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The effect of low-dose marine n-3 fatty acids on the biosynthesis of pro-inflammatory 5-lipoxygenase pathway metabolites in overweight subjects: A randomized controlled trial ☆, ☆☆

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ABSTRACT

Introduction: Marine n-3 polyunsaturated fatty acids (PUFA) have a variety of anti-inflammatory properties. This study evaluated the effect of n-3 PUFA in a low, but recommended cardioprotective dosage on the formation of 5-lipoxygenase pathway metabolites in overweight subjects.
Materials and methods: Fifty subjects were randomized to 1.1 g of n-3 PUFA or olive oil for 6 weeks.
Results: Leukotriene B_4 formation decreased by 14% in the n-3 PUFA group which proved to be significant within the group ($p=0.005$) but not between groups ($p=0.25$). The formation of 5-hydroxyeicosatetraenoic acid (5-HETE) did not differ significantly between the groups. In the n-3 PUFA group, both 5-hydroxyeicosapentaenoic (5-HEPE) acid and leukotriene B_5 increased significantly compared to the control group ($p<0.001$).
Conclusion: In conclusion, we did not observe any significant net anti-inflammatory effect on the 5-lipoxygenase pathway from a daily supplement of 1.1 g marine n-3 PUFA for 6 weeks.

1. Introduction

Marine n-3 polyunsaturated fatty acids (PUFA) have been recognized to lower the risk of cardiovascular disease (CVD). In line with this, the American and European Societies of Cardiology have recommended that all patients with CVD should consume 1 g of marine n-3 PUFA daily [1,2]. A variety of mechanisms by which n-3 PUFA may reduce CVD have been proposed, including a lowering of blood pressure, improved endothelial function, lowered triglyceride levels, decreased platelet aggregability and anti-arrhythmic effects. In addition, much attention has been given to the anti-inflammatory actions of n-3 PUFA since atherosclerosis is widely accepted to be an inflammatory disease [3]. Interestingly, n-3 PUFA, in particular eicosapentaenoic acid (EPA), seem to exert some anti-inflammatory actions through the lipoxygenase pathway (Fig. 1) which has recently been linked to the development of atherosclerosis and plaque instability by effects on leukocyte chemotaxis, vascular inflammation and subsequent matrix degeneration [4]. Furthermore, since the 5-lipoxygenase pathway has been identified as a potential causal pathway in subclinical inflammation and insulin resistance mediated by obesity [5], overweight subjects seem to be a highly relevant group for evaluating possible anti-inflammatory mechanisms for the prevention of CVD. Dietary n-3 PUFA is associated with an increase in the EPA-derived Leukotriene (LT) B_5 and a decrease in the far more pro-inflammatory arachidonic acid (AA)-derived LTB_4. However, previous studies have used n-3 PUFA in higher doses than current recommendations for prevention of CVD, and the majority has been carried out in healthy or low-CVD risk subjects and often in the absence of a control group [6–13].

In this study, we investigated whether a supplement of marine n-3 PUFA in a clinically relevant cardioprotective dosage of approximately 1 g daily alters the biosynthesis of LTB_4, LTB_5 and other less known metabolites (5-hydroxyeicosatetraenoic acid (5-HETE) and 5-hydroxyeicosapentaenoic acid (5-HEPE)) from the 5-lipoxygenase pathway in subjects with abdominal obesity. Furthermore, we evaluated whether these changes in biosynthesis were accompanied by changes in the content of EPA, docosahexaenoic acid (DHA) and AA of neutrophil membranes.

