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Modulation of blood oxylipin levels by long-chain omega-3 fatty acid supplementation in hyper- and normolipidemic men

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ABSTRACT

Introduction: Long chain omega-3 polyunsaturated fatty acids (LC n-3 PUFA) such as EPA and DHA have been shown to possess beneficial health effects, and it is believed that many of their effects are mediated by their oxygenated products (oxylipins). Recently, we have shown that serum levels of several hydroxy, epoxy, and dihydroxy FAs are dependent on the individual status of the parent FAs in a cohort of normo- and hyperlipidemic subjects. So far, the effect of an increased dietary LC n-3 PUFA intake on hydroxy, epoxy, and dihydroxy FA levels has not been investigated in subjects with mild combined hyperlipidemia.
Subjects and methods: In the present study, we compared oxylipin patterns of 10 hyperlipidemic (cholesterol >200 mg/dl; triglyceride >150 mg/ml) and 10 normolipidemic men in response to twelve weeks of LC n-3 PUFA intake (1.14 g DHA and 1.56 g EPA). Levels of 44 free hydroxy, epoxy and dihydroxy FAs were analyzed in serum by LC-MS. Additionally, oxylipin levels were compared with their parent PUFA levels in erythrocyte membranes; a biomarker for the individual PUFA status.
Results: Differences in the oxylipin pattern between normo- and hyperlipidemic subjects were minor before and after treatment. In all subjects, levels of EPA-derived oxylipins (170–4800 pM) were considerably elevated after LC n-3 PUFA intake (150–1400%), the increase of DHA-derived oxylipins (360–3900 pM) was less pronounced (30–130%). The relative change of EPA in erythrocyte membranes is strongly correlated ($r \geq 0.5$; $p < 0.05$) with the relative change of corresponding epoxy and dihydroxy FA serum levels. The effect on arachidonic acid (AA)-derived oxylipin levels (140–27,100 pM) was inconsistent.
Discussion and conclusions: The dietary LC PUFA composition has a direct influence on the endogenous oxylipin profile, including several highly biological active EPA- and DHA-derived lipid mediators. The shift in oxylipin pattern appears to be dependent on the initial LC PUFA status particularly for EPA. The finding that also levels of other oxylipins derived from ALA, LA or AA are modified by LC n-3 PUFA intake might suggest that at least some of the effects of EPA and DHA could be mediated by a shift in the entire oxylipin profile.

1. Introduction

Long chain omega-3 polyunsaturated fatty acids (LC n-3 PUFA) such as eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) have been shown to possess health beneficial effects such as triglyceride-lowering, anti-inflammatory and anti-arrhythmic effects conferring cardiovascular protective qualities [1]. Accordingly, many national heart and nutrition associations recommend increased consumption of fatty fish or LC n-3 PUFA supplements to prevent cardiovascular disease (CVD). However, the molecular mediators of these effects are not well characterized. It is believed that many actions of LC n-3 PUFAs are mediated by their oxidative products – the oxylipins. Oxylipins function as potent lipid mediators regulating various biological processes such as cell proliferation, apoptosis, tissue repair, blood clotting, blood vessel permeability, inflammation, immune cell behavior and other biological functions [2,3]. Oxylipins are formed via enzymatic conversion by cyclooxygenases (e.g. COX-2), lipoxygenases (e.g. 12-LOX, 15-LOX and 5-LOX) and cytochrome P450s (CYPs, e.g. CYP2J2) [2,4,5] and are further converted by the soluble epoxide hydrolase (sEH) [6–8] and other enzymes. Many conversions are multistep reactions requiring several enzymes. In addition, oxylipins can be also formed by non-enzymatic autoxidation of LC PUFAs.

LC PUFAs such as arachidonic acid (AA, 20:4 n-6), EPA and DHA are incorporated into membrane phospholipids, where they modulate membrane fluidity, microdomain assembly and lipid raft signaling as well as the activity of ion channels and other

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membrane proteins [9]. Increasing the dietary intake of LC n-3 PUFAs, e.g. via fish oil supplementation, results in increasing EPA and DHA percentage as well as decreasing AA percentage in erythrocyte membranes, while it is unlikely that the total amount of fatty acids in the erythrocytes is altered by the treatment [10–12]. Therefore the relative percentage of LC PUFAs on total fatty acids in erythrocyte membranes was used to assess the long term nutrition status of LC PUFAs [13]. After release by phospholipase A2 (PLA2) and other enzymes, AA, EPA and DHA become accessible to oxylipin biosynthesis by COX, LOX, and CYP enzymes [9]. The competition between AA, EPA and DHA for the production of classical COX- and LOX-derived oxylipins has been described earlier [14]. However, these LC PUFAs are also the precursors of oxylipins such as hydroxy, epoxy and dihydroxy FAs, of which some metabolites are highly potent lipid mediators [2,15,16]. While LOX form regio- and stereo-selective hydroperoxides, which serve as precursors for leukotrienes and hepoxylins and can be reduced to hydroxy FA (HETE, HODE, HOTRrE, HEPE, HDoHE) [17], some CYPs dominantly form epoxy FA (EpOME, EpETrE, EpODE, EpETE, and EpDoPE) [2,4,5], which can be hydrolyzed to the corresponding dihydroxy FA (DiHOME, DiHETrE, DiHODE, DiHETE, and DiHDPE,) by sEH [6–8]. The effect of LC n-3 PUFA supplementation and an elevated LC n-3 PUFA status on concentrations of many n-3- as well as n-6-derived hydroxy, epoxy and dihydroxy FAs in humans is unclear. For example, in vitro studies have shown that CYP epoxygenases not only accept AA as substrate but also EPA and DHA [2,15,18]. Hence, AA, EPA and DHA compete for the same enzymes to form epoxides. There is increasing evidence that EPA- and DHA-derived epoxides are active lipid mediators similarly to anti-inflammatory and analgesic epoxides of AA [19]. In vitro and in vivo studies disclosed anti-hypertensive, anti-thrombotic, anti-atherosclerotic and anti-angiogenic properties of LC n-3 PUFA-derived epoxides [16,20]. A recent study showed that 17 (18)-EpETE and 19(20)-EpDPE act as anti-arrhythmic agents, suppressing the Ca^{2+}-induced rate of spontaneous beating of neo-natal rat cardiomyocytes, at low nanomolar concentrations [2]. Moreover, DHA-derived epoxides can inhibit angiogenesis, tumor growth and metastasis [20].

