA-IR, HOMA-B, and QUICKI were calculated. Microalbuminuria was quantified by laser nephelometry (Image MA Reagent Kit, Beckman Coulter Co., U.S.). Activities of the antioxidant enzymes glutathione peroxidase (GPx), glutathione reductase (GR) and paraoxonase-1 (PON1) were determined in erythrocytes (Kodydková et al. 2009). As an indicator of oxidative stress, conjugated dienes in LDL (LDL-CD) were determined (Ahotupa et al. 1996). The content of fatty acids (FA) in plasma phospholipids was analyzed by capillary gas chromatography (Tvrzická et al. 2002).

For statistical analyses, the software STATISTICA CZ ver.7.1 (StatSoft Inc., Tulsa, U.S.) was used. Non-normally distributed variables were transformed logarithmically or by square roots. The significance of differences between groups was assessed with ANCOVA, within groups with paired t-tests. As there were stated many hypotheses in Table 2, we used the Benjamini-Hochberg (exploratory Simes) procedure for adjusting p in multiple test procedure in the Table 2. This allowed us to still reject null hypotheses with p<0.05. Relationships between changes in sdLDL and other variables we analyzed with Pearson (Spearman) correlation coefficients. Statistical significance was defined as p<0.05.

RESULTS

The baseline clinical parameters (the beginning of the run-in period) of the studied groups are presented in Table 1. The basic clinical parameters did not change during the run-in period, except for slight decrease in body weight (group S: 91.5±9.7 kg vs. 88.6±14.9 kg; group N: 86.8±12.8 kg vs. 85.2±13.1 kg, both p<0.05). Further weight changes were not significant.

Main characteristics of the patients at the end of the placebo and active treatment periods are shown in Table 2 separately for Group S (with statins), Group N (no statins), and for all subjects (Group S + Group N). As expected, the treatment with n-3 PUFA caused a substantial decrease in plasma triacylglycerols (−28%, p<0.001) and VLDL-C (−27%, p<0.001). These changes were accompanied by a mild increase in plasma HDL-C (+4%, p<0.01) and sdLDL-C (−16%, p<0.05). The concentration of LDL-C in plasma increased (+8.8%, p<0.01).

Furthermore, we observed a trend toward reduction in plasma concentrations of lipoprotein Lp(a) (−10%). Total homocystein (tHcy) decreased only slightly. However, in a subgroup of 31 patients with basal values of tHcy > 14 μmol/l, mean values of tHcy decreased significantly, from 19.3±5.9 to 17.9±5.7 μmol/l (p<0.01).

Importantly, a significant decrease in microalbuminuria was found in all groups.

The marker of oxidative stress – conjugated dienes in LDL – slightly decreased (−6.3%). From the antioxidant enzymes, we found a higher activity of paraoxonase-1 (+5%, p<0.05), while the activity of glutathione reductase decreased (−11%, p<0.001) and that of glutathione peroxidase did not change. The administration of PUFA n-3 did not interfere with the metabolism of glucose, as could be seen from the unchanged values of plasma glucose, insulin, and Hba1c, as well as of HOMA-IR, HOMA-B, and QUICKI indexes (data not shown).

Analysis of fatty acids in plasma phospholipids showed a significant decrease in the sum of n-6 PUFA (38.70±1.89 vs. 32.77±2.36, p<0.001, placebo vs. PUFAn-3 stage, in mol%) after the treatment with n-3 PUFA, especially a decrease in linoleic acid (18:2n-6, p<0.001), dihomo-γ-linolenic acid (20:3n-6) (3.41±0.66 vs. 2.52±0.60, p<0.001) and arachidonic acid (20:4n-6) (11.95±2.34 vs. 10.26±1.70, p<0.001). We also found a decrease in the content of monounsaturated fatty acids (11.70±1.43 vs. 10.85±1.33, p<0.001), e.g. palmitoleic acid (16:1n-7) (0.53±0.17 vs. 0.46±0.12, p<0.001) and oleic acid (18:1n-9) (9.52±1.24 vs. 8.80±1.03, p<0.001). The content of saturated FAs (44.06±1.05 vs. 45.61±1.66, myristic acid (14:0) and palmitic acid (16:0), was slightly higher (p<0.001). The marked increase in n-3 PUFA (5.54±1.25 vs. 10.77±2.15) was mainly due to a fourfold increase in EPA (1.00±0.65 vs. 3.91±1.31) and an almost twofold increase in DHA (3.48±0.82 vs. 5.31±0.97); the increase in docosapentaenoic acid (22:5n-3, 0.88±0.16 vs. 1.38±0.27) was

