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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[1,*], **Yewon Cheon**[1], **Keturah F. Faurot**[3], **Beth MacIntosh**[4], **Sharon F. Majchrzak-Hong**[2], **J. Douglas Mann**[5], **Joseph R. Hibbeln**[2], **Amit Ringel**[2], and **Christopher E. Ramsden**[2,3]

[1]Brain Physiology and Metabolism Section, Laboratory of Neuroscience, National Institute on Aging, National Institutes of Health, Bethesda, MD, USA

[2]Section on Nutritional Neurosciences, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, MD, USA

[3]Department of Physical Medicine and Rehabilitation, Program on Integrative Medicine, University of North Carolina, Chapel Hill, NC, USA

[4]Nutrition Research and Metabolism Core, North Carolina Translational Clinical Sciences Institute, University of North Carolina-Chapel Hill, USA

[5]Department of Neurology, Program on Integrative Medicine, University of North Carolina, Chapel Hill, NC, USA

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, change 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

[*]Corresponding author. Ameer Y. Taha, PhD, 9000 Rockville Pike, Building 9, 1S-126, BPMS, National Institute on Aging, National Institutes of Health, Bethesda, MD 20892, ameer.taha@nih.gov, Phone: 301-394-8393, Fax: 301-402-0074.

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 PUFA for 12 weeks further increases n-3 PUFA plasma concentrations, but also reduces AA.

Keywords

Linoleic acid (LA); lowering; Eicosapentaenoic acid (EPA); docosahexaenoic acid (DHA); Arachidonic acid; omega-6 (n-6); omega-3 (n-3); polyunsaturated fatty acids; esterified; unesterified; plasma; lipids; fish; migraine

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].

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 to relate efficacy in 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 at Chapel Hill (UNC) 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, sixty-seven subjects with chronic headaches were randomized to either a low n-6 PUFA (L6) diet or a high n-3 plus low n-6 PUFA (H3-L6) 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-hour 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 2000g 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 (0.14 nmol/μl) for 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 re-extracted with 2 ml 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 and phospholipid classes 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 to 6, absolute baseline fatty acid concentrations in plasma phospholipids, triglycerides, cholesteryl esters and unesterified fatty acids did not differ

significantly between the groups (P > 0.05 by Mann-Whitney U test). Baseline absolute concentrations for esterified and unesterified fatty acids are comparable to a previous report in humans exposed to similar North American intakes of LA [31]. The percent composition in the various lipid pools are presented in Supplementary Tables 1 to 4, and are comparable to values in other studies [32, 33].

3.2. Phospholipid fatty acid concentrations

Table 3 shows median phospholipid fatty acid concentrations. Compared to baseline, dietary n-6 lowering (L6 group) decreased absolute concentrations (nmol / ml) of LA (12%) and n-6 DPA (22:5n-6; 26%), and increased concentrations of oleic acid (18:1n-9; 9%), α-LNA (20%), EPA (33%), n-3 DPA (22:5n-3; 21%) and DHA (9%). In the H3-L6 group, absolute concentrations of LA, AA, docosatetraenoic acid (DTA, 22:4n-6) and n-6 DPA were reduced significantly compared to baseline by 19%, 20%, 28% and 50%, respectively, whereas α-LNA, EPA, n-3 DPA and DHA concentrations were increased by 39%, 271%, 25% and 112%, respectively. The changes in LA, AA, EPA, DTA, n-6 DPA and DHA concentrations relative to baseline were higher in the H3-L6 than the L6 group, as evidenced by the statistically significant differences between the L6 and H3-L6 groups at 12 weeks (P < 0.05 by Mann-Whitney U test, final column in Table 3).

3.3. Triglyceride fatty acid concentrations

Within triglycerides, only the LA concentration was changed significantly (−29%) compared to baseline in the L6 group (Table 4). The H3-L6 intervention produced a comparable reduction in triglyceride LA concentration (−24%), with concurrent reductions in n-6 DPA and AA (−25 to −29%), and increased α-LNA, EPA, n-3 DPA and DHA concentrations (+57 to 216%). At 12 weeks, EPA and DHA concentrations in the H3-L6 group were significantly higher than in the L6 group (P < 0.05 by Mann-Whitney U test, final column in Table 4).