2. Materials and methods

2.1. Study design

The study design has been described in detail elsewhere [14]. In short, we conducted a parallel double-blinded randomized controlled trial in which 50 overweight subjects were allocated to

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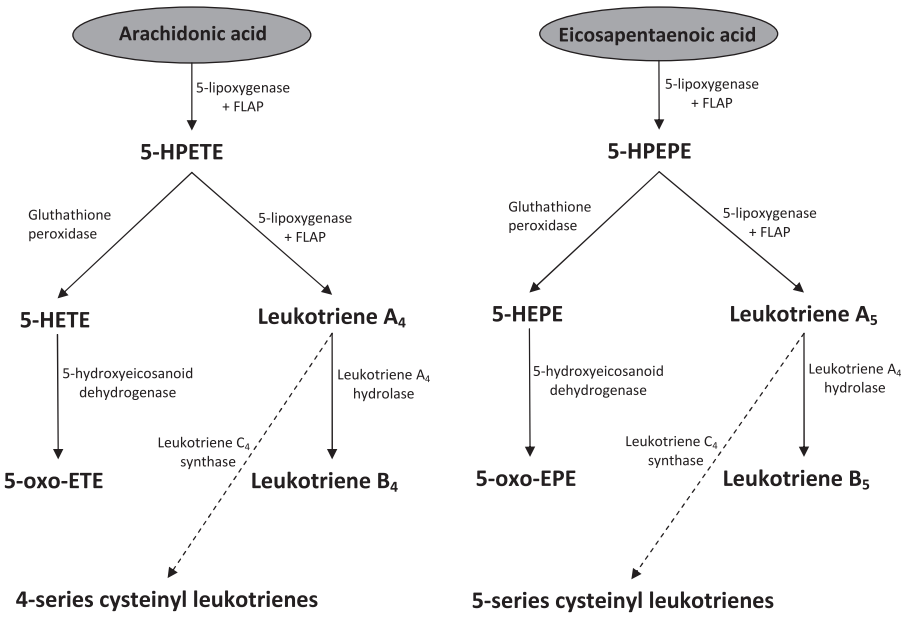

Fig. 1. Lipoxygenase pathway metabolites from arachidonic acid (AA) and eicosapentaenoic acid (EPA). 5-HPETE = 5-hydroperoxyeicosatetraenoic acid, 5-HPEPE = 5-hydroperoxypentaenoic acid, 5-HETE = 5-Hydroxyeicosatetraenoic acid, 5-HEPE = 5-hydroxyeicosapentaenoic acid, 5-oxo-ETE = 5-oxo-eicosatetraenoic acid, 5-oxo-EPE = 5-oxo-eicosapentaenoic acid, FLAP = 5-lipoxygenase activating protein.

a 6-week supplementation with approximately 1.1 g marine n-3 PUFA (640 mg EPA and 480 mg DHA) or 2 g olive oil daily. Blood samples were collected at baseline, after 1 day and after 6 weeks of supplement. All samples were drawn after at least 10 h of fasting. Daily intake of fish was recorded at baseline using food frequency questionnaires. From these data, we calculated the corresponding daily intake of n-3 PUFA using mean values for n-3 PUFA content in fatty and lean fish, respectively [15]. The inclusion criteria were: age 30–75 years, postmenopausal status and waist circumference (WC) $>$ 94 cm for men, $>$ 80 cm for women (identifying subjects being both overweight and having a central fat distribution [16]). The exclusion criteria were any one of the following: a daily intake of anti-inflammatory drugs (except ASA), estrogen/progesterone analogues, acute disease, dysregulated diabetes mellitus (HBA1c $\geq$ 8%) and severe renal insufficiency (GFR $<$ 30 mL/min). Participants taking fish oil supplements were required to undergo a 12-week wash-out period before inclusion. Approval of the study was obtained from the regional research ethics committee, and written informed consent was obtained from all subjects. Compliance was assessed by capsule returns and by neutrophil membrane fatty acid analysis. The study was reported at clinicaltrials.gov (NCT00885053).