| Tab. 1. Baseline clinical and laboratory data of the patients. |
|---------------------|-----------------|-----------------|---|
| number              | S statin treatment | N without therapy | *p-value |
| gender (male/female)| 24/12           | 15/9            | 0.96**  |
| age (years)         | 55.0±9.4         | 48.6±13.0       | 0.047*** |
| body weight (kg)    | 91.5±9.7         | 86.8±14.9       | 0.57    |
| fat mass (% of body weight, bioimpedance) | 30.2±7.0         | 23.4±6.4        | 0.16    |
| waist circumference (cm) | 105±10          | 97±12           | 0.39    |
| TC (mmol/l)         | 5.70±1.40        | 5.57±0.72       | 0.54    |
| TAG (mmol/l)        | 3.61±5.01        | 2.66±1.52       | 0.33    |
| HDL-C (mmol/l)      | 1.21±0.23        | 1.15±0.29       | 0.51    |
| LDL-C (mmol/l)      | 3.14±0.89        | 3.35±1.03       | 0.46    |
| apo A1 (g/l)        | 1.34±0.21        | 1.30±0.25       | 0.68    |
| apo B (g/l)         | 1.19±0.23        | 1.19±0.24       | 0.96    |
| glucose (mmol/l)    | 5.29±0.69        | 5.04±0.81       | 0.43    |
| bilirubin (μmol/l)  | 10.48±4.96       | 13.40±7.73      | 0.19    |

* ANCOVA, age adjusted, ** χ² test with Yates’ correction, *** unpaired t-test; BMI - body mass index
also significant (all p<0.001). All the abovementioned changes were similar in both subgroups. We did not observe the stearidonic acid (18:4n-3) and its immediate metabolite, 20:4n-3, in the analyzed plasma lipid classes, although the used method is able to determine both fatty acids. When comparing the effects of n-3 PUFA, no significant difference could be found between Group S and Group N, with the only exception of tHcy (+1.00±3.28 μmol/l in Group S, -1.34±3.12 μmol/l in Group N, p<0.01).

The correlation analysis applied to the data of all patients revealed several significant relationships. After placebo, plasma triacylglycerols correlated positively with sLDL-C (r=0.623; p<0.001). The decreases in sLDL-C induced by n-3 PUFA correlated positively with the decreases in triacylglycerols (r=0.333; p<0.05), as well as with the placebo values of sLDL-C (r=0.561; p<0.001). No significant correlation was found between the decreases in sLDL-C after n-3 PUFA and the placebo values of triacylglycerols (r=0.244; p<0.075). The decreases in triacylglycerols after n-3 PUFA correlated positively with the placebo values of sLDL-C (r=0.567; p<0.001), but not with the increases in the plasma concentration of n-3 PUFAs (r=0.146; p<0.225).

**DISCUSSION**

The change in body weight observed during the run-in period is probably caused by more strict adherence to dietary recommendations. However, it was not accompanied by the change in plasma TAG. The principal dietary recommendation for control of atherogenic dyslipidemia is decreased energy intake and body weight. This effect was seen in both studied subgroups.
The increased production of VLDL in the liver, especially of the subfraction VLDL-1 (Sf 100-400), plays a key role in the pathogenesis of atherogenic dyslipidemia. In patients with the metabolic syndrome, an excessive flux of fatty acids from the adipose tissue was shown, together with an increase of de novo lipogenesis. The resulting hypertriacylglycerolemia is causally associated with a decreased amount of HDL₂ particles in the circulation, and with a considerable increase in atherogenic LDL particles, especially in sdLDL fraction (Chan et al. 2006b). As a marker of sdLDL, the hypertriacylglycerolemic waist can be used (Gazi et al. 2006). In apparently healthy normolipidemics, sdLDL particles can be found in some individuals (Oravec et al. 2011). While low plasma concentrations of HDL-C are associated with cardiovascular risk, the value of increased HDL-C concentrations cannot be simply interpreted (Sethi et al. 2010).

Several experimental and clinical studies have shown that the lipoprotein metabolism can be beneficially influenced by n-3 PUFA, the natural ligands for several transcription factors (e.g. PPARγ and SREBP-1c). N-3 PUFA improve insulin sensitivity in muscles and adipose tissues (Nakamura et al. 2004), stimulate the expression of lipoprotein lipase, while inhibiting hormone sensitive lipase (Harrir et al. 2009). Moreover, they stimulate mitochondrial and peroxisomal oxidation of fatty acids (Chapman et al. 2011), suppress the secretion of VLDL-1 and accelerate the conversion of VLDL to large LDL particles (Bays et al. 2008; Calabresi et al. 2000), decrease the catabolism of apoprotein AI, and increase the proportion of HDL₂ (Chan et al. 2006a).