3.4. Cholesteryl ester fatty acid concentrations

In cholesteryl ester, n-6 lowering for 12 weeks increased palmitic acid, oleic acid, α-LNA, EPA and DHA concentrations by 7–45%, and decreased LA concentration by 9% compared to baseline (Table 5). The H3-L6 intervention significantly reduced AA by 13% (but not LA) and increased palmitic acid, oleic acid, α-LNA, EPA and DHA concentrations by 21%, 90%, 365% and 99%, respectively (P < 0.001), compared to baseline. At 12 weeks, α-LNA and EPA were significantly higher in the H3-L6 compared to the L6 group (P < 0.05 by Mann-Whitney U test, final column in Table 5).

3.5. Unesterified fatty acid concentrations

Compared to baseline, the EPA concentration within unesterified fatty acids was significantly increased (+30%) in the L6 group at 12 weeks (Table 6). This increase was much higher in the H3-L6 group (+199%; P < 0.001). n-3 DPA and DHA were significantly higher by 19% and 221%, respectively, in the H3-L6 group at 12 weeks compared to baseline (P<0.01). Statistical comparison of the medians at 12 weeks by the Mann-Whitney

U test indicated that EPA and DHA were significantly higher in the H3-L6 compared to the L6 group.

4. Discussion

Dietary n-6 PUFA lowering (the L6 intervention) for 12 weeks did not significantly alter the AA concentration (nmol/ml) in any esterified or unesterified plasma lipid fraction of headache patients, but did increase the n-3 PUFA concentration of both esterified and unesterified plasma lipids. By contrast, dietary LA lowering with concurrent increase in dietary EPA and DHA (the H3-L6 intervention) significantly reduced plasma AA concentration, and produced significantly greater increases in n-3 PUFA concentrations in esterified and unesterified plasma lipids.

4.1. The L6 intervention

Findings in the L6 group are consistent with previous reports showing that dietary LA lowering for 8–12 weeks in humans without concurrent increases in dietary n-3 PUFA significantly increased n-3 EPA, DPA and DHA concentrations in various circulating lipid pools [34, 18, 24]. Increases in plasma esterified EPA, n-3 DPA and DHA concentrations produced by the L6 intervention could reflect increased hepatic synthesis-secretion from α-LNA, because dietary LA lowering might enhance the elongation-desaturation of α-LNA by reducing LA substrate availability and subsequent competition between LA and α-LNA for liver conversion into their respective longer-chain PUFAs [35]. This interpretation is consistent with evidence of increased hepatic desaturase and elongase transcription [35] and increased plasma and tissue EPA, n-3 DPA and DHA concentrations in rats fed a low LA diet [36, 16]. Since dietary LA also competes with n-3 EPA and DHA for esterification within liver phospholipids [37], reduced competition for esterification also may have contributed to the observed increase in n-3 PUFA concentration of phospholipids, triglycerides and cholesteryl esters in the L6 group.

The failure of the L6 intervention to significantly reduce the AA concentration in any plasma lipid pool is consistent with our erythrocyte assays from the same trial showing no change in AA percent composition [19, 24]. Interestingly, this L6 intervention produced significant reductions in the absolute concentrations of DTA and n-6 DPA (products of AA elongation / desaturation) in plasma phospholipids, despite the lack of reduction in AA. This discrepancy suggests that a diet-induced reduction in AA synthesis may be offset by homeostatic mechanisms that maintain AA concentrations, such as increased adipose mobilization of AA by hormone-sensitive lipase [38] or reduced metabolic conversion of AA into bioactive mediators [24]. Reduced AA metabolism is consistent with our report that the L6 intervention significantly reduced several bioactive hydroxy-eicosatetraenoic acid (HETE) derivatives of AA in plasma, without altering plasma or erythrocyte AA content [19, 24].

Plasma reductions in esterified AA concentration in the L6 group might become evident during a study sufficiently long to reduce adipose tissue AA stores, which may take years in view of 1–2 year fatty acid half-lives in human adipose tissue [39]. The disconnect between

diet-induced changes in AA and AA-derived bioactive mediators suggests that simply measuring fatty acid concentrations may not adequately reflect metabolic state.