2.2. Separation of neutrophils

Neutrophils were separated from anticoagulated (K-EDTA 1.6 mg/mL) blood layered on top of PolymorphprepTM (AXIS-SHIELD PoC AS, Rodeloekka, Norway) and separated by a one-step centrifugation technique at 450*g* for 40 min. Neutrophils were harvested and washed twice in tissue culture medium (RPMI 1640, Sigma-Aldrich, Ayrshire, UK), at ambient temperature and centrifuged for 10 min at 520*g*. Subsequently, neutrophils were counted and red cells eliminated by addition of ice-cold 0.2% saline for 35 s. Next, 1.6% ice-cold saline was added in order to obtain an isotonic 0.9% concentration, followed by centrifugation at 300*g* at 5 °C for 5 min which was repeated once. Neutrophils were then washed in a phosphate buffer containing glucose and human albumin (PBS) and resuspended in the PBS adjusting the concentration to 1×10^7 neutrophils/mL PBS.

2.3. Analysis of fatty acids from neutrophils

Membrane fatty acids of neutrophils were extracted according to the method of Van Kuijk [17]. The cell suspension of 250 μL (1×10^7 neutrophils/mL) was mixed with 1.0 mL dichloromethane and 1.0 mL methanol containing butylated hydroxytoluene (10 mg/100 mL) to avoid oxidation. Next, another 1.0 mL dichloromethane and 1.0 mL water were added, mixed and centrifuged for 4 min at 1800*g*. The lower organic phase was collected, and extraction of the supernatant was repeated. The combined lipid phase was dried under nitrogen at 25 °C. The lipid sediment was dissolved in 250 μL heptane at 50 °C and methylated for 2 min using 12.5 μL 2M potassium hydroxide in methanol according to IUPAC standards [18]. After centrifugation, the heptane phase was collected, and the fatty acid composition was analyzed by gas chromatography using a Varian 3900GC supplied with a CP-8400 auto sampler, flame ionization detector and CP-sil 88 capillary column (Varian, Middleburg, The Netherlands). Split injection mode and temperature programming were employed, and helium was used as carrier gas. Commercially available standards (Nu-chek-Prep, Inc. MN, US) were used to recognize the individual fatty acids. The fatty acid contents were expressed as a percentage of total fatty acids.

2.4. Measurement of leukotrienes, 5-HETE and 5-HEPE

The neutrophil suspension was preheated to 37 °C and stimulation of cells was initiated with $CaCl_2$, $MgCl_2$ and calcium ionophore (A23187) at a final concentration of 10 μmol/L. The reaction was stopped after 10 min by addition of ice-cold ethanol. The mixtures were centrifuged at 4 °C at 700*g* and the supernatant was stored at −80 °C for later analysis. The ethanol mixture was thawed and centrifuged. LTB_4, LTB_5, 5-HETE and 5-HEPE were extracted from the mixture using C18 cartridges (Sep-Pak VAC RC, Waters Co., Milford, MA 01757, US).

Table 1
Baseline characteristics.

	n-3 PUFA, $n=25$	Controls, $n=25$
Sex		
Male	12 (48%)	12 (48%)
Female	13 (52%)	13 (52%)
Age (years)		
	58.0 ± 7.4	55.4 ± 9.5
n-3 PUFA intake (g/day)[a]		
	0.53 (0.11–0.86)	0.47 (0.19–0.81)
BMI (kg/m^2)		
	30.8 ± 4.2	29.5 ± 3.3
Waist circumference (cm)		
Male	108 ± 7.7	106 ± 7.7
Female	100 ± 12.8	95 ± 6.9
Blood pressure (mmHg)		
Systolic	142 ± 19.9	139 ± 14.6
Diastolic	82 ± 9.3	83 ± 8.3
Smoking		
Smokers	5 (20%)	3 (12%)
Non-smokers	20 (80%)	22 (88%)
Medications		
ASA	15 (60%)	3 (12%)
Insulin	1 (4%)	0 (0%)
Oral anti-diabetic	3 (12%)	0 (0%)
β-blocker	13 (52%)	3 (12%)
Other anti-hypertensives	17 (44%)	19 (48%)
Statin	18 (72%)	17 (68%)
Medical history		
Dyslipidemia	19 (76%)	17 (68%)
Hypertension	10 (40%)	13 (52%)
DM2	4 (16%)	0 (0%)
MI/PCI/CABG	5 (20%)	2 (8%)
Stroke	2 (8%)	2 (8%)
Blood chemistry (fasting)		
P-cholesterol (mmol/L)	5.1 ± 1.3	5.4 ± 1.0
P-LDL (mmol/L)	3.0 ± 1.1	3.3 ± 0.9
P-HDL (mmol/L)	1.3 ± 0.4	1.3 ± 0.4
P-triglycerides (mmol/L)	1.9 ± 1.3	2.0 ± 1.9
P-glucose (mmol/L)	6.3 ± 1.3	5.7 ± 0.5
P-hsCRP (mg/L)	2.3 ± 2.5	2.3 ± 2.5
P-creatinine (μmol/L)	81 ± 22	75 ± 15

