Eur J Nutr (2009) 48:1–5
DOI 10.1007/s00394-008-0758-z

REVIEW

Maria Weinkouff Pedersen
Wolfgang Koenig
Jeppe Hagstrup Christensen
Erik Berg Schmidt

The effect of marine n-3 fatty acids in different doses on plasma concentrations of Lp-PLA_2 in healthy adults

Received: 21 April 2008
Accepted: 4 November 2008
Published online: 21 November 2008

M. Weinkouff Pedersen
Prof. E. Berg Schmidt (✉)
Dept. of Cardiology and Center for Cardiovascular Research
Aalborg Hospital,
Århus University Hospital
Aalborg, Denmark
E-Mail: ebs@rn.dk

Prof. W. Koenig
Dept. of Internal Medicine II-Cardiology
University of Ulm, Medical Center
Ulm, Germany

Prof. J. Hagstrup Christensen
Dept. of Nephrology and Center for Cardiovascular Research
Aalborg Hospital,
Århus University Hospital
Aalborg, Denmark

Abstract *Background* Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) is an emerging independent risk factor for cardiovascular disease (CVD). Lp-PLA_2 can be modified by lipid lowering drugs, but it is unknown whether diet can reduce plasma levels of Lp-PLA_2. *Aim of the study* The aim of the trial was to study the effect of marine n-3 polyunsaturated fatty acids (PUFA) on plasma Lp-PLA_2 levels in healthy subjects. *Methods* Sixty healthy subjects were randomized to a moderate dose (2 g) of n-3 PUFA, a high dose (6.6 g) of n-3 PUFA or olive oil (control) daily for 12 weeks. Plasma Lp-PLA_2 was measured at baseline and after the interventions. *Results* Plasma Lp-PLA_2 levels were unchanged in all three groups before and after the supplements. Neither did the results differ between groups. There was no correlation between the content of n-3 PUFA in platelets or granulocytes or plasma Lp-PLA_2. *Conclusion* Marine n-3 PUFA had no effect on plasma levels of Lp-PLA_2 in healthy adults and relatively young people.

Key words omega-3 fatty acids – coronary heart disease – Lp-PLA_2 – fish

Introduction

There is some evidence that fish consumption may decrease the risk of cardiovascular disease (CVD) [18, 26, 27]. This is believed to be due to the content in fish of long-chained n-3 polyunsaturated fatty acids (PUFA), primarily eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), although other components in fish may also contribute [12]. There are several mechanisms by which n-3 PUFA may exert their beneficial effects including an effect on plasma lipids and lipoproteins, effects on blood pressure, platelet and leukocyte reactivity and an antiarrhythmic effect [18, 27]. It is, however, puzzling that even very small amounts of n-3 PUFA (not shown to elicit the above-mentioned beneficial effects on risk factors for CVD) apparently may protect against CVD [13, 14, 18, 27].

The evidence is rapidly emerging that lipoprotein-associated phospholipase A_2 (Lp-PLA_2) is a novel marker for the risk of CVD [1, 7, 10, 19, 25]. We have previously found that the content of EPA in adipose tissue inversely correlated to plasma levels of Lp-PLA_2 in subjects admitted to elective coronary angiography [28]. We here report the effect of dietary supplementation with marine n-3 PUFA in different doses on plasma levels of Lp-PLA_2 in healthy subjects. We also studied the correlation between the content of EPA and DHA in platelets and granulocytes and plasma Lp-PLA_2.

EJN 758

Subjects

Sixty healthy subjects from the medical staff, students and bank employees (25 women and 35 men) with a mean age of 38 years (range 21–57 years) were enrolled in the study. All volunteers were free of medications and without known acute or chronic illnesses. Seventeen of the subjects were smokers (8 women and 9 men).

Design of the study

The subjects were randomized to a supplement of 10 capsules per day for 12 weeks. One group (high dose n-3 PUFA) received 10 capsules of fish oil delivering a total of 6.6 g of n-3 PUFA (3.0 g EPA and 2.9 g DHA), another group (moderate dose n-3 PUFA) received 3 capsules of fish oil (2.0 g of n-3 PUFA) + 7 capsules of olive oil (4.9 g), while the third group (control) was allocated to 10 capsules of olive oil (7.0 g). The oils were given in the form of 10 externally identical capsules, three with the morning meal from one box and seven with the evening meal from another box in order to maintain the blinding of participants. The fish oil used was Pikasol®, a re-esterified triacylglycerol delivered by Pronova Biocare, Norway. Subjects were asked to maintain their habitual diets throughout the trial, but no formal dietary registration was undertaken. Compliance was investigated by determining the relative content of EPA and DHA in platelets and granulocytes at baseline and after the supplements.

The present study was a substudy with the main study focusing on the effect of n-3 PUFA in different doses on heart rate variability [8] and serum levels of high sensitive C-reactive protein[20].

All participants gave informed signed consent. The study was approved by the local Ethics Committee and in accordance with Helsinki Declaration for Ethical Standards.

Blood sampling and analyses

Blood was sampled at baseline and after the supplements in the morning after at least 10 h of fasting. Plasma was frozen at −80°C for later analysis with samples from each subject investigated in the same analytical run.

Plasma lipids and lipoproteins were determined by routine laboratory methods with LDL cholesterol calculated by the Friedewald formula. Apolipoprotein B was measured using antibodies from DAKO on an Advia 1650 (Bayer Diagnostics, NY, USA). Platelet-rich plasma was harvested after centrifugation for 15 min. at 180*g* and then centrifuged for 10 min. at 1,500*g*. The platelets were washed twice in 0.9% sodium-chloride and the pellet resuspended in 0.5 ml sodium-chloride. Granulocytes were harvested from whole blood drawn into K-EDTA. Blood was layered on top of a solution of Hypaque-Ficoll (Nycomed, Oslo, Norway). After discontinuous gradient centrifugation, the granulocytes were harvested into RPMI, red blood cells were haemolysed, and the granulocytes were washed in isotonic phosphate buffer and resuspended in 0.5 ml sodium-chloride [29].

Lipids from platelets and granulocytes were extracted and fatty acids methylated and analysed using a CP-9002 Gaschromatograph equipped with CP-Wax 58 CB capillary column, using temperature programming from 40 to 190°, and constant flow. Results are given at weight % of total fatty acids [29].

Plasma (previously unthawn) was shipped on dry ice to Ulm, Germany, for analysis of Lp-PLA_2 and measured with an ELISA test kit (PLAC^{T}M) supplied by diaDexus Inc. (San Francisco, CA, USA) [15]. The detection limit of Lp-PLA_2 in this assay is 2 ng/ml. The interassay of variation (CV) was below 7%.

Statistical analysis

Differences among the groups (high dose n-3 PUFA, moderate dose n-3 PUFA and control) were tested by one-way analysis of variance. If significant differences were found when comparing the three groups, Tukey's test was applied. Correlations between plasma lipids and lipoproteins, the cellular content of EPA and DHA, and Lp-PLA_2 were tested by simple linear regression analysis using the SPSS software package, version 11.0 (SPSS, Chicago, IL, USA). Bonferroni-corrected correlations were used if multiple correlations were made. A P value < 0.05 (two-tailed) was considered statistically significant.

Results

Before supplementation

The three intervention groups were comparable before supplementation regarding gender, age, body mass index, smoking, and plasma lipids and lipoproteins (Table 1). Univariate correlation analysis revealed positive associations between Lp-PLA_2 and the following parameters: (1) Body mass index ($r = 0.38$, $P < 0.01$), (2) Total cholesterol ($r = 0.29$, $P = 0.02$), and 3) LDL cholesterol ($r = 0.26$, $P = 0.04$). In contrast, Lp-PLA_2 was not significantly associated with the content of EPA or DHA in platelets or granulocytes (Table 2). Baseline levels of plasma