Apart from COX or LOX metabolites, the endogenous levels of LC n-3 PUFA-derived oxylipins, especially epoxy and dihydroxy FAs in human blood, are poorly examined. Human studies examining comprehensive oxylipin profiles are rare [21–25]. Only two studies investigated the effects of LC n-3 PUFA supplementation on endogenous hydroxy, epoxy and dihydroxy FA profiles and showed that LC n-3 PUFA treatment is able to affect oxylipin profiles [21,24]. However, both pilot studies focused on healthy subjects and the analysis of the sum of bound (esterified) and free oxylipins in plasma. In order to understand the role of oxylipins in health and disease, it is necessary to investigate oxylipin profiles and their variability by LC n-3 PUFA treatment, in different health states. The intention of this work is to compare free (non-esterified) oxylipin profiles of healthy subjects and subjects with mild combined hyperlipidemia after dietary LC n-3 PUFA supplementation. In a recent publication we showed that free hydroxy, epoxy and dihydroxy FA levels in serum of subjects with combined hyperlipidemia were not different from healthy subjects [26]. By contrast, serum levels of several hydroxy, epoxy, and dihydroxy FA are dependent on the individual status of the parent FA (as measured by the relative FA level in erythrocyte membranes) suggesting that free oxylipin levels can be directly influenced by the diet. The correlation was apparent for EPA in erythrocyte membranes and the serum concentration of EPA metabolites. In the present paper, we show the effect of a 12 week LC n-3 PUFA supplementation on free oxylipin levels in normo- and hyperlipidemic subjects of the same cohort from our first study. In addition, oxylipin levels were correlated with parent FA levels in erythrocyte membranes. The aim was to elucidate if and how responding oxylipin levels depend on the lipidemic state of the subjects or their baseline FA status.

2. Materials and methods

This investigator initiated study was designed and conducted according to the principles of the Good Clinical Practice Guidelines laid down in the Declaration of Helsinki and was approved by the Freiburger ethic committee.

2.1. Subjects

Subjects participated in another study aiming to compare baseline serum oxylipins concentrations of 20 normolipidemic with 20 hyperlipidemic subjects. For description of recruitment and screening procedure please see [26]. Only men were selected to compile a comparable study collective and to prevent the possible influence of hormonal changes on expression of genes coding for LC PUFA metabolizing enzymes which could hamper the attribution of observed effects to treatment. The following exclusion criteria for study participation were applied: Female; body-mass-index > 35; smoker; intake of any corticosteroids, lipid-lowering or anti-inflammatory drugs; diagnosed chronic, cardiovascular or liver diseases; gastrointestinal disorders; blood coagulation disorders and intake of coagulation-inhibiting drugs; renal failure; periodic intake of laxatives; ingestion of supplements enriched with LC n-3 PUFAs, phytosterols, polyglucosamines, other lipid-binding ingredients or daily eating of fatty fish; allergy to fish or fish oil; participation in another clinical study < 30 days before the start of the study or at the same time. 10 normolipidemic and 10 hyperlipidemic men aged between 24 and 48 years were randomly selected to take part in the intervention period with fish oil capsules. The serum lipid levels of the hyperlipidemic group (HG; total cholesterol [TC] > 200 mg/dl; LDL > 130 mg/dl; TG > 150 mg/dl) were significantly higher compared to the normolipidemic group (NG; < 200 mg/dl; LDL < 130 mg/dl; TG < 150 mg/dl), while HDL levels were lower, respectively (Table S1). Likewise, the HG had a higher mean weight and BMI, while differences in age were not significant. All included subjects gave their written informed consent to take part in the study.

2.2. Study design

To realize a comparable mean age between both groups, the group formation was performed by stratified allocation according to subject's age. Subjects ingested six fish oil capsules per day for a period of twelve weeks. Each soft gelatin capsule contained fish oil concentrate (840 mg/ capsule) containing 252 mg EPA and 168 mg DHA as re-esterified triglycerides. The total amount of LC PUFAs (C20:5, C22:6, C18:3, C18:4; C20:4, C21:5, C22:5) was 504 mg. Additionally, each soft gelatin capsule contained 6 mg α-tocopherole. The daily LC n-3 PUFA intake was 3.0 g with 1.14 g DHA and 1.56 g EPA. The subjects were instructed to take three capsules in the morning and three in the evening together with food and a glass of water. Usual exercise and dietary habits should be maintained throughout the intervention time. Moreover, subjects were requested to abstain from eating fatty fish during the intervention period. During the two visits, fasting blood was collected from subjects. Additionally, subjects completed a questionnaire to obtain information about changes in medication, diet (e.g. changes in weekly fish intake, preferred fish dishes or species, respectively) and lifestyle habits (e.g. physical activity), as well as the tolerability of the capsules. The subjects' compliance was