Continuous variables are reported as means ± SD and categorical variables as numbers and percentage of each group.
BMI = Body mass index, ASA = Acetylsalicylic acid, DM2 = Diabetes mellitus type 2, MI = Myocardial infarction, PCI/CABG = Percutaneous coronary intervention/coronary artery bypass grafting.

[a] Daily intake of n-3 PUFA is positively skewed and thus reported as medians and interquartile ranges.

Triflouroacedic acid (TFA) was added and Prostaglandin B2 (PGB2) was added as internal standard. The cartridges were conditioned and equilibrated using methylformiate, 100% ethanol and water. The acidified sample mixture was loaded to the cartridge and washed with 15% ethanol, water and hexane, eluted with methylformiate and dried under nitrogen. The sediment was dissolved in the mobile phase (31% H_2O, 27% methanol, 42% acetonithrile and 0.025% trifluoroacetic acid) and analyzed by high pressure liquid chromatography (Dionex Ultimate LPG-3400A) on an Acclaim RSLC 2.1 mm × 100 mm C18 column (Dionex Corporation, Sunnyvale, CA, US). Concentrations were calculated using the internal standard and response factors. The response factors were calculated by analysis of a non-stimulated neutrophil suspension after addition of known amounts of standards of LTB_4, LTB_5, 5-HETE and 5-HEPE as well as an internal standard (PGB2). Samples were extracted and analyzed using high performance liquid chromatography and eventually, the recovery factors were calculated.

2.5. Statistics

Differences between and within the groups were analyzed at baseline, after 1 day, and after 6 weeks of supplement using repeated measures analysis of variance (ANOVA), including multivariable analyses adjusting for age, sex, waist circumference, ASA consumption, beta-blocker use, diabetes and plasma glucose levels. Assumptions of normality were checked and found fulfilled, using Q–Q plots of the residuals. Variance homogeneity requirements were examined by means of scatter plots of residuals against predicted values and Levene's robust test statistic over treatment and time. If outcome variables did not meet the demand for variance homogeneity, we performed repeated measures ANOVA with correction for lack of sphericity. This did not alter any conclusions. Comparison of relative changes in LTB_4 and 5-HETE levels and likewise in LTB_5 and 5-HEPE levels within the n-3 PUFA group was done by analysis of logarithmically transformed data. All model assumptions were then fulfilled. p-Values < 0.05 (two-tailed) were considered statistically significant. The STATA 11 software package (Stata Corp., College Station, TX, USA) was used for all analyses.

3. Results

All results are given in Table 2, but below we only comment on the changes from baseline to 6 weeks of supplement as there were no significant changes from baseline to day 1.

