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Dietary omega-6 fatty acid lowering increases bioavailability of omega-3 polyunsaturated fatty acids in human plasma lipid pools

Ameer Y. Taha [a,*], Yewon Cheon [a], Keturah F. Faurot [c], Beth MacIntosh [d], Sharon F. Majchrzak-Hong [b], J. Douglas Mann [e], Joseph R. Hibbeln [b], Amit Ringel [b], Christopher E. Ramsden [b,c]

[a] Brain Physiology and Metabolism Section, Laboratory of Neuroscience, National Institute on Aging, National Institutes of Health, Bethesda, MD, USA
[b] Section on Nutritional Neurosciences, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, MD, USA
[c] Department of Physical Medicine and Rehabilitation, Program on Integrative Medicine, University of North Carolina, Chapel Hill, NC, USA
[d] Nutrition Research and Metabolism Core, North Carolina Translational Clinical Sciences Institute, University of North Carolina, Chapel Hill, USA
[e] Department of Neurology, Program on Integrative Medicine, University of North Carolina, Chapel Hill, NC, USA

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ABSTRACT

Background: Dietary linoleic acid (LA, 18:2n-6) lowering in rats reduces n-6 polyunsaturated fatty acid (PUFA) plasma concentrations and increases n-3 PUFA (eicosapentaenoic (EPA) and docosahexaenoic acid (DHA)) concentrations.
Objective: To evaluate the extent to which 12 weeks of dietary n-6 PUFA lowering, with or without increased dietary n-3 PUFAs, alters unesterified and esterified plasma n-6 and n-3 PUFA concentrations in subjects with chronic headache.
Design: Secondary analysis of a randomized trial. Subjects with chronic headache were randomized for 12 weeks to (1) average n-3, low n-6 (L6) diet; or (2) high n-3, low n-6 LA (H3–L6) diet. Esterified and unesterified plasma fatty acids were quantified at baseline (0 weeks) and after 12 weeks on a diet.
Results: Compared to baseline, the L6 diet reduced esterified plasma LA and increased esterified n-3 PUFA concentrations (nmol/ml), but did not significantly change plasma arachidonic acid (AA, 20:4n-6) concentration. In addition, unesterified EPA concentration was increased significantly among unesterified fatty acids. The H3–L6 diet decreased esterified LA and AA concentrations, and produced more marked increases in esterified and unesterified n-3 PUFA concentrations.
Conclusion: Dietary n-6 PUFA lowering for 12 weeks significantly reduces LA and increases n-3 PUFA concentrations in plasma, without altering plasma AA concentration. A concurrent increase in dietary n-3 PUFAs for 12 weeks further increases n-3 PUFA plasma concentrations and reduces AA.
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1. Introduction

Linoleic acid (LA, 18:2n-6) is a major constituent of the North American diet, accounting for approximately 7% of daily caloric intake and 20% of total dietary fatty acids (~16 g LA/day) [1]. This intake is more than three-fold higher than the historic norm of 2%, owing mainly to the increased consumption of seed oils containing 20–54% LA of total fatty acids [1]. The biochemical and health implications of this change are not fully understood.

The mammalian liver can convert LA to longer chain n-6 PUFAs, particularly arachidonic acid (AA, 20:4n-6), by elongation–desaturation *via* Δ5 and Δ6 desaturases and elongases-2 and -5. LA competes with alpha-linolenic acid (α-LNA, 18:3n-3) for elongation–desaturation enzymes that convert α-LNA to longer chain n-3 PUFAs including eicosapentaenoic acid (EPA, 20:5 n-3), n-3 docosapentaenoic acid (n-3 DPA 22:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) [2,3], which have several putative health benefits [4–6]. LA and AA are precursors to bioactive LA oxidation products [7] and eicosanoids [8], respectively, which have been implicated in pathological conditions such as non-alcoholic steatohepatitis, Alzheimer disease and asthma [9–11]. By contrast, n-3 EPA, DPA and DHA can be converted into anti-inflammatory and pro-resolving lipid mediators [12,5,13,14].

Abbreviations: AA, arachidonic acid; cPLA$_2$, calcium-dependent phospholipase A$_2$; COX-2, cyclooxygenase; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EDTA, ethylenediaminetetraacetic acid; EPA, eicosapentaenoic acid; HETE, hydroxyeicosatetraenoic acid; iPLA$_2$, calcium-independent phospholipase A$_2$; LA, linoleic acid; α-LNA, α–linolenic acid; PUFA, polyunsaturated fatty acid

* Correspondence to: 9000 Rockville Pike, Building 9, 1S-126 BPMS, National Institute on Aging, National Institutes of Health Bethesda, MD 20892.
Tel.: +1 301 394 8393; fax: +1 301 402 0074.
E-mail address: ameer.taha@nih.gov (A.Y. Taha).

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In rodents, dietary LA lowering has been shown to reduce the absolute concentration of AA (nmol per ml plasma or g tissue), and to increase EPA, DPA and DHA concentrations in plasma and numerous tissues [15,2,16,17]. However, comparatively few human trials have evaluated the biochemical effects of lowering dietary LA. To our knowledge, there are no human data indicating that altering dietary LA changes circulating AA concentrations [18–20]. By contrast, dietary LA lowering in humans was reported to increase α-LNA conversion to EPA and DHA [21], to increase the EPA and DHA content of erythrocytes [19] and to increase EPA in plasma phospholipids [18]. In these human studies, data were expressed as percent composition (% of total fatty acids), which may not necessarily reflect changes in absolute concentrations because a change in the concentration of one fatty acid can reflect a change in the opposite direction of another [22,23]. The effects of dietary n-6 PUFA lowering in humans on absolute n-3 and n-6 PUFA concentrations have not been reported for unesterified and esterified (phospholipids, triglycerides, cholesteryl esters) plasma lipid fractions.

