Prostaglandins, Leukotrienes and Essential Fatty Acids 111 (2016) 17–24

Contents lists available at ScienceDirect

Prostaglandins, Leukotrienes and Essential Fatty Acids

journal homepage: www.elsevier.com/locate/plefa

Original research article

Bioavailability of long chain omega-3 polyunsaturated fatty acids from phospholipid-rich herring roe oil in men and women with mildly elevated triacylglycerols

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ARTICLE INFO

Article history:
Received 20 July 2015
Received in revised form
6 January 2016
Accepted 12 January 2016

Keywords:
Omega-3 fatty acids
DHA
EPA
phosphatidylcholine
fish oil

ABSTRACT

This randomized, single-blind, crossover trial assessed the bioavailability of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and docosapentaenoic acid (DPA) from two different sources, each examined over a 12 h period following consumption of a single serving and after 2-weeks of daily supplementation. Thirty-two adults with fasting triacylglycerol (TAG) concentrations between 100 and 399 mg/dL were randomly assigned, with stratification by sex and age, to receive 12 capsules/day containing either phospholipid (PL)-rich herring roe oil (Romega® 30, 628 mg/day EPA; 1810 mg/day DHA; 137 mg/day DPA) or TAG-rich fish oil (575 mg/day EPA; 1843 mg/day DHA; 259 mg/day DPA) each for a 2-week period separated by a 4 week washout. The net incremental area under the curve from 0 to 12 h for EPA, DHA, and EPA+DHA in plasma phosphatidylcholine (PC) were significantly higher ($p < 0.01$ for all) after Romega 30 supplementation compared to fish oil. Similar results were observed when the data for the Romega 30 condition were normalized to fish oil EPA and DHA intakes ($p < 0.001$ for all). After the 2-week supplementation period, fasting plasma PC EPA+ DHA was elevated by ~2.8 to 3.0-fold relative to baseline in both conditions ($p < 0.0001$ for each), but there was no significant difference in the change from baseline ($p = 0.422$) between Romega 30 (baseline = 62.2 ± 3.8 μg/mL vs. end of study = 172.9 ± 11.7 μg/mL) and fish oil (baseline = 62.0 ± 3.4 μg/mL vs. end of study = 185.4 ± 11.2 μg/mL) conditions. Similar results were observed for each individual LC n-3 PUFA in plasma PC after 2 weeks of supplementation. These data demonstrate that PL-rich herring roe is a well-tolerated and bioavailable source of LC n-3 PUFA.

1. Introduction

Consumption of the long-chain omega-3 polyunsaturated fatty acids (LC n-3 PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) has been shown to provide a wide range of health benefits [1–5]. As a result, a number of scientific organizations and authoritative bodies around the world, including the Food and Agriculture Organization (FAO) of the United Nations and World Health Organization (WHO), have put forth dietary intake recommendations for EPA and DHA with an acceptable macronutrient distribution range of 250 mg/day to 2 g/day of EPA+DHA [6]. The United States Institute of Medicine (IOM) has not established a specific Dietary Reference Intake or Adequate Intake level for EPA and DHA, but the 2010 Dietary Guidelines for Americans recommend consuming 8 ounces of seafood per week for the general public, equivalent to 1750 mg/week (250 mg/day) of EPA+DHA [7]. An analysis of the U.S. National Health and Nutrition Examination Survey (NHANES 2003–2008) indicates most American adults (men and women ≥ 19 years of age) do not meet these general intake recommendations [8]. Indeed, these data suggest that usual intakes (median [25th and 75th percentile]) from foods and dietary supplements combined are 18 (11–28) mg/day for EPA and 39 (20–77) mg/day for DHA. Furthermore, current

Abbreviations: DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LC n-3 PUFA, long-chain omega-3 polyunsaturated fatty acids; PC, phosphatidylcholine; PL, phospholipid; TAG, triacylglycerol

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http://dx.doi.org/10.1016/j.plefa.2016.01.007
0952-3278/

supplies of n-3 LC-PUFA from wild or farm-raised fish may be insufficient to meet recommended intake levels of EPA and DHA [9]. Thus, a growing demand for EPA and DHA will need to be met with novel, particularly under-utilized, sources.

In most commercially available fish oils, EPA and DHA are predominately in the triacylglycerol (TAG) form with a smaller proportion as ethyl esters or phospholipids (PL). Evidence suggests the bioavailability of EPA and DHA differs depending on the source and processing of the oil. Indeed, there is some evidence from clinical trials in humans to suggest that EPA and DHA in the PL form may be more bioavailable than EPA and DHA in the TAG form [10–12]. However, this issue remains controversial [13]. Currently, the primary PL form of EPA and DHA is derived from oil extracted from Antarctic Krill, having a DHA:EPA ratio in the range of 1:1.8–1:2.5.

EPA and DHA bioavailability from PL-rich herring roe relative to other sources, such as TAG-rich fish oil, remains to be determined. Therefore, the objective of this study was to compare the acute and sub-chronic (2 week) bioavailability of EPA and DHA from PL-rich immature herring roe oil and TAG-rich fish oil supplements in generally healthy men and women with elevated TAG. Immature herring roe are currently an under-utilized, but potentially important, source of proteins [14] and lipids [15] for humans. The roe are available in high quantities (in the EU $>15{,}000$ metric tons/year) as by-products from the sustainable herring fishery industry.