3.1. Subject characteristics

Table 1 summarizes the subject characteristics. Fifty patients were enrolled, but 1 patient (control group) dropped out due to gastrointestinal discomfort. The mean age was 57 years (range 35–72). Subjects were overweight or obese with a mean BMI of 30.2 and mean waist circumference of 108 cm (men) and 97 cm (women). The median daily fish intake was comparable in the two groups. The mean high sensitivity CRP levels were identical in the two groups. Since there were differences between the groups, which theoretically could be associated with the inflammatory status (ASA consumption, diabetes, glucose levels, use of beta-blockers), an analysis adjusted for these covariates together with the pre-study selected covariates was made, but this did not alter the point estimates (data not shown).

3.2. Neutrophil membrane fatty acids

Both EPA and DHA contents in neutrophil membranes increased significantly in the n-3 PUFA group compared to the changes in controls (Table 2). Likewise, membrane AA content decreased significantly in the treatment group compared to the control group.

3.3. Formation of leukotriene B_4 and B_5

Fig. 2 and Table 2 illustrate changes in leukotriene formation in the two groups during the study. After 6 weeks, LTB_4 levels decreased by 35 (14%) ng/10^7 cells (95% CI: −59; −10) in the n-3 PUFA group which was significant within the group ($p=0.005$). The difference between the changes in LTB_4 formation in the groups was, however, not significant ($p=0.26$) since LTB_4 formation decreased (non-significantly) by 15 (6%) ng/10^7 cells (95% CI: −39; 10) in the control group. LTB_5 levels increased significantly by 5 (59%) ng/10^7 cells (95% CI: 4; 6) in the treatment group and decreased non-significantly by 1 (10%) ng/10^7 cells (95% CI: −2; 1)

Table 2
Neutrophil membrane fatty acid content and 5-lipoxygenase metabolites at baseline, day 1 and day 42.

	Baseline		p	Day 1		p^*	Day 42		p^*
	Controls	n-3 PUFA		Controls	n-3 PUFA		Controls	n-3 PUFA	
Membrane fatty acid content									
EPA (%)	0.57 ± 0.06	0.68 ± 0.06	0.19	0.55 ± 0.06	0.68 ± 0.06	0.71	0.59 ± 0.06	1.36 ± 0.06	< 0.001
DHA (%)	1.33 ± 0.09	1.40 ± 0.09	0.59	1.33 ± 0.09	1.42 ± 0.09	0.31	1.32 ± 0.09	1.65 ± 0.09	< 0.001
EPA+DHA (%)	1.90 ± 0.13	2.08 ± 0.13	0.31	1.83 ± 0.13	2.10 ± 0.13	0.39	1.92 ± 0.13	3.01 ± 0.13	< 0.001
AA (%)	13.27 ± 0.20	13.36 ± 0.19	0.74	13.27 ± 0.20	13.38 ± 0.19	0.95	13.38 ± 0.20	12.17 ± 0.19	< 0.001
5-Lipoxygenase metabolites									
LTB_4 (ng/10^7 cells)	240 ± 12	240 ± 12	0.96	245 ± 12	235 ± 12	0.60	226 ± 12	205 ± 12	0.26
LTB_5 (ng/10^7 cells)	8 ± 1	9 ± 1	0.37	8 ± 1	8 ± 1	0.49	7 ± 1	14 ± 1	< 0.001
5-HETE (ng/10^7 cells)	353 ± 17	350 ± 18	0.88	348 ± 17	346 ± 18	0.97	349 ± 17	328 ± 18	0.46
5-HEPE (ng/10^7 cells)	50 ± 6	58 ± 6	0.34	50 ± 6	55 ± 6	0.68	48 ± 6	117 ± 6	< 0.001

Fatty acids are expressed as means ± SE. p-Values are for the hypothesis of no difference between the groups at baseline.
EPA=Eicosapentaenoic acid; DHA=Docosahexaenoic acid; AA=Arachidonic acid; LTB_4=Leukotriene B_4; LTB_5=Leukotriene B_5; 5-HETE=5-hydroxyeicosatetraenoic acid; 5-HEPE=5-hydroxyeicosapentaenoic acid.