We recently reported that the combination of increasing dietary n-3 fatty acids with concurrent reduction in n-6 LA produced statistically significant, clinically relevant improvements in headache frequency, intensity and quality of life in chronic headache patients [24], a condition with reported elevations of AA-derived mediators in blood and saliva [25,24,26]. Blood collected from this trial provides a unique opportunity to evaluate the effects of targeted alterations of dietary n-3 and n-6 fatty acids on plasma esterified and unesterified fatty acid concentrations [19,24], which could be used as biomarkers of dietary treatment effects.

In the present study, we sought to evaluate the effects of dietary n-6 lowering with or without concurrent increases in dietary n-3 PUFA on unesterified and esterified plasma lipid fractions, using plasma samples from a completed dietary trial in patients with chronic headaches [19,24]. We tested the following hypotheses: (1) an average n-3, low n-6 (L6) dietary intervention would increase n-3 PUFA and decrease n-6 AA absolute concentrations in esterified and unesterified plasma lipid pools; and (2) a high n-3, low n-6 LA (H3–L6) dietary intervention would produce significantly greater increases in circulating n-3 PUFA concentrations and reductions in AA concentrations.

2. Materials and methods

2.1. Patients and dietary methods

A detailed description of the dietary methods and procedures of the main trial have been published [19,24,27]. The trial was conducted at The University of North Carolina (UNC) at Chapel Hill from April 2009 to November 2011. Subjects signed informed consent prior to participation. Trial procedures were approved by the UNC Institutional Review Board. This trial is registered under ClinicalTrials.gov (NCT01157208). In brief, 67 subjects with chronic headaches were randomized to either a low n-6 PUFA (L6) or a high n-3 plus low n-6 PUFA (H3–L6) diet to be maintained for 12-weeks. Nutrient compositions of the two interventions are shown in Table 1. The interventions were designed to be equally credible and to provide equivalent: (1) amounts of study foods; (2) macronutrient and caloric intake; (3) interactions with the study investigators and dietitian; and (4) intensity and breadth of dietary advice and intervention materials [19]. A registered dietitian provided intensive counseling at randomization and at 2-week intervals. Foods meeting nutrient targets were provided to participants for two meals and two snacks per day. Detailed intervention-specific web-based materials were also provided to reinforce dietitian advice and complement the study food provision. To assess nutrient intakes six unannounced telephone-administered 24-h recalls were administered for each participant – three during the baseline phase and three in the final four weeks of the intervention phase – as previously described [19].

Fifty-six of the 67 randomized participants completed the 12-week intervention phase, with 55 providing pre- and post-intervention plasma samples (28 in the L6 group and 27 in the H3–L6 group). Baseline demographics and clinical characteristics were comparable in the two groups (Table 2); 87% of randomized subjects were female. At baseline, participants averaged 23 headache days per month and 10 headache hours per day, and reported taking an average of six different headache-related medications per subject.

2.2. Sample collection

Fasting whole blood, drawn at baseline and again after 12 weeks of dietary intervention, was collected into ethylenediaminetetraacetic acid (EDTA) tubes. Samples were immediately centrifuged at 2000*g* for 15 min at room temperature, and plasma aliquots were stored in a −80 °C freezer until analysis. Sample preparation and analyses were performed by investigators who were blinded to the study protocol and clinical data.

2.3. Analysis of plasma esterified and unesterified fatty acids

Total lipids were extracted from 200 μl of plasma in 3 ml of 2:1 chloroform/methanol following the addition of unesterified heptadecaenoic acid (17:0) as an internal standard of unesterified fatty acids. KCl (0.5 M, 0.75 ml) was then added to separate the aqueous phase. The bottom chloroform layer was separated and the remaining aqueous phase was re-extracted with 2 ml of chloroform. The pooled chloroform extracts containing total lipids were dried down and separated into neutral lipid subclasses (cholesteryl esters, triacylglycerol, unesterified fatty acids, and total phospholipids) using silica gel-60 thin layer chromatography plates (EM Separation Technologies, Gibbstown, NJ, USA), in a heptane:diethylether:glacial acetic acid (60:40:3, by vol) solvent system [28]. Authentic standards of neutral lipids were run on separate lanes on the plates to identify lipid bands under ultraviolet light, after spraying with 0.03% 6-p-toluidine-2-naphthalene sulfonic acid in 50 mM Tris–HCl buffer (pH 7.4) (w/v). The bands were scraped into test tubes and methylated with 1% H_2SO_4 in methanol for 3 h at 70 °C [29]. Before methylation, di-17:0 PC was added to each tube as an internal standard for phospholipids, triglycerides and cholesteryl esters. The prepared fatty acid methyl esters (FAMEs) were analyzed by a gas-chromatography system (6890N, Agilent Technologies, Palo Alto, CA, USA) equipped with an SPTM-2330 fused silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) (Supelco, Bellefonte, PA, USA) and a flame ionization detector as previously described [30]. Fatty acid concentrations were calculated by proportional comparison of peak areas of samples to the area of the 17:0 internal standard.

2.4. Data analysis

Non-parametric analyses were employed due to the presence of non-normal distributions. Pre-to-post intervention comparisons were tested with the Wilcoxon Signed-Rank test for matched pairs. A Mann Whitney *U* test was used to compare differences in fatty acid concentrations between the two groups at baseline and at 12 weeks. Statistical significance was accepted at $P \leq 0.05$.

3. Results

3.1. Baseline fatty acid concentrations

As shown in Tables 3–6, baseline total plasma fatty acid concentrations (nmol/ml) in phospholipids, triglycerides, cholesteryl