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Changes in relative and absolute concentrations of plasma phospholipid fatty acids observed in a randomized trial of Omega-3 fatty acids supplementation in Uganda

Xiaoling Song[a,*], Pho Diep[a], Jeannette M. Schenk[a], Corey Casper[a,b], Jackson Orem[b,c], Zeina Makhoul[a], Johanna W. Lampe[a], Marian L. Neuhouser[a]

[a] Public Health Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA
[b] Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA
[c] Uganda Cancer Institute, Kampala, Uganda

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ABSTRACT

Expressing circulating phospholipid fatty acids (PLFAs) in relative concentrations has some limitations: the total of all fatty acids are summed to 100%; therefore, the values of individual fatty acid are not independent. In this study we examined if both relative and absolute metrics could effectively measure changes in circulating PLFA concentrations in an intervention trial. 66 HIV and HHV8 infected patients in Uganda were randomized to take 3 g/d of either long-chain omega-3 fatty acids (1856 mg EPA and 1232 mg DHA) or high—oleic safflower oil in a 12-week double-blind trial. Plasma samples were collected at baseline and end of trial. Relative weight percentage and absolute concentrations of 41 plasma PLFAs were measured using gas chromatography. Total cholesterol was also measured. Intervention-effect changes in concentrations were calculated as differences between end of 12-week trial and baseline. Pearson correlations of relative and absolute concentration changes in individual PLFAs were high (> 0.6) for 37 of the 41 PLFAs analyzed. In the intervention arm, 17 PLFAs changed significantly in relative concentration and 16 in absolute concentration, 15 of which were identical. Absolute concentration of total PLFAs decreased 95.1 mg/L (95% CI: 26.0, 164.2; P=0.0085), but total cholesterol did not change significantly in the intervention arm. No significant change was observed in any of the measurements in the placebo arm. Both relative weight percentage and absolute concentrations could effectively measure changes in plasma PLFA concentrations. EPA and DHA supplementation changes the concentrations of multiple plasma PLFAs besides EPA and DHA.Both relative weight percentage and absolute concentrations could effectively measure changes in plasma phospholipid fatty acid (PLFA) concentrations.

1. Introduction

Epidemiologic studies of the relationship between circulating phospholipid fatty acids (PLFA) and disease outcomes have expressed each fatty acid as either weight or molar percentage of total fatty acids analyzed [1–3]. Using such relative concentrations, the total of all fatty acids are summed to 100%; therefore, the values of individual fatty acid are not independent. This has led to debate, along with the suggestion to use absolute concentrations of fatty acids [4–8]. The interdependent nature of the relative metric could be especially problematic when studying changes in concentrations: when certain fatty acids maintain their absolute concentrations while others increase, their relative concentrations would decrease because all fatty acids have to add up to 100%.

In the present study, we used samples from an EPA (eicosapentaenoic acid, 20:5n3) plus DHA (docosahexaenoic acid, 22:6n3) supplementation trial in human immunodeficiency virus (HIV) infected patients and measured changes of individual plasma PLFA concentration in both relative (weight percentage) and absolute terms. The hypothesized intervention effects were that plasma phospholipid EPA and DHA concentrations would increase and that EPA and DHA supplementation may influence both the relative and absolute concentrations of other plasma PLFAs. In this unique study population, we had an opportunity to test whether one metric vs. the other may be

Abbreviations: CV, coefficient of variation; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; KS, Kaposi's sarcoma; GC, gas chromatography; HOSO, high—oleic safflower oil; PLFA, phospholipid fatty acid; QC, quality control; UCI, Uganda Cancer Institute

* Correspondence to: Fred Hutchinson Cancer Research Center, 1100 Fairview Ave N, Mail Box M5-A864, Seattle WA 98109, USA.
E-mail address: xsong2@fredhutch.org (X. Song).

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more sensitive at picking up intervention-effect changes in plasma PLFAs.

2. Materials and methods

2.1. Study population and study samples

The Uganda Omega-3 trial was a 12-week randomized, double-blind, placebo-controlled trial among men and women infected with both human immunodeficiency virus (HIV) and human herpesvirus 8 (HHV8). Participants were recruited between June and November 2012 from the Uganda Cancer Institute (UCI)/Hutchinson Center Cancer Alliance in Kampala, Uganda. Individuals with Kaposi's sarcoma (KS) were also recruited by collaborating physicians during routine clinic visits to the UCI. Exclusions criteria included pregnancy, age of less than 18, and active disease, i.e. KS-positive individuals receiving chemotherapy and KS-negative individuals receiving antiretroviral therapy. Participants were block randomized by KS status to receive either the omega-3 supplement or placebo. The intervention group consumed 8 capsules/day of a fish oil preparation, which contained 3.1 g of EPA (1.86 g) and DHA (1.23 g), plus 2.5 g other fatty acids naturally present in fish oil; while the placebo group consumed 5.6 g high—oleic safflower oil (HOSO) in identical packaging and appearance. Participants were instructed to take 4 capsules with meal twice a day. The EPA+DHA dose used in the study was considered safe to consume without physician supervision [9]. HOSO, which contains about 78% of oleic acid [10], was used as control because changes in PLFA profile after intake of 5.6 g/d of HOSO were expected to be small. Both active and placebo capsules were supplied by Capsugel Liquids Group (Greenwood, South Carolina). A flavorant was added to the omega-3 fatty acid capsules to mask the fish scent. All capsules contained mixed tocopherols (to prevent oxidation) and other excipients necessary for the stability of the supplement. Non-fasting plasma samples were collected at baseline and at the end of the 12-week trial, stored locally at −80 °C, and shipped on dry ice to the Fred Hutchinson Cancer Research Center for lab analyses. Non-fasting blood does not affect the analytes in the present study—plasma phospholipid fatty acids reflect the intake of past 1–2 weeks [11] and circulating total cholesterol changes minimally in response to food intake [12,13]. The study was approved by the Institutional Review Board of the Fred Hutchinson Cancer Research Center in Seattle, USA; the School of Medicine Research and Ethics Committee at Makerere College of Health Sciences in Kampala, Uganda; the Uganda National Council for Science and Technology; and the Uganda National Drug Authority. All participants provided written, informed consent.