* p-Values are for the hypothesis of no difference between the changes in the groups after 1 day and 6 weeks of supplementation.

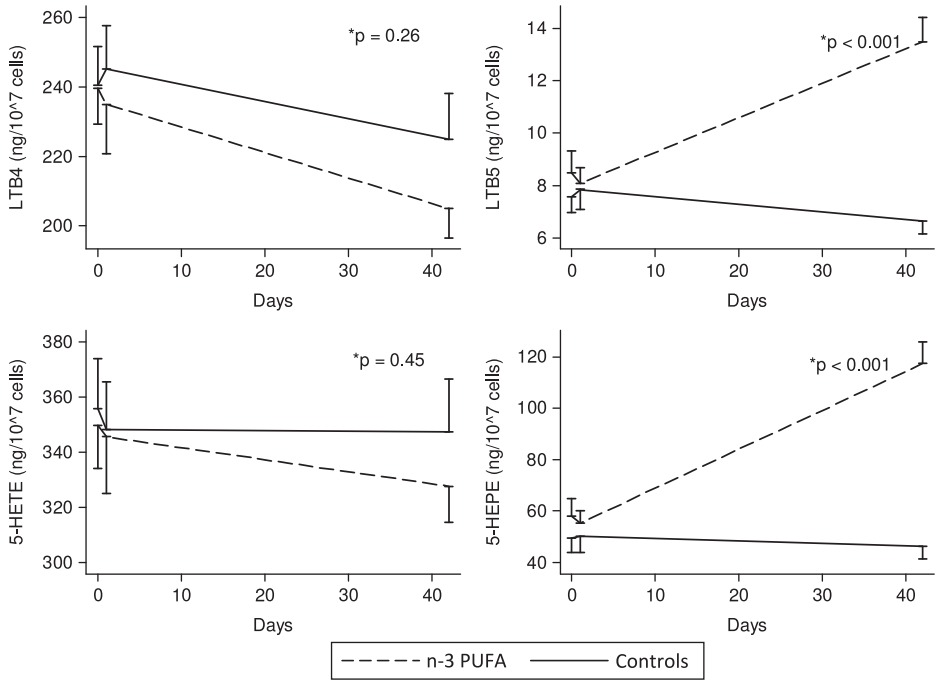

Fig. 2. Formation of 5-lipoxygenase metabolites.Values represent means at baseline, day 1 and after 6 weeks. *p-Values are for the hypothesis of no difference between changes in the groups after 6 weeks of supplementation. LTB4=Leukotriene B4; LTB5=Leukotriene B5; 5-HETE=5-hydroxyeicosatetraenoic acid; 5-HEPE=5-hydroxyeicosapentaenoic acid.

in the control group. The difference between these changes in LTB_5 formation in the two groups was highly significant ($p < 0.001$).

3.4. Formation of 5-HETE and 5-HEPE

The changes in 5-HETE formation were non-significant between the groups after 6 weeks of supplementation (Fig. 2 and Table 2). In the n-3 PUFA group we observed a marked rise in 5-HEPE formation after 6 weeks of supplementation, with a significant difference between the groups (Fig. 2). In the n-3 PUFA group the relative increase in 5-HEPE formation (103%) was significantly ($p=0.008$) higher than the corresponding relative increase in LTB_5 formation (59%). The relative decrease in LTB_4 formation (14%) was larger than the relative decrease in 5-HETE formation (6%), but this difference did not reach statistical significance ($p=0.25$).

4. Discussion

To our knowledge, no previous studies have evaluated the incorporation of marine n-3 PUFA into neutrophil membranes and the concomitant changes in ex vivo LTB_4 and LTB_5 biosynthesis in overweight subjects. Furthermore, we evaluated the formation of two other lipoxygenase pathway metabolites (5-HETE and 5-HEPE), whose biological functions have only been sparsely described. No effect was found after a single dose (acute effect) of marine n-3 PUFA on any measured variable, and results are discussed only with respect to the effect of supplementation for 6 weeks (short term effect).

EPA and DHA were incorporated into the phospholipids of neutrophil membranes during 6 weeks of supplement. This incorporation of n-3 PUFA occurred partly at the expense of AA, whose membrane content decreased in an almost equivalent manner in the n-3 PUFA group. This mechanism of exchange in

membrane PUFA has often been reported [19,20], but far from all studies have in fact found a decrease in membrane AA content but rather a decrease in membrane n-6/n-3 PUFA ratio caused by an increase in n-3 PUFA alone [21]. Since lipoxygenase and cyclooxygenase pathways are initiated at membrane level by cytosolic phospholipase A_2, it is of mechanistic importance for future studies to establish whether membrane AA is, in fact, exchanged for n-3 PUFA.

In order to achieve an anti-inflammatory response mediated by a fish-oil induced change in leukotriene formation, it is probably essential that the formation of the AA-derived LTB_4 is suppressed, because even the EPA-derived LTB_5 has pro-inflammatory capacity, although it is much less pronounced compared to LTB_4 [22,23]. In the current study, we observed a statistically significant 14% decrease in LTB_4 formation within the n-3 PUFA group which, however, failed to remain significant when compared to controls. Concerning LTB_5 formation, we observed a highly significant increase in the n-3 PUFA group compared to controls. This finding confirms that EPA was indeed incorporated into the cell membranes of overweight subjects to an extent in which EPA is used as an alternative substrate for the 5-lipoxygenase pathway. Previous human studies evaluating the effect of n-3 PUFA supplementation on leukotriene formation have reported a significant decrease in LTB_4 formation in the range of 20–70% [6–9,12,24–26] and negative studies are rare [10]. However, these studies are heterogeneous regarding subject characteristics, sample size and n-3 PUFA dosage. It is noteworthy that most studies reporting a significant decrease in LTB_4 formation have a sample size of no more than 22 subjects [6–9,11,12,24,25]. This indicates a strong negative association between n-3 PUFA intake and LTB_4 formation. Since the present study and another study [10], both with larger sample sizes and control groups, did not find significant suppression of LTB_4 levels, this association may be overestimated. There could be several reasons for this. Firstly, many studies are suffering from the lack of a control group [6,7,11,12,24,25] which more easily leads to significant results as demonstrated in the present study, where a significant decrease was seen within the n-3 PUFA group, but not between groups. Secondly, most of the studies used a higher dosage of n-3 PUFA [7–9,11,12,25,26]. Thirdly, publication bias favoring publication of positive results is not unlikely.

In the present study we observed a non-significant 6% decrease in 5-HETE formation in the n-3 PUFA group. Since 5-HETE only possesses 1–2% of the chemotactic activity of LTB_4 in both in vitro and in vivo studies [12,27], this metabolite could appear to be unimportant. However, further metabolism of 5-HETE to 5-oxo-ETE catalyzed by 5-hydroxyeicosanoid dehydrogenase (Fig. 1) may indeed constitute a clinically relevant biological activity as 5-oxo-ETE is 100 times more potent than 5-HETE in activating neutrophils and exerts its actions independently of LTB_4 [28]. Although marine n-3 PUFA may not suppress the biosynthesis of 5-HETE and 5-oxo-ETE, both EPA and DHA have been reported to be antagonists of the OXE receptor [29] which might account for a biologically relevant mechanism by which marine n-3 PUFA inhibit atherosclerosis and CVD.

In the present study we found that the relative increase in 5-HEPE formation was significantly higher than the rise in LTB_5 formation and similarly, the relative decrease in 5-HETE was smaller than the decrease in LTB_4 in the n-3 PUFA group. This is in line with the observations by Lee et al. [30] who hypothesized that EPA influenced the metabolism of AA by a dose-dependent stimulation and subsequent inactivation of 5-LO exerted by 5-HEPE and an inhibition of LTA_4 hydrolase exerted by LTA_5. Thus, these EPA metabolites could be of functional importance. 5-HEPE has no clinically relevant chemotactic activity neither in vitro nor in vivo [12,27], but 5-HEPE is further metabolized to 5-oxo-EPE which is 10 fold less potent in stimulating neutrophils compared to 5-oxo-ETE [31]. The findings from previous studies that both 5-oxo-EPE and 5-HEPE are much less potent chemotactic agents compared to the AA-derived analogues, together with the above mentioned n-3 PUFA mediated antagonism of the OXE receptor, further support the evidence that dietary EPA and DHA can modulate the inflammatory response through the 5-lipoxygenase pathway.

Body mass index and especially waist circumference are associated with low-grade inflammation in addition to increased cardiovascular risk. Recent evidence from animal studies has suggested that the 5-lipoxygenase pathway is an important contributor to this low-grade inflammation and may also decrease insulin sensitivity in dietary induced obesity [5]. These findings have very recently been supported by Giouleka et al. who reported an increased LT formation in obese asthmatics compared to non-obese asthmatics [32]. In the present study, mean CRP levels were 2.3 mg/L which corresponds to a moderate (not high) CVD risk group as proposed by guidelines from the American Heart Association [33]. We did expect somewhat higher levels of CRP in these overweight subjects and this apparent lack of low grade inflammation might possible explain our results. In line with this, we also found no effect on other inflammatory markers (IL-6, TNF-α, hsCRP) from low-dose n-3 PUFA supplementation in the same group of patients as previously reported [14]. Overall, the present study suggests that a daily dose of 1 g n-3 PUFA is not sufficient to inhibit this pathway in a clinically relevant magnitude in overweight subjects. However, potent drugs inhibiting the 5-lipoxygenase pathway are already in clinical use, and clinical trials evaluating these drugs in the secondary prevention of CVD in obese patients would be of major interest.

The main strength of this study is attributed to the randomized controlled design comparing two groups consisting of subjects with a clinically relevant phenotype. Moreover, this study evaluated a clinically relevant dosage of marine n-3 PUFA, as recommended by the European Society of Cardiology and the American Heart Association for the secondary prevention of CVD. Several limitations of this study should also be acknowledged. All of the enrolled subjects were overweight which means that they all have a fairly large volume of distribution. This could underestimate the effects of the relatively low dose n-3 PUFA supplementation used in this study. In continuation hereof, this study may have been underpowered to detect a difference in LTB_4 formation between the groups, despite the positive results from smaller studies. Lastly, the randomization allocated especially ASA and beta-blocker users in an unexpected uneven way. Since EPA, in the presence of ASA, can be converted to resolvin E1, which has recently been described as a competitive inhibitor of the LTB_4 actions through the BLT1 receptor [34], the different use of ASA between groups could be of particular importance when evaluating a possible anti-inflammatory effect of low-dose n-3 PUFA on the 5-lipoxygenase pathway. However adjustment for intake of ASA did not change the results. Likewise, other unmeasured variables with potential anti-inflammatory properties, like high intakes of fruits and vegetables, could also be unevenly distributed between the groups and thereby contribute to residual confounding.

In conclusion, a 6-week daily supplement of 1.1 g of marine n-3 PUFA in overweight subjects was readily incorporated into neutrophils, partly at the expense of AA. This resulted in a significant increase in the EPA-derived LTB_5 and 5-HEPE, but not a concomitant significant decrease in the AA-derived LTB_4 and 5-HETE. A single dose of 1.1 g n-PUFA did not affect any of the measured variables. Overall, this study does not provide evidence for a net anti-inflammatory effect on lipoxygenase metabolites from a daily supplement of 1.1 g n-3 PUFA in overweight subjects.

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