4.2. The H3-L6 intervention

The more marked increase (>10-fold) in esterified n-3 PUFAs in the H3-L6 diet compared to the L6 diet group was likely due to competition between dietary (preformed) n-3 EPA and DHA and other n-6 PUFAs for esterification. Since hepatic synthesis of EPA and DHA from α-LNA can be inhibited by dietary EPA and /or DHA supplementation in humans and rodents [3, 40, 41], the contribution of α-LNA to the increased long-chain plasma n-3 PUFA concentrations in the H3-L6 group likely was small compared to that of preformed dietary EPA and DHA. Consistent with this interpretation, clinical trials of α-LNA supplementation for up to one year did not change plasma DHA concentrations [42, 43].

The decrease in AA concentration in plasma phospholipid, triglyceride and cholesteryl esters in the H3-L6 group compared to the L6 group likely reflects reduced AA synthesis-secretion, since the consumption of EPA and DHA likely reduces the activity of elongase and desaturase enzymes involved in both AA and EPA synthesis [3, 41]. The observed reduction in AA concentration may also reflect competition of ingested EPA and DHA with endogenous AA for incorporation into phospholipids, triglycerides or cholesteryl esters within liver [44].

Adipose tissue unesterified fatty acid release and circulating esterified fatty acids secreted by the liver are the two principle sources of circulating unesterified fatty acids, the species that are preferentially incorporated into the brain [45, 46]. In this study, unesterified plasma EPA was increased in the L6 group, and unesterified plasma EPA, n-3 DPA and DHA were increased in the H3-L6 group, consistent with the increases in their respective esterified concentrations. By contrast, unesterified n-6 PUFA concentrations did not change in either group, despite being reduced within their esterified pools. LA presently accounts for 14–18% of total adipose fatty acids in the North American population [47], compared to about 6% in 1961 [48, 49], suggesting that a prolonged period of LA-lowering may be necessary to sufficiently reduce adipose stores and decrease LA in the unesterified fatty acid pool. Clearly, a different and more rapid homeostatic state was attained for n-3 PUFAs, which are much less abundant in adipose tissue (< 2%) compared to n-6 PUFAs (~20%) [47].

4.3. Potential implications for brain PUFA metabolism and neuroinflammation

In rats, dietary LA lowering from 3.5% to 0.3% energy for 15 weeks increased n-3 PUFA concentrations and decreased plasma unesterified and esterified LA and AA concentrations [17]. These changes were accompanied by decreased expression and activity of AA-releasing calcium-dependent phospholipase A_2 ($cPLA_2$) IVA and AA-metabolizing cyclooxygenase-2 (COX-2), and increased expression and activity of DHA-releasing calcium-independent phospholipase A_2 ($iPLA_2$ VIA) [50]. Since $cPLA_2$ and COX-2 are upregulated during neuroinflammation, and increased $iPLA_2$ VIA reflects increased DHA metabolism into bioactive pro-resolving metabolites, it is possible that that the H3-L6 intervention (and to a lesser extent the L6 intervention) would attenuate neuroinflammation. This may explain the efficacy of the H3-L6 diet in reducing headache frequency in patients

with chronic migraine [24]. Future trials are needed to determine the efficacy of n-6 PUFA lowering and n-3 supplementation on brain PUFA metabolism and neuroinflammation in humans.

4.4. Limitations

The present trial was conducted in a population with high n-6 LA and low n-3 EPA+DHA consumption at baseline. The magnitudes of changes in plasma lipids may differ in populations with different dietary characteristics. Since this study was conducted in a predominantly female patient population with chronic headaches, the observed biochemical changes are not necessarily generalizable to men or neurologically healthy individuals.

4.5. Conclusion

In conclusion, 12 weeks of dietary n-6 lowering increased concentrations of n-3 PUFAs in plasma esterified and unesterified lipids, and a combination of dietary LA lowering with concurrent increases in EPA and DHA further increased circulating n-3 PUFA levels and reduced esterified AA and other n-6 PUFA concentrations in a patient population with chronic headaches.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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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

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Table 1

Diet fatty acid composition:

Changes pre-post diet intervention								
	L6 diet (n=28)			H3-L6 diet (n=27)			Between Diets	
	Pre	Post		Pre	Post		Pre	Post
Variable	Median (25%, 75%)	Median (25%, 75%)	P value	Median (25%, 75%)	Median (25%, 75%)	P value	P value	P value
Total energy (kcal)	1997 (1487, 2253)	1859 (1411, 2199)	0.52	1707 (1377, 1880)	1596 (1293,1959)	0.89	0.03	0.09
Total protein (en%)	15.7 (13.8, 16.8)	15.2 (13.7, 17.0)	0.13	16.1 (13.5, 19.6)	17.2 (15.1, 20.0)	0.25	0.62	0.01
Total fat (en%)	33.6 (29.6, 40.1)	30.4 (26.8, 34.3)	0.05	33.4 (29.1, 36.4)	30.7 (27.3, 34.0)	0·08	0.38	0.84
LA 18:2 (en%)	7.4 (5.7, 9.6)	2.4 (2.0, 2.9)	<0.001	6.4 (5.3, 7.4)	2.5 (2.2, 3.9)	<0.001	0.03	0.15
α-LNA 18:3 (en%)	0.7 (0.6, 0.9)	0.7 (0.6, 0.9)	0.96	0.6 (0.5, 0.9)	1.6 (1.3, 2.0)	<0.001	0.32	<0.001
AA 20:4 (mg)	106 (57, 159)	48* (18, 74)	<0.001	110 (66, 176)	114* (69, 195)	0.75	0.64	<0.001
EPA + DHA (mg)	43 (25, 73)	76* (19, 264)	0.32	47 (20, 71)	1482* (374, 2558)	<0.001	0.96	<0.001
Total SFA (en%)	10.5 (9.1, 11.9)	14.0 (12.0, 17.2)	<0.001	10.5 (9.8, 11.7)	12.9 (9.9, 14.5)	0.16	0.99	0.06
Total Trans (en%)	0.9 (0.7, 1.2)	0.6 (0.5, 0.84)	0.005	1.1 (0.9, 1.4)	0.5 (0.3, 0.7)	<0.001	0.07	0.06
Total MUFA (en%)	11.8 (10.1, 13.2)	10.4 (8.0, 12.6)	0.008	12.1 (11.0, 13.5)	9.3 (8.4, 11.4)	<0.001	0.82	0.51
Total PUFA (en%)	8.1 (6.4, 10.6)	3.5 (3.1, 4.3)	<0.001	7.4 (5.9, 8.5)	6.0 (5.1, 7.1)	0.1	0.05	<0.001

Adapted from [19]. Diet fatty acid composition pre and post intervention, expressed as energy % (en%) or mg.

SFA, saturated fatty acids; Trans, trans fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; LA, linoleic acid; α-LNA, α-linolenic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

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.

Table 2

Baseline characteristics of 67 patients with chronic headaches

	H3-L6 diet n = 33	L6 diet n = 34
Age, y, mean (SD)	41 (13.4)	42 (11.1)
Female, n (%)	28 (84.8)	30 (88.2)
White race, n (%)	28 (84.8)	30 (88.1)
Married, n (%)	19 (57.6)	19 (55.9)
Education, n (%)		
High school or college	20 (60.6)	19 (59.3)
Master's degree or higher	13 (40.6)	13 (39.4)
Employment, n (%)		
Employed/student	26 (78.8)	23 (69.7)
Retired/Caretaker	3 (9.1)	3 (9.1)
Disabled/unemployed	4 (12.1)	7 (20.0)
Headache days per month, mean (SD)	23.3 (20.9, 25.8)	23.2 (20.2, 25.8)
Headache hours per day, mean (SD)	10.2 (8.4, 12.3)	9.8 (8.1, 11.8)
Number of different headache-related medications, mean (SD)	6.4 (3.4)	5.6 (3.3)

Adapted from reference [24]., number of subjects;%, proportion of subjects; SD, standard deviation.

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Table 3

Pre- and post-intervention phospholipid fatty acids concentrations (nmol/ml)

	Changes pre-post diet intervention									
	L6 diet (n=28)				H3-L6 diet (n=27)				Between Diets	
	Pre	Post			Pre	Post			Pre	Post
Variable (nmol/ml)	Median (25%, 75%)	Median(25%, 75%)	% Change	P value	Median(25%, 75%)	Median (25%, 75%)	% Change	P value	P value	P value
LA	838.58 (765.18,984.43)	742.13 (643.09,820.44)	−11.50	<0.001	763.38(721.40,919.94)	620.52 (550.79,753.91)	−18.71	<0.001	0.178	0.030
AA	382.21 (306.80,440.84)	333.04 (280.54,445.66)	−12.87	0.088	356.18 (292.04,421.84)	286.64 (255.68,365.97)	−19.53	<0.001	0.960	0.043
DTA	13.63 (9.69,15.40)	11.85 (9.26,15.18)	−13.06	0.072	11.80 (9.81,14.31)	8.44 (7.06,9.68)	−28.49	<0.001	0.439	0.001
n-6 DPA	10.79 (6.08,12.83)	8.00(6.46,11.47)	−25.82	0.007	9.03 (6.01,10.64)	4.48* (3.67,5.68)	−50.43	<0.001	0.201	<0.001
α-LNA	7.57 (6.19,9.04)	9.06 (6.62,13.01)	19.73	0.020	6.92 (5.39,10.32)	9.65 (5.75,13.42)	39.47	0.038	0.769	0.853
EPA	20.35 (11.78,25.18)	27.09 (20.31,35.68)	33.10	0.001	17.28 (13.44,22.40)	64.13 (37.50,80.09)	271.08	<0.001	0.400	<0.001
n-3 DPA	21.81 (18.18,26.82)	26.39 (18.88,31.81)	21.01	0.021	19.99 (14.61,22.81)	25.06 (20.78,33.78)	25.34	0.001	0.110	0.814
DHA	76.84 (56.10,98.64)	83.86 (66.91,113.49)	9.13	0.011	71.18 (61.61,109.20)	150.72 (112.18,181.80)	111.74	<0.001	0.920	<0.001
Palmitic acid	1134.30 (939.56,1326.62)	1172.08 (1046.50,1293.27)	3.33	0.524	1121.47 (927.88,1352.70)	1131.08 (940.30,1379.20)	0.86	0.325	0.853	0.933
Oleic acid	334.48 (289.70,396.40)	365.06 (307.76,425.62)	9.14	0.036	332.96 (279.61,407.08)	354.82 (284.22,409.46)	6.57	0.904	1.000	0.274
Total fatty acids	3700 (3254,4162)	3681 (3252,4008)	−0.52	0.733	3495.03 (3153,3971)	3504 (3110,3953)	0.26	0.456	0.556	0.409

Plasma phospholipid fatty acid concentrations (nmol/ml) pre and post intervention.

LA, linoleic acid; AA, arachidonic acid; DTA, docosatetraenoic acid; n-6 DPA, n-6 docosapentaenoic acid; α-LNA, α-linolenic acid; EPA, eicosapentaenoic acid; n-3 DPA, n-3 docosapentaenoic acid; DHA, docosahexaenoic acid.

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.

Table 4

Pre- and post-intervention triglyceride fatty acids concentrations (nmol/ml)