2. Patients and methods

2.1. Study design

This was a randomized, controlled, crossover study consisting of one screening visit followed by two single-blinded, 2-week treatment periods each separated by a 4-week washout. Eligible subjects were randomly assigned by sex and age to receive 12 capsules/day containing oil high in EPA and DHA PL (Romega® 30; Arctic Nutrition, Ørsta, Norway) providing 628 mg/day EPA, 1810 mg/day DHA, and 137 mg/day n-3 docosapentaenoic acid (DPA), or 12 capsules/day containing oil from a blend of two different fish oils high in EPA and DHA TAG providing 575 mg/day EPA, 1843 mg/day DHA, and 259 mg/day DPA for 2 weeks (first treatment period) and then, after a 4-week washout, crossed over to receive the opposite study product for 2 weeks (second treatment period). Romega 30 is oil extracted from Norwegian herring immature roe, mixed 50/50 with fish oil TAG to adjust viscosity, that overall contains 45% n-3 PUFA (g/100 g product basis) with a DHA:EPA ratio of approximately 3:1, basis the exact amounts described above [15]. In addition, 32% of the total lipids are PL, of which most (89%) are in the form of phosphatidylcholine (PC) with minor amounts of lysoPC [15]. Approximately 30% of the EPA and DHA present in the product are esterified to PL (mainly PC), with the rest esterified to TAG. Two commercially processed fish oils high in EPA and DHA TAG were mixed in a precise proportion blended to approximate the EPA and DHA content of Romega 30.

This study was conducted at Biofortis Clinical Research (d.b.a. Biofortis, Inc., Addison, IL) according to Good Clinical Practice Guidelines as described in the United States 21 Code of Federal Regulations and the Declaration of Helsinki 2000. The protocol was approved by Quorum Review Institutional Review Board (Seattle, WA) before the study was initiated. All participants provided signed informed consent and authorization for disclosure of protected health information before any study specific procedures were carried out.

On the first day of each 2-week treatment period, an acute 12 h test was conducted, wherein subjects consumed all 12 capsules with a standardized low-choline, DHA-, EPA-free breakfast (capsules and breakfast consumed within 15 min). Blood samples were obtained via an indwelling venous catheter (or venipuncture if the catheter failed) at $t=-0.5$, 4, 8, 10, and 12 h $\pm$ 5 min, (where $t=0$ h was the start of study product and breakfast consumption) for measurements of the plasma PC and plasma sphingomyelin EPA, DHA, and DPA fatty acid profiles over time (plasma sphingomyelin results are not reported herein). Standardized, low-fat, low-choline, DHA-, EPA- free lunch and dinner meals were provided immediately following the $t=4$ h and the $t=8$ h time points ($\pm$ 5 min), each consumed in its entirety within 20 min. All meals were based on each subject's estimated energy needs for weight maintenance using 30 kcal/kg body weight per day. Subjects replicated 24 h food intake prior to the second acute 12 h test visit (week 6) by following 24 h diet records taken prior to the first acute bioavailability test day. Study product capsules and the standardized breakfast meal were administered within $\pm$ 30 min of the $t=0$ h time established during the first acute 12 h test day. Breakfast, lunch, and dinner menus were also replicated.

For the two weeks after each acute 12 h test visit, subjects were instructed to consume four capsules immediately prior to each meal (breakfast, lunch and dinner separated by at least 3 h) each day. Subjects were also instructed to maintain their habitual dietary practices, physical activity patterns, and body weight, to limit alcohol intake to one drink per day (1 drink = 12 oz beer, 5 oz wine, or 1½ oz distilled spirits), and to avoid fish and seafood, foods rich in choline, other EPA- or DHA-containing foods and supplements. Subjects were also asked to avoid exercise for 24 h, and tobacco for 1 h, prior to all test visits. On the last day of each 2-week test period, fasting (12 $\pm$ 1 h) blood samples were obtained for measurement of EPA, DHA, and DPA plasma fatty acid profiles described for the acute 12 h test. Compliance with capsule consumption was assessed by counting the unused study capsules returned to the clinic and by evaluation of a study product consumption diary completed by each subject.

2.2. Subjects

Men and non-pregnant, non-lactating women, 18–59 years of age (inclusive), each with a body mass index (BMI) of 18.50–29.99 kg/m^2 and a fasting TAG concentration of 100–399 mg/dL, who were in good general health on the basis of medical history and routine laboratory tests were eligible for the study. In addition, subjects were required to be willing to refrain from consumption of all fish and seafood (including shellfish), foods rich in choline (subjects provided with a list of choline-rich foods and supplements), fatty acid-containing supplements, and/or EPA-, DHA-containing foods and supplements ($\leq$ 1.0 g/day) 14 days prior to randomization. The use of any medications, dietary supplements, or fortified foods with lipid-altering effects was excluded for at least 4 weeks before study entry, as was use of weight-loss drugs or programs and a recent body weight change greater than 4.5 kg. Additionally, individuals were excluded from participation if they used non-study related omega-3-acid ethyl ester drug(s) or dietary supplement(s) containing $\geq$ 1.0 g/day of EPA, DHA, or a combination of EPA and DHA within 4 months of screening. Individuals with a known allergy or sensitivity to omega-3 fatty acids, fish, other seafood, or any ingredient in the study products or meals were also excluded.

Additional exclusion criteria included resting systolic blood pressure of at least 160 mmHg and/or a diastolic blood pressure of at least 100 mmHg, and history or presence of clinically important endocrine (including type 1 or 2 diabetes mellitus), cardiovascular (including, but not limited to history of myocardial infarction, peripheral arterial disease, stroke), pulmonary (including