Table 1 Baseline characteristics of subjects in the three intervention groups

	Moderate dose (2.0 g n-3 PUFA)	High dose (6.6 g n-3 PUFA)	Control (olive oil)
Women/men (*n*)	8/12	8/12	9/11
Age (years)	36.5 (11)	37.0 (10)	37.9 (10)
Body mass index (kg/m^2)	24.1 (3)	24.8 (3)	24.6 (4)
Current smokers (*n*)	6	7	4
Lipids and lipoproteins			
Total cholesterol (mmol/l)	5.0 (1.1)	5.1 (0.9)	5.1 (1.2)
LDL cholesterol (mmol/l)	3.0 (1.0)	3.3 (0.9)	3.2 (1.1)
HDL cholesterol (mmol/l)	1.5 (0.4)	1.3 (0.3)	1.3 (0.3)
Triglycerides (mmol/l)	1.2 (0.7)	1.1 (0.6)	1.3 (1.3)
Apolipoprotein B (g/l)	0.8 (0.4)	0.9 (0.3)	0.8 (0.4)

Values are means (SD) or exact figures

Table 2 Univariate correlation coefficients (Spearman's *r*) between Lp-PLA$_2$ and body mass index, plasma lipids and lipoproteins and the content of n-3 PUFA (EPA and DHA) in platelets and granulocytes

	r	*P*
Body mass index	0.38	0.003
Total cholesterol	0.29	0.02
LDL cholesterol	0.26	0.04
HDL cholesterol	−0.05	NS
Triglycerides	0.09	NS
Apolipoprotein B	−0.08	NS
EPA (platelets)	−0.04	NS
DHA (platelets)	0.08	NS
EPA (granulocytes)	−0.01	NS
DHA (granulocytes)	−0.12	NS

NS not statistically significant

Lp-PLA$_2$ were slightly higher ($P = 0.05$) in subjects randomized to control than in the two other groups (Table 3).

Dietary supplementation

The dietary supplements were well tolerated and no subject dropped out of the study. Subjects receiving 2.0 or 6.6 g n-3 PUFA daily for 12 weeks had a highly significant increase in granulocyte and platelet concentrations of EPA and DHA with the largest increase occurring in the 6.6 g group, whereas no changes were observed among controls (Table 3). A decrease in plasma triglycerides was observed after both doses of n-3 PUFA, whereas other plasma lipids and lipoproteins were unaffected by supplementation with n-3 PUFA (previously reported in [8]). Dietary supplementation had no effect on plasma Lp-PLA$_2$ levels in any of the three groups (Table 3), nor were there any significant differences between groups.

Discussion

Lp-PLA$_2$, formerly named platelet activating factor acetylhydrolase (PAF-AH), is produced from macrophages, lymphocytes and mast cells. About 80% is bound in the circulation to LDL, 15–20% in HDL, while the reminder is found in VLDL [7]. Because of its ability to degrade platelet-activating factor the enzyme was previously thought to protect against CVD [19]. Lp-PLA$_2$ remains latent in LDL, but with oxidation of the LDL particle, Lp-PLA$_2$ cleaves the oxidized phosphatidylcholine to lysophosphatidylcholine and oxidized free fatty acids, both proinflammatory compounds [19]. This may, in contrast to early findings, indicate a proatherogenic effect of Lp-PLA$_2$, which is further supported by a number of observations in humans. Thus, Lp-PLA$_2$ activity positively correlated with carotid intima-media thickness in a large population-based cohort [25] and with coronary atherosclerosis in patients with angina pectoris [28]. More importantly, Lp-PLA$_2$ was shown to be an independent risk factor for coronary events in a nested case-control study from the West of Scotland Coronary Primary Prevention Study (WOSCOPS), where there was an adjusted increase in coronary events by 60% in the highest quintile of Lp-PLA$_2$ compared to the lowest quintile [23]. Since then, several other prospective studies in humans

Table 3 The concentration of the n-3 fatty acids EPA and DHA in granulocytes and platelets and Lp-PLA$_2$ before and after supplementation in the three groups

	Moderate dose (2.0 g n-3 PUFA)			High dose (6.6 g n-3 PUFA)			Control (olive oil)		
	Before	After	Difference	Before	After	Difference	Before	After	Difference
n-3 PUFA in granulocytes									
EPA (%)	0.56 (0.5)	1.82 (0.89)	1.24 (0.8)*	0.58 (0.2)	4.07 (1.0)	3.49 (1.0)*	0.51 (0.2)	0.55 (0.3)	0.04 (0.3)
DHA (%)	1.48 (0.4)	1.85 (0.6)	0.36 (0.4)*	1.58 (0.5)	2.14 (0.4)	0.56 (0.4)*	1.54 (0.4)	1.54 (0.5)	0.01 (0.4)
n-3 PUFA in platelets									
EPA (%)	0.72 (0.3)	2.06 (0.6)	1.33 (0.6)*	0.85 (0.3)	4.66 (1.3)	3.81 (1.3)*	0.73 (0.3)	0.74 (0.4)	0.01 (0.2)
DHA (%)	2.42 (0.4)	2.81 (0.4)	0.39 (0.3)*	2.66 (0.5)	3.57 (0.5)	0.91 (0.4)*	2.45 (0.5)	2.40 (0.6)	0.05 (0.4)
Lp-PLA$_2$ (ng/ml)	197 (75)	216 (123)	1 (139)	199 (56)	206 (170)	8 (181)	316 (248)**	277 (222)	39 (200)

Values are mean (SD)
*$P < 0.01$; **$P = 0.05$ (compared to moderate and high dose before)

have reported Lp-PLA$_2$ to be an independent risk factor for CVD [1, 2, 5, 11, 16, 21, 24], although not confirmed by all studies [3, 22].

In WOSCOPS [16], treatment with 40 mg of pravastatin for 1 year was associated with a lowering of Lp-PLA$_2$ by 17% indicating that Lp-PLA$_2$ levels can be modified by statin treatment. Recently, fenofibrate and orlistat, given alone or in combination to obese patients with metabolic syndrome, was also reported to lower Lp-PLA$_2$ activity [9]. Furthermore, specific inhibitors showed a regression of atheroma size in animal models [4]. This was recently confirmed in a placebo-controlled study of 330 patients with angiographically documented coronary artery disease treated with darapladib (an oral Lp-PLA$_2$ inhibitor) or placebo for 12 months [30]. There was no effect on the primary endpoints, plaque deformability or plasma levels of C-reactive protein, but a beneficial change in intraplaque composition with a prevention of necrotic core expansion indicating a reduction in plaque vulnerability.

It is at present unknown whether diet may influence Lp-PLA$_2$, but a candidate for this might be long-chained marine n-3 PUFA, because these fatty acids are incorporated into LDL particles and may affect oxidation of LDL [18]. Also, we have previously reported a correlation (albeit weak) between the content of EPA (but not DHA) in adipose tissue and plasma Lp-PLA$_2$ concentrations in patients admitted to elective coronary angiography for suspected coronary artery disease [28]. However, in the present study, supplementation with a moderate daily dose of 2 g n-3 PUFA or a high dose of 6.6 g n-3 PUFA had no effect on levels of Lp-PLA$_2$. The set-up enabled us to detect a 10% effect of n-3 PUFA supplements on plasma Lp-PLA$_2$ levels with a power of >80%. Furthermore, there was no indication of any correlation between cellular (granulocytes and platelets) incorporation of EPA and DHA and plasma Lp-PLA$_2$.

Body mass index was positively correlated to plasma Lp-PLA$_2$, which has been found by others [2], but not uniformly [11, 16, 23, 25]. In line with previous findings we observed a significant correlation between Lp-PLA$_2$ and plasma total cholesterol and LDL cholesterol [2, 11, 16, 22, 23, 25].

Our study was performed in a limited number of subjects, all healthy and relatively young. We can therefore not exclude an effect of n-3 PUFA on plasma Lp-PLA$_2$ levels in (older) patient groups, by other doses or longer periods of treatment with n-3 PUFA. Still, n-3 PUFA may—despite having no effect on circulating Lp-PLA$_2$ levels—because of their anti-inflammatory effects [6, 29] counteract proatherosclerotic leukocyte activation and inflammatory responses elicited by Lp-PLA$_2$ [31]. Finally, n-3 PUFA may also oppose [32] plaque instability suggested to be promoted by Lp-PLA$_2$ [17, 30, 33].

Further studies evaluating the effect of diet and other lifestyle measures on Lp-PLA$_2$ are warranted.

Conflict of interest There are no conflicts of interest.

Funding No funding has been received.

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