	Changes pre-post diet intervention									
	L6 diet (n=28)				H3-L6 diet (n=27)					
	Pre	Post			Pre	Post			Between Diets	
Variable (nmol/ml)	Median (25%, 75%)	Median (25%,75%)	% change	P	Median(25%, 75%)	Median (25%, 75%)	% change	P	Pre	Post
LA	613.48 (346.72, 784.84)	436.37 (315.04, 652.69)	−28.87	0.002	471.49 (304.00, 689.71)	356.24 (259.04, 504.20)	−24.44	0.002	.201	0.130
AA	37.20 (21.98, 51.70)	28.20 (20.20, 43.64)	−24.18	0.210	38.63 (24.79, 53.45)	28.90 (20.44, 34.58)	−25.18	0.024	.840	0.686
DTA	5.33 (4.02, 7.45)	4.78 (3.15, 7.32)	−10.38	0.648	5.59 (3.57, 6.95)	4.96 (3.43, 5.62)	−11.28	0.191	.986	0.880
DPAn6	2.30 (1.79, 3.75)	2.26 (1.89, 4.52)	−1.57	0.545	2.79 (1.76, 3.80)	1.98 (1.65, 2.66)	−29.05	0.035	.670	0.138
α-LNA	36.52 (18.22, 52.86)	32.87 (22.45, 42.24)	−10.00	0.316	21.71 (14.98, 46.36)	34.01 (19.27, 56.43)	56.66	0.034	.219	0.419
EPA	4.57 (2.70, 6.69)	5.71 (3.53, 7.11)	24.99	0.080	3.94 (2.71, 6.52)	12.44 (5.99, 22.62)	216.01	0.000	.703	0.000
DPAn3	4.84 (4.08, 8.06)	5.73 (3.72, 8.66)	18.29	0.486	3.85 (2.44, 5.89)	7.77 (4.70, 11.23)	101.95	0.000	.058	0.132
DHA	7.56 (3.85, 11.56)	8.91 (4.42, 13.77)	17.84	0.187	6.45 (3.11, 10.96)	20.40 (14.17, 45.56)	216.31	0.000	.614	0.000
Palmitic acid	786.35 (384.74, 992.81)	720.41 (501.79, 1089.82)	−8.39	0.387	587.49 (449.54, 1013.38)	584.31 (421.25, 988.54)	−0.54	0.564	.662	0.337
Oleic acid	985.83 (554.29, 1159.58)	945.73 (668.89, 1239.75)	−4.07	0.295	816.74 (495.32, 1203.88)	766.35 (472.01, 1223)	−6.17	0.564	.449	0.207
Total fatty acids	2887 (1689, 3617)	2696 (1876, 3482)	−6.61	0.750	2359. (1582, 3269)	2106 (1514, 3353)	−10.74	0.349	.480	0.232

Plasma triglyceride fatty acid concentrations (nmol/ml) pre and post intervention.

LA, linoleic acid; AA, arachidonic acid; DTA, docosatetraenoic acid; n-6 DPA, n-6 docosapentaenoic acid; α-LNA, α-linolenic acid; EPA, eicosapentaenoic acid; n-3 DPA, n-3 docosapentaenoic acid; DHA, docosahexaenoic acid.

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.

Table 5

Pre- and post-intervention cholesteryl ester fatty acid concentrations (nmol/ml)

	Changes pre-post diet intervention									
	L6 diet (n=28)				H3-L6 diet (n=27)					
	Pre	Post			Pre	Post			Between Group	
Variable (nmol/ml)	Median (25%, 75%)	Median (25%, 75%)	% change	P	Median (25%, 75%)	Median (25%, 75%)	% change	P	Pre	Post
LA	1618.47 (1457.38, 1922.56)	1467.89 (1301.19, 1711.10)	−9.30	0.001	1570.69 (1281.42, 1706.78)	1429.18 (1166.21, 1628.61)	−9.01	0.118	0.096	0.297
AA	199.99 (149.98, 278.79)	172.10 (144.89, 252.91)	−13.95	0.088	195.71 (142.57, 268.79)	169.98 (148.48, 211.88)	−13.15	0.017	0.960	0.533
α-LNA	15.63 (12.74, 18.82)	22.67 (18.19, 31.76)	45.06	0.001	14.55 (9.62, 18.09)	27.70 (22.63, 44.07)	90.33	0.000	0.184	0.049
EPA	14.85 (9.47, 20.21)	21.27 (15.16, 29.41)	43.18	0.004	12.34 (8.74, 17.09)	57.37 (28.41, 82.16)	365.03	0.000	0.259	0.000
DHA	13.59 (7.47, 82.53)	17.60 (10.77, 80.28)	29.52	0.018	11.22 (8.01, 73.77)	22.33 (19.60, 98.22)	99.06	.0002	0.699	0.106
Palmitic acid	351.34 (306.49, 429.57)	376.40 (309.70, 433.11)	7.13	.0210	344.34 (305.11, 403.35)	404.04 (308.79, 455.38)	17.34	0.000	0.590	0.490
Oleic acid	492.68 (427.56, 592.96)	556.90 (499.60, 652.53)	13.03	0.045	491.97 (425.77, 555.70)	594.52 (453.05, 680.24)	20.85	0.000	0.544	0.724
Total fatty acids	2808 (2549, 3507)	3140 (2532, 3433)	11.83	0.400	2827 (2460, 3162)	2903 (2598, 3414)	2.69	0.118	0.391	0.775

Plasma cholesteryl ester fatty acid concentrations (nmol/ml) pre and post intervention.

LA, linoleic acid; AA, arachidonic acid; α-LNA, α-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

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.

Table 6

Pre- and post-intervention unesterified fatty acid concentrations (nmol/ml)

	Changes pre-post diet intervention									
	L6 diet (n=28)				H3-L6 diet (n=27)					
	Pre	Post			Pre	Post			Between Group	
Variable (nmol/ml)	Median (25%,75%)	Median (25%,75%)	%Change	P	Median (25%,75%)	Median (25%,75%)	%Change	P	Pre	Post
LA	57.99 (36.51, 78.58)	54.91 (45.33, 73.23)	−5.30	.964	57.89 (29.73, 80.62)	52.47 (40.33, 65.35)	−9.36	0.325	0.736	0.400
DGLA	2.19 (1.55, 2.74)	2.05 (1.34, 2.53)	−6.53	.501	1.70 (1.05, 2.94)	1.79 (1.37, 2.28)	5.61	0.829	0.413	0.400
AA	1.82 (1.59, 2.72)	2.05 (1.53, 2.80)	12.75	.767	2.14 (1.42, 2.87)	2.22 (1.70, 2.62)	3.73	0.773	0.749	0.724
DTA	3.91 (3.66, 4.45)	4.00 (3.58, 4.36)	2.10	.509	3.74 (3.28, 4.17)	3.93 (3.59, 4.42)	5.08	0.195	0.035	0.711
n-6 DPA	0.23 (0.14, 0.31)	0.22 (0.18, 0.26)	−4.94	.975	0.20 (0.18, 0.29)	0.18 (0.15, 0.21)	−8.03	0.125	0.947	0.114
EPA[1]	0.22 (0.16, 0.32)	0.29 (0.21, 0.36)	29.60	.039	0.18 (0.16, 0.25)	0.53 (0.37, 0.82)	198.52	0.001	0.303	0.001
n-3 DPA	0.38 (0.19, 0.57)	0.36 (0.22, 0.55)	−4.94	.639	0.35 (0.23, 0.45)	0.41 (0.35, 0.63)	18.68	0.007	0.560	0.187
DHA	0.90 (0.70, 1.70)	1.10 (0.75, 1.32)	22.77	.354	0.77 (0.60, 1.54)	2.47 (1.30, 3.39)	220.73	0.001	0.594	0.001
Palmitic acid	90.84 (68.31, 111.64)	88.14 (70.80, 119.86)	−2.97	.616	99.22 (59.46, 115.91)	96.93 (71.12, 118.07)	−2.31	0.981	0.762	0.827
Oleic acid	114.74 (92.16, 172.58)	135.61 (103.45, 182.08)	18.19	.246	115.78 (65.72, 168.16)	133.33 (96.25, 160.89)	15.16	0.904	0.775	0.400
Total fatty acids	335.99 (260.61, 446.12)	356.64 (275.96, 478.34)	6.14	.480	369.52 (214.80, 440.40)	366.26 (274.80, 418.77)	−0.88	0.962	0.814	0.567

Plasma unesterified fatty acid concentrations (nmol/ml) pre and post intervention.

[1]Unesterified α-LNA is not reported because it was not detected in most samples.

LA, linoleic acid; AA, arachidonic acid; DTA, docosatetraenoic acid; n-6 DPA, n-6 docosapentaenoic acid; α-LNA, α-linolenic acid; EPA, eicosapentaenoic acid; n-3 DPA, n-3 docosapentaenoic acid; DHA, docosahexaenoic acid.

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.