2.2. Plasma phospholipid fatty acids (PLFA) analysis

The plasma PLFA assay was described previously [14]. Briefly, total lipids were extracted from plasma using the method of Folch [15]. An internal standard, 1,2-dihenarachidoyl-*sn*-glycero-3-phosphocholine (21:0 PC, Avanti Polar Lipids, Inc., Alabaster, Alabama), was added to each plasma sample before lipid extraction. Phospholipids were separated from other lipids by one dimensional thin layer chromatography [16]. Fatty acid methyl esters of the phospholipids were prepared by direct transesterification [17] and separated using gas chromatography (Agilent 5890 Gas Chromatograph with flame ionization detector, Supelco fused silica 100 m capillary column SP-2560). This GC method quantified 41 known fatty acids for this study. Relative concentration of each fatty acid was expressed as a weight percentage of total PLFAs analyzed. Absolute concentration (mg/L) of each fatty acid was calculated by comparing its peak area to that of the internal standard: $\text{mg/L}_{21:0} * (\text{peak area count}_{\text{fatty acid of interest}} / \text{peak area count}_{21:0})$. A lab quality control (QC) sample (a pooled plasm) was included with each batch of study samples. When measured in weight percentage, coefficients of variation (CVs) of the 41 PLFAs in the QC samples ranged from 0.3% to 20.9%; when measured in mg/L, from 2.2% to 21.8%. All 32 relatively abundant PLFAs (> 0.1%) in the QC sample had CVs less than 6% whether measured in weight percentage or mg/L except for 18:1n8c, which had CV of 13.6% measured in weight percentage and 14.6% in mg/L.

2.3. Other laboratory assays

Total plasma cholesterol was measured on a Roche Cobas Mira Plus Chemistry Analyzer, using reagents from Sekisui Diagnostics (Lexington, MA) and following the manufacturer's instructions.

2.4. Statistical analyses

Baseline means and standard deviations in the intervention arm and in the placebo arm were calculated for each plasma PLFA, measured as relative weight percentage and absolute concentrations. Intervention-effect changes in concentrations were calculated as differences between end of 12-week trial and baseline. Pearson correlations were used to measure agreement between relative and absolute changes for each fatty acid; Spearmen correlations were nearly identical to Pearson correlations and are therefore not given in results. One-sample *t*-tests were used to evaluate whether change in each fatty acid concentration differed significantly from zero. Paired *t*-tests were used to compare baseline vs. end of trial (12-week) total cholesterol, and absolute concentration of total PLFAs analyzed. Bonferroni correction was used to account for multiple comparisons of 41 fatty acids; otherwise statistical tests are two-sided with significance level set at $P < 0.05$. Analyses were performed using SciPy Stack version 0.12.0 downloaded from SciPy.org or StataSE 13 (StataCorp LP, College Station, TX).

3. Results

Participants in the intervention and placebo arms were well balanced in terms of age, sex, BMI, KS status, total cholesterol, and total plasma PLFAs analyzed (Table 1). Concentrations of individual PLFAs at baseline expressed as both relative weight percentage and absolute concentrations were not significantly different between the two arms after correction for multiple comparisons (Table 2; *P* values not shown).

Pearson correlations of relative and absolute concentration changes in individual PLFAs were high except for 4 saturated fatty acids (16:0, 18:0, 20:0, and 24:0), which had correlation coefficients < 0.6 (Table 3). After the 12-week EPA+DHA supplementation, EPA and DHA increased significantly in both relative and absolute concentrations (Table 3, intervention arm): EPA had 5.7 and 5.1 mean fold

Table 1
Participants characteristics at baseline in the Uganda Omega-3 Trial.

	intervention arm (n =33)	placebo arm (n =33)
Age	38.6 ± 9.4	39.8 ± 8.9
BMI	23.2 ± 3.8	23.8 ± 6.3
Sex		
Male	17 (51.5%)	20 (60.6%)
Female	16 (48.5%)	13 (39.4%)
KS Status		
KS Positive	27 (81.8%)	28 (84.8%)
KS Negative	6 (18.2%)	5 (15.2%)
Total plasma cholesterol (mg/dl)	165.0 ± 46.0	151.9 ± 36.9
Total plasma PLFAs analyzed (mg/L)	1264.8 ± 280.4	1246.0 ± 269.2

Data are means ± SD or frequency (%).
KS, Kaposi's sarcoma.