Plasma non-esterified fatty acids (NEFA) contribute the largest fraction to VLDL-TAG production in hypertriacylglycerolemic, insulin resistant patients. In these patients an increased delivery of NEFA is caused by diminished FA trapping in adipose tissue and by desinhibition of hormone-sensitive lipase (HSL). Chronic low-grade adipose tissue inflammation, a hallmark of obesity and insulin resistant states, is connected with activation of HSL-mediated lipolysis and increasing lipolytic response to catecholamines. Because plasma NEFA are supposed to be the primary source of FA for VLDL-TAG production, the decrease in plasma NEFA is supposed to be the primary mechanism for the hypertriacylglycerolemic effect of n-3 PUFA. In addition to above-mentioned hypertriacylglycerolemic effects, these FA can counteract the action of cytokines (e.g. TNF-α, IL-6) on HSL-lipolysis by down-regulation of inflammatory cytokines secreted from adipose tissue macrophages (Kalupahana et al. 2011; Shearer et al. 2012).

In this study, we treated hypotriacylglycerolemic patients with n-3 PUFA (EPA + DHA + stearidonic acid ethyl esters) in a daily dose of 3.0 g (less than the usual therapeutic dose of 4.0 g) for 6 weeks. In the above cited GISSI-Prevenzione Trial (Marchioli et al. 2002), n-3 PUFA in a daily dose of 1.0 g was administered for 12 months, in the JELIS Study (Yokoyama et al. 2007), EPA in a daily dose of 1.8 g for up to 5 years. We found a significant decrease in plasma triacylglycerols and VLDL triacylglycerols.

Among the pleiotropic effects of n-3 PUFA, antioxidative, antiinflammatory and antithrombotic properties have been described (Robinson & Stone 2006). An elevated level of oxidative stress can be presumed in all patients with the metabolic syndrome (Poudyal et al. 2011). After the treatment with n-3 PUFA, CD-LDL tended to decrease. Simultaneously, there was an increase in the activity PON1, an antioxidative enzyme associated with HDL, which protects LDL particles from oxidation (Soran et al. 2009). Decreased activity of GR and unchanged activity of GPx implicated a reduced demand for the recycling of glutathione (Kodydková et al. 2009).

Oxidative stress was shown to be causally associated with urinary excretion of albumin. Microalbuminuria as a consequence of increased glomerular permeability frequently occurs in patients with diabetes and/or arterial hypertension. Renoprotective effects of n-3 PUFA have repeatedly been described (Shapiro et al. 2011). In patients with diabetic nephropathy treated with the combination of a statin and fibrate, the supplementation of n-3 PUFA decreased plasma triacylglycerols, homocystein, and microalbuminuria (Zeman et al. 2006).

In a previous study we have shown that patients with the metabolic syndrome have a characteristic fatty acid pattern in the principal plasma lipid classes (Zák et al. 2007). By comparison with healthy subjects, the content of saturated and monounsaturated fatty acids is significantly higher, the content of linoleic acid markedly decreased, probably as a result of oxidative stress. In this study, we found several beneficial effects of the treatment with n-3 PUFA, in the first place a significant decrease of all n-6 acids examined, as well as of the n-6/n-3 ratio. Together with the expected high values of EPA and DHA, the content of several other n-3 acids was also markedly increased. The third supplemented fatty acid, stearidonic acid, as well as its immediate metabolite, 20:4n-3, were not observed in fatty acid profiles. These fatty acid are readily metabolized into EPA in humans (James et al. 2003).

A decreased content of n-3 PUFA (particularly EPA and DHA) in plasma phospholipids and cholesteryl esters was shown to be an independent risk factor for ischemic heart disease. Low concentrations of n-3 PUFA in erythrocytes (less than 4 molar %) are associated with an increased cardiovascular risk, while values higher than 8 molar % are protective (Harris & Jacobson 2009). In our study, the sum of n-3 PUFA in plasma phospholipids more than doubled after the treatment with EPA and DHA. This result gives support to the opinion that hypertriacylglycerolemic patients with the metabolic syndrome
can benefit from the treatment with n-3 PUFA, which improves lipid metabolism and decreases the residual cardiovascular risk.

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Conflict of interest

The authors state that there are no conflicts of interests to be disclosed.

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