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A sixteen-week three-armed, randomized, controlled trial investigating clinical and biochemical effects of targeted alterations in dietary linoleic acid and n-3 EPA+DHA in adults with episodic migraine: Study protocol

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Authors' contributions

Drs. Mann, Faurot, Palsson, Suchindran, Gaylord, MacIntosh, Barrow, Hibbeln, and Ramsden participated in study design. Dr. Mann, Dr. Faurot, Ms. MacIntosh, Dr. Palsson, Ms. Lynch, and Ms. Johnston have collected data and implemented the intervention. Dr. Suchindran and Dr. Faurot have provided statistical advice and input. All authors have participated in drafting the manuscript, and have read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Ethics approval and consent to participate

Ethics approval was provided by the Human Research Ethics Committee of the University of North Carolina (IRB# 13–3284) and all participants have provided written informed consent.

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Abstract

Migraine is a prevalent neurological disorder, affecting over 16% of adult women and 7% of adult men in the U.S., causing significant pain, disability, and medical expense, with incomplete benefits from conventional medical management. Migraine, as a chronic pain syndrome, provides a practical model for investigating the impact of dietary modifications in omega-3 (n-3) and omega-6 (n-6) fatty acids. This paper reports the protocol of a trial to assess whether targeted dietary modifications designed to increase n-3 eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), with or without concurrent reduction in n-6 linoleic acid (LA), will alter nociceptive lipid mediators and mediate decreases in frequency and severity of migraine. This prospective, randomized, controlled trial in 153 male and female adult subjects, ages 18–99, with diagnosed and actively managed episodic migraine tests the efficacy, safety, and biochemical effects of targeted, controlled alterations in dietary omega-3 and omega-6 fatty acids. Participants are masked to diet hypotheses and all assessors are masked to treatment assignment. Following a four-week baseline period, participants with migraine headache frequency of 5–20 per month are randomized to one of three intensive dietary regimens for 16 additional weeks followed by a less intensive observation period. Dietary intervention arms include: 1) increased n-3 EPA+DHA with low n-6 linoleic acid (H3 L6); 2) increased n-3 EPA+DHA with usual US dietary intake of n-6 linoleic acid (H3 H6); and 3) usual US dietary content of n-3 and n-6 fatty acids (L3 H6). During the actual intervention, subjects receive content-specific study oils and foods sufficient for two meals and two snacks per day, as well as dietary counseling. Biochemical and clinical outcome measures are performed at intervals throughout this period. This randomized controlled trial is designed to determine whether targeted alterations in dietary n-3 and n-6 fatty acids can alter nociceptive lipid mediators in a manner that decreases headache pain and enhances quality of life and function in adults with frequent migraines.

Trial registration: NCT02012790

Keywords

Migraine; Omega-3; Omega-6; Linoleic acid; Lipid mediator; Oxylipin; pain; Headache; Diet; RCT

1. Introduction

Migraine is a common cause of chronic pain and the most prevalent neurologic disorder, [1,2] affecting over 16% of adult women and 7% of adult men. It is a major cause of

disability in the U.S., with loss of work and medical expenses adding up to billions of dollars per year [1]. Patients with migraine often receive only limited benefit from conventional medical management. While abortive therapy for acute migraine has improved substantially [2], preventative strategies—particularly for patients with more frequent headaches—often provide only modest benefit [3]. Conventional treatments rely heavily on medications that may provide only limited or transient relief, target symptoms rather than addressing the underlying causes of pain, and are associated with significant negative side effects and costs [4–11]. Medication side effects include medication overuse syndromes, as well as weight gain, fatigue, alopecia, tremor, constipation, cognitive impairment, and depression [1,5,8–10,12].

Migraine provides a practical model for investigating causes and treatments for chronic pathological pain syndromes because it shares underlying biochemical mechanisms with other chronic pain syndromes, such as peripheral and central sensitization [13], as well as neurologic features such as autonomic (flushing, chills, etc.), gastrointestinal (nausea, vomiting, diarrhea), sensory (photophobia, phonophobia, osmophobia and allodynia) and cognitive changes [14]. In addition, migraine is an extensively researched clinical condition with reliable self-report measures that are sensitive to change with treatment[15].

1.1. Dietary modulation of nociceptive mediators and physical pain

N-6 and n-3 fatty acids regulate multiple inflammation and pain-related biochemical pathways as major components of meninges, myelin, glia, skeletal muscle, and neuronal cell membranes [16]. N-6 and n-3 fatty acids can be converted to lipid mediators with pro- and anti-nociceptive properties (e.g. prostanoids [17], octadecanoids [18–20], endocannabinoids [21], n-3 monoepoxides [22], and resolvins [23–25]). With a few notable exceptions (e.g. lipoxins and epoxyeicosatrienoic acids), lipid mediators derived from n-6 fatty acids have pro-nociceptive properties, while mediators derived from n-3 fatty acids have anti-nociceptive and pro-resolvin properties [17–24].

Our preliminary study, in a chronic daily headache (CDH) population (in which 88% of subjects had either chronic migraine or mixed headache with significant migraine features) sought to establish the feasibility and preliminary efficacy of targeted reductions in dietary n-6 LA, with and without concurrent increases in n-3 EPA and DHA. Results from this two armed, randomized pilot trial, with 67 CDH patients, demonstrated the feasibility of using intensive dietitian support and food provision in community dwelling patients to elicit targeted changes in dietary n-6 and n-3 fatty acids [26], and that the targeted changes in dietary intakes, erythrocyte fatty acids, and nociceptive lipid mediators could be achieved. Moreover, the high n-3 EPA + DHA, low n-6 LA (H3-L6) diet intervention produced a large, statistically significant and clinically-relevant improvement in headache frequency, intensity and other clinical outcomes compared to baseline, and compared to the Low n-6 only (L6) group. Those clinical effects were accompanied by statistically significant reductions in vasoactive abortive medication use, as well as total use of acute and adjunctive medications. The H3-L6 intervention produced marked increases in anti-nociceptive derivatives of EPA and DHA, and significant reductions in pronociceptive derivatives of n-6 LA and AA, while the L6 group had comparatively modest, but statistically significant biochemical changes

and improvement in headache-related clinical outcomes compared to baseline. However, these changes were significantly weaker than those seen in subjects undergoing the combined H3-L6 intervention [27].

In the previous study, both trial arms involved lowering of dietary n-6 LA to about 2.5% of calories, a level not usually found in modern U.S. diets. The current study seeks to examine the role that dietary n-6 LA lowering may play in the observed biochemical and clinical effects and to clarify the effects that can be achieved by increasing dietary EPA+DHA alone, without concurrent LA lowering. It is essential to determine whether LA lowering is necessary for clinical benefit since LA lowering is challenging in a U.S. population due to widespread addition of LA to packaged and restaurant foods. In the current, three-armed RCT, we address this knowledge gap by 1) comparing the effects of a H3-L6 intervention to a High n-3 intervention without a concurrent reduction in n-6 LA (H3-H6); 2) adding a control group consuming average U.S. amounts of n-3 and n-6 fatty acids (L3-H6). We also extend the active intervention phase to 16 weeks to determine the longer-term biochemical and clinical effects of these interventions.

2. Materials and methods

2.1. Study aims

This is a 3-arm, parallel-group, randomized, controlled 16-week trial to evaluate the biochemical effects and therapeutic efficacy of two dietary interventions in patients with episodic migraine compared with a control diet group.

The project's primary objective is to assess whether targeted dietary modifications designed to increase dietary n-3 EPA and DHA, with or without concurrent reduction in n-6 LA, can increase analgesic derivatives of n-3 EPA and DHA, and improve headache-related clinical outcomes. This modified double-blind randomized controlled trial design will randomize 153 participants to one of three 16-week dietary interventions:

Diet A: High n-3 EPA+DHA, Low n-6 LA (H3-L6) (See Preliminary Data)

Diet B: High n-3 EPA+DHA, High n-6 LA (H3-H6)

Diet C: Low n-3 EPA+DHA, High n-6 LA (L3-H6, average U.S. in-take; control group)

Target intakes of n-3 EPA+DHA and n-6 LA for each diet are provided in Table 1. The control amounts of LA (7.2% of energy (%E), Diet B and Diet C) and EPA+DHA (150 mg per 200 cal, Diet C) are comparable to average US intakes of these nutrients. The target dose selected for lowering of dietary LA (1.8%E, Diet A) was selected to be consistent with estimated intakes in historical diets comprised of un-processed foods prior to the widespread addition of seed oils in industrialized diets. The target for increasing EPA+DHA (to 1500 mg per 2000 cal, Diet A and Diet B) was selected based on the levels that were achievable in our pilot study [27]; these intakes resulted in major biochemical changes and appear to have reduced pain.

Specific Aim 1. is to assess the efficacy of the dietary interventions in inducing the predicted changes in circulating n-3 and n-6 derivatives.

Hypothesis 1. (1a) Compared to Diet C, Diet A will produce significant increases in anti-nociceptive derivatives of n-3 EPA and DHA (*Primary Biochemical Outcome, 17-hydroxy DHA*) and reductions in pronociceptive n-6 AA-derived and LA-derived mediators. (1b) Diet B (High n-3, High n-6 LA) will result in values for n-3 and n-6 derivatives intermediate between Diet A and Diet C.

Specific Aim 2. is to compare the clinical efficacy of the dietary interventions in adults with migraine.

Hypothesis 2. (2a) Compared to Diet C, Diet A will produce significant improvement in the Headache Impact Test score—a headache-specific quality of life measure (*Primary Clinical Outcome*). (2b) Additionally, as measured in the daily Headache Diary, Diet A will result in a significantly steeper rate of decrease in headache hours per day as compared with Diet C. (2c) Diet B will result in changes in clinical outcomes intermediate between Diet A and Diet C.

A secondary objective, **Specific Aim 3**, is to test our model of the proposed causal chain (Fig. 4) linking changes in n-3 and n-6 fatty acids and their bioactive derivatives to headache endpoints.

Hypothesis 3. (3a) Increases in erythrocyte and free fatty acid n-3 EPA and DHA and (3b) their anti-nociceptive derivatives will be positively associated with improvement in headache-specific quality-of-life and mean headache hours per day; (3c) reductions in n-6 LA and AA, and (3d) their pro-nociceptive derivatives will be positively associated with headache-specific quality-of-life and mean headache hours per day.

2.2. Overview of recruitment and study procedures

Ethics approval for the study was provided by the Human Research Ethics Committee of the University of North Carolina (IRB# 13–3284), and all participants have provided written informed consent.

Approximately 2250 patients with episodic migraine are being recruited from central North Carolina clinics and practices, including headache clinics. Self-referrals are accepted unless the patient is not under the care of a physician who is managing headaches. Physicians in the community are informed of the study by direct contact and informational handouts so that they may inform their patients of the study and provide them with contact information and/or permission to have members of the research team contact them.

Potential participants are screened to achieve a pre-intervention sample size of a minimum of 51 subjects per group for each of the three study arms. We expect at least 42 of the 51 in each group to complete the 16-week intervention, which will provide adequate power to test both the primary clinical and biochemical aims (Specific Aims 1 and 2).

In addition to history, physical examination, and documentation of inclusion criteria, participants complete baseline assessment instruments that measure headache characteristics and their impact on quality-of-life. Participants who are compliant with all aspects of data collection, and who experience migraine headaches between 5 and 20 days per month, are eligible for the study. After an initial 4- to 6-week baseline phase for monitoring usual dietary intake, diary adherence, and headache characteristics, 153 participants are planned to be randomized to 1 of the 3 study diets (Table 1, Fig. 1), to be maintained for 16 consecutive weeks. All participants will continue to receive usual medical care for their headaches throughout the 4- to 6-week baseline phase, and the 16-week intervention period. Eligibility is based on inclusion and exclusion criteria (Table 2).

2.2.1. Participant flow—At the end of the baseline phase, participants who qualify are randomized to one of the following arms: 1) High n-3, Low n-6 (H3-L6) Diet A; 2) High n-3, High n-6 (H3-H6) Diet B; or 3) the Low n-3, High n-6 (L3-H6) Diet C. After randomization, participants attend an initial, in-depth dietary counseling session administered by the registered dietitian. Participants receive detailed instruction in the specific dietary intervention per group assignment. Participants in each group attend five blood draw visits and six follow-up sessions with the dietitian. At each visit, the dietitian reviews and records dietary adherence per diet history, daily food checklists and subject self-report, assesses diet education needs, provides tailored diet counseling and dispenses research food supply. Visits occur every 2 weeks for the first 4 weeks of the active intervention and then every 3 weeks for the remainder of the 16-week intervention. At the 16-week visit, participants are asked to continue following the diet for the final six weeks of the study, but without regular intensive diet counseling and research food supply. The study concludes at a 22-week visit in which participants undergo their final blood draw and meet with the study team for final assessments and unmasking (Fig. 2).

2.2.2. Modified double-blind design—A modified double-blind study design is applied to ensure maximal masking of participants, investigators and staff. Masking is maintained in the random allocation procedure, and the research staff—including investigators, referring and research clinicians and laboratory personnel—are masked to treatment assignment for the full duration of the trial. Only the dietitian, by necessity, is not masked in order to administer the dietary interventions. Participants receive counseling one at a time, not in groups; the dietitian meets with each participant individually and provides them with diet information on only their assigned diet. The three study diets were designed to produce controlled alterations in (1) n-6 LA; and (2) n-3 EPA+DHA. The diets were carefully designed to look and taste similar, by using as many common products as possible and only differing in the added visible oils and/or animal sources of fat and protein. No details of the other diets are provided and participants are masked to hypotheses associated with diet assignments throughout the study.

2.3. Intervention protocol

2.3.1. Interventions, administration, and duration—The study intervention consists of providing food appropriate to the study arm assignment at two and three week intervals, sufficient for two meals and two snacks per day for the entire 16 weeks of the

active intervention. In addition, participants receive in depth diet counseling by a registered dietitian experienced in research, reinforcing dietary choices in accordance with their arm assignments. They have continuous access to comprehensive web-based information on best food choices, grocery shopping, dining out, and detailed menus and recipes. Participants are asked to self-monitor their dietary intake by tracking their intake on a daily food checklist that targets the foods to consume regularly and the foods to avoid. Blood samples are collected at the start of the intervention, then at 4, 10 and 16 weeks after the start of the intervention, and 6 weeks post-intervention.

All participants receive: 1) tailored dietitian-administered counseling consistent with the group assignment; 2) access to a password-protected project website including a headache diary for daily completion along with intervention-specific informational materials; and 3) food items throughout the 16-week period of active intervention - the equivalent of two meals and two snacks per day - with precisely quantified fatty acid composition, formulated to meet nutrient intake targets specific for each of the assignment groups.

Throughout the 22 weeks of active intervention and post-intervention phases, participants have access to a website as a user-friendly strategy to complement and reinforce dietitian advice. Online diet education materials include the diet guidelines, allowed food list, how to read food labels, detailed grocery shopping guides for 9 local grocery stores, dining out guide identifying allowable foods at local restaurants, 7-day meal plan and 75 recipes with the ability for the dietitian to add more.

2.3.1.1. Food supply.: Each participant visits the Research Kitchen located on UNC campus every two to three weeks during the 16-week intervention to obtain specific food items with targeted nutrient compositions. Food items include cooking oils, salad dressings, mayonnaise, portable dressing packets, prepared snack foods, prepared breakfast foods, unprepared frozen food items, ingredients for home preparation of meals and snacks, and several packaged food items.

All foods have been formulated, or specifically selected, to ensure that participants meet nutrient intake targets, specific to their diet assignment. Study-provided oil blends, formulated to meet the diet targets, are prepared and packaged into bottles marked only with their diet assignment. Salad dressings and study-provided foods are prepared with the oil blend. Fish and poultry are specifically selected for their EPA+ DHA content. Other grocery staples such as frozen fruits, frozen vegetables, bread, dairy and snack foods are provided to encourage intake of packaged foods with added oils. Nutrient analysis of more than 80 relevant food items is performed by the NIH-NIAAA Section of Nutritional Neurosciences (SNN) and used to develop the diet intervention. For foods not provided by the Research Kitchen, participants rely on their training by the dietitian and website diet guides for shopping and choosing foods in restaurants.

All intervention related visits take place in a clinical research space with adequate privacy and well trained, highly skilled personnel.

Food is dispensed from the Research Kitchen located on the UNC Medical Campus. Portable coolers on wheels are supplied to all participants to transport food items home. Participants are not required to return unused food portions.

A study folder and database is maintained to document participation in all aspects of the project including attendance at clinic visits, maintenance of headache diary, cooperation with the dietitian, and reporting of adverse events.

2.4. Assessments

Participants record headache characteristics, medication use, and psychological status using a daily, web-based headache diary throughout the 4 to 6-week baseline phase, 16-week intervention phase, and the 6-week post-intervention period. Diary access and updates can be made using a personal computer with internet access, a smart phone, or other compatible portable device that connects to the internet. During times when participants do not have internet access they are allowed to fill out paper diaries; paper diaries are then given to study staff for manual data entry.

Blood samples are collected for biochemical measurement during five visits: the start of the intervention phase, then after 4, 10, and 16 weeks of diet exposure in the intervention, and 6 weeks post-intervention (22 weeks). At each of these five visits, participants use the research clinic computer to complete the following online questionnaires: 1) Headache Impact test (HIT-6); 2) PROMIS-29 Profile, for assessment of anxiety, depression, fatigue, sleep disturbance, and physical and social role functioning; 3) The Total Pain Scale that looks at the comorbid pain assessment with severity index; 4) Satisfaction with Care, 5) Changes in Health; 6) Nicotine, Caffeine, and Alcohol scale; and 7) Hunger scale. Participants complete the Migraine Disability Assessment Score (MIDAS) at start of the intervention, at completion of the intervention phase (16 weeks), and at completion of the post-intervention phase (22 weeks). In addition, participants complete the Diet Acceptability Form at Weeks 4, 10, 16, and 22.

Dietary intake is assessed by unannounced, telephone administered, 24-h dietary recalls on two non-consecutive days during the baseline phase and repeated during the intervention phase and post-intervention phase. Hence, six total 24-h dietary recall attempts are administered per subject by trained interviewers at the Nutritional Epidemiology Core of NORC and analyzed using the Nutrient Data System for Research (NDSR). Nutrient analyses of study foods are reconciled with the NDSR database to improve 24-h dietary recall accuracy.

2.4.1. Eligibility screening—Baseline evaluation for those who screen into the study includes headache and medical history with a physical exam and study enrollment, and evaluation of inclusion/exclusion criteria. During the four to six-week baseline period, participants who record at least five headache days and complete at least 80% of diaries are eligible to join the study. Participants who meet inclusion/exclusion criteria and attend the first intervention visit are then randomized.

2.4.2. Baseline assessments—The 4–6 week baseline phase assessments include the following:

- Daily completion of the online or paper headache diary, recording hourly headache occurrence, intensity, duration, medication use, sleep and general health status;
- Two, non-consecutive 24-h dietary recall attempts;
- Vitals that include weight, height, blood pressure, pulse
- Online questionnaire that includes the following: 1) Headache Impact test (HIT-6); 2) PROMIS-29 Profile, for assessment of anxiety, depression, fatigue, sleep disturbance, and physical and social role functioning; 3) The Total Pain Scale that looks at the comorbid pain assessment with severity index; 4) Demographics; 5) Nicotine, Caffeine, and Alcohol scale; and 6) Hunger scale

2.4.2.1. Biochemical outcome measures.: The primary biochemical hypothesis is that plasma 17-Hydroxy DHA will increase significantly in Diet A (H3-L6) relative to the control diet (average US intakes of n-3 and n-6). 17-hydroxy DHA is a pathway marker and precursor to two families of potent bioactive mediators with anti-nociceptive properties (D series resolvins and protectins).

Secondary biochemical hypotheses postulate that other anti-nociceptive derivatives of EPA and DHA will increase and that pro-nociceptive derivatives of AA and LA will decrease in Diet A relative to Diet C, and that these diet-induced changes will correlate with improvements in pain-related clinical endpoints. Following study completion, LA, AA, EPA and DHA derived mediators, pathway markers and inactivation products will be quantified, as previously described [28]. Briefly, following protein precipitation solid phase extraction will be performed using a polymer-based reversed phase Strata X cartridge (33 μm, 200 mg/6 mL, Phenomenex, PA), followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The LC-MS/MS comprises a Shimadzu chromatographic system with two LC-20AD pumps, a SIL-20AC autosampler (4 °C), a CTO-20AC column oven (30 °C), and a CBM-20A controller (Shimadzu Scientific Instruments, Columbia, MD), and using a ZorBAX RRHD Eclipse Plus C18 column (100 mm × 4 mm; 1.8 μm). The UPLC system is coupled to a Qtrap 5500 mass spectrometer (AB Sciex, USA) with an electrospray ionization source, and operated in negative ion mode. Quantitative analysis will be performed using scheduled multiple reaction monitoring (sMRM) scans, with simultaneous profiling of targeted oxylipins derived from n-6 and n-3 fatty acids that serve as oxylipin precursors, as previously described [28]. A list of the oxylipins and their precursor fatty acids that we plan to quantify are shown in Table 3. Pathways used for biosynthesis of selected derivatives of LA, AA, and DHA are shown in Fig. 3. Key analytes include: 17-hydroxy-DHA (Primary biochemical outcome; 18-HEPE (Pathway marker and precursor for E-series resolvins)); as well as bioactive LA-derived oxylipins (e.g. 9- and 13-HODE, 13-hydroxy-9,10-epoxy-octadecadienoic acid, 11-hydroxy-12,13-epoxy-octadecadienoic acid); AA-derived eicosanoids (e.g. 5-, 12-and 15-HETE, PGE2, LTB4) and lipoxins (LXA4). In an exploratory manner, we will also attempt to quantify plasma concentrations of individual E-series and D-series resolvins, maresins, protectin D1 and other bioactive lipid mediators.

2.4.2.2. Clinical outcome measures.

- **Headache-specific Quality of Life: The Headache Impact Test (primary clinical outcome). (HIT–6)**[29]. The HIT-6 is a validated questionnaire designed "to measure the impact headaches have on a person's ability to function on the job, at school, at home, and in social situations" [29]. It has good internal reliability. The scale consists of six items that cover various content areas relevant to headache-related disability: pain, social functioning, role functioning, vitality, cognitive functioning, and psychological distress. The scores range from 36 to 78, with higher scores indicating greater negative impact. A decrease of 2.3 HIT points (95% CI,0.3–4.3) over six weeks among patients with chronic headache corresponds to a "somewhat better" self-reported rating on a global clinical change scale [30]. Participants complete this questionnaire at baseline, at the start of the intervention, at weeks 4, 10, and 16 of the intervention, and at week 22 of the post-intervention phase.

- **Headache Diary: Frequency, Intensity, Duration.** Participants are instructed to maintain a daily record of their headaches usinga headache diary throughout the entire 26 weeks of the study. This diary was used successfully in our previous clinical trial involving participants with chronic headache, with participants willing and able to complete daily diaries with minimal loss of data [27]. Participants record their headache duration, frequency, intensity (rating as "none", "mild", "moderate" and "severe") and sleep for each hour of the day. Diaries are completed on a secure website via a computer or smart-phone interface. For those participants unwilling to use electronic diaries, paper diaries are provided. In the analyses, numbers of hours of any headache will be calculated along with numbers of hours of moderate to severe headache for use in long-itudinal models.

- **Perceived Changes in Headache and Overall Health.** This measure consists of two questions, on a Likert format scale, about participants' perceived change over the last four weeks in their headache condition and overall health. Possible answers are much better, somewhat better, about the same, somewhat worse, much worse, and don't know. This measure provides subjective outcome data regarding symptom change. Participants complete this brief questionnaire at the start of the intervention, at weeks 4, 10, and 16 of the intervention, and at week 22 of the post-intervention phase.

- **Headache–Related Disability: Migraine Disability Assessment Score (MIDAS).** Disability, defined as the consequences of illness on ability to work and function, is measured using the MIDAS. Derived from the Headache Impact Test, MIDAS is a 7-item questionnaire that assesses the number of days during the previous three months that respondents missed work or school, experienced decreased productivity at work or home, or missed social engagements because of headaches [31]. Test-retest reliability is acceptable, with Spear-man's correlation coefficient ranging from 0.67 to 0.73. Cronbach's alpha is 0.83 [32]. The MIDAS is highly correlated with headache diaries and physician

assessments of patient disability and is useful for stratifying patients by degree of disability [33]. Participants complete this questionnaire at the start of the intervention, at week 16 of the intervention, and at week 22 of the post-intervention phase.

- **Headache Diary: Medication Use for Headache and Cost Analysis.** Participants use the headache diary to record the name, dose and amount of all medications, including herbal remedies and supplements, taken for prevention or relief of headache. On the day of enrollment, the study coordinator enters the name and dose of each medication/supplement taken either daily or intermittently for headache. In the daily diary, participants choose from their list of medications to indicate how many doses were taken during that day. They are also able to add new medications to the list. In the analyses, cost for prescription and non-prescription medications and remedies will be estimated by pricing the retail cost at two local pharmacies or health food stores and averaging the result for each medication. The total cost of analgesic medication and total medication and amounts used will be calculated.
- **Satisfaction with Care.** At baseline, on completion of the usual-care phase and at the final visit, participants are asked how satisfied they have been with their care including, at the end of the study, their specific intervention, using a 5 point Likert satisfaction scale.
- **Impact on Other Pain Syndromes (Whole Body Pain Scale).** Participants indicate on this questionnaire how often in the past 7 days they have experienced pain in 28 anatomical sites that cover the entire body, and how much of their total waking time they experienced any body pain in the past 7 days. Participants complete this questionnaire at baseline, at the start of the intervention, at weeks 4, 10, and 16 of the intervention, and at week 22 of the post-intervention phase.
- **Patient Reported Outcomes Measurement Information System (PROMIS) Short Form PROMIS-29 Profile.** PROMIS measures were developed from existing items in multiple measures of self-reported health by a collaborative network of researchers at the National Institutes of Health and several universities [34]. Using item response theory, candidate items were evaluated in several domains, including physical function, social functioning, psychological distress, pain, and fatigue and T scores were calculated for population norms [34]. Across all domains except fatigue, short forms of items were highly correlated with the full item bank ($r \geq 0.95$) and reliability coefficients remained high across domains (≥ 0.80). Domains were correlated in expected directions with legacy measures (e.g., SF-36, FACIT-fatigue, Brief Pain Inventory, Pittsburgh Sleep Quality Index, Mood and Anxiety Symptom Questionnaire, FACIT-Functional Well-Being scale)[34]. Participants complete this questionnaire at baseline, at the start of the intervention, at weeks 4, 10, and 16 of the intervention, and at week 22 of the post-intervention phase.
- **Demographic and Clinical Characteristics.** The participant's age, gender, ethnicity, religion, occupation, employment status, level of education, income,

type of medical insurance, date of onset of headache, presence of other medical conditions and diagnoses are recorded at the time of the baseline interview. The medical history and physical exam performed by the PI become part of this data set. All participants are interviewed concerning their headache symptoms. Medical records are reviewed as needed to aid in assessing diagnostic criteria for the major forms of migraine using ICHD–II.

- **Expectations of Benefit/Credibility.** This instrument provides an assessment of credibility of each intervention using an adaptation of a validated credibility scale previously developed for psychological studies [35,36] for purposes of comparing the two treatment arms. This instrument has been successfully adapted for use in other conditions such as chronic pain and migraine headache [37]. It is administered at the end of the randomization visit after each subject receives instruction in the diet.
- **Changes in Usual Care.** The participants are interviewed at the start of the diet, at weeks 4, 10, and 16 of the intervention, and at week 22 of the post-intervention phase to document any changes in conventional treatment that occurred during the study. This includes all forms of therapy for headache and the development of any new medical conditions or significant changes in existing conditions. Medical records are accessed, if necessary, with the consent of the participant.

2.4.2.3. Process measures.

- **BiochemicalAssessment.** Participants have blood drawn at the end of the baseline phase(the randomization visit), after 4, 10, and 16 weeks of diet exposure, and 6 weeks post active intervention for assessment of fatty acids, fatty acid derived lipid mediators, pathway markers and inactivation products, and other bioactive mediators and biomarkers that may be altered by the study diets. Blood samples are collected by a Clinical Translation Research Center (CTRC) phlebotomist, immediately processed to plasma and erythrocytes by the study coordinator, barcoded and immediately stored in a −80 °C freezer, and transferred to the Bioanalytical Core Laboratory. At completion of the study, batched plasma samples will be delivered to the NIH/NIA Lipid Mediators, Inflammation and Pain Unit, Laboratory of Clinical Investigation and for analysis of precursor fatty acids, lipid mediators, pathway markers and inactivation products. Other biochemical assessments will be performed through the UNC Bioanalytical Core Laboratory, using ELISA methodology.
- **Erythrocyte Fatty Acids.** Fasting (>10 h) blood are collected at the conclusion of the run-inphase, and after 4, 10, and 16-weeks of diet exposure and at 6 weeks post-intervention (Week 22), in EDTA tubes and immediately centrifuged at 2960 rpm for 15 min. Erythrocyte aliquots are prepared and stored at −80 °C until analysis. Following Bligh/Dyer extraction [38], aliquots are heated at 100 °C for one hour with methanol containing 14% boron trifluoride to generate FAME, which is then extracted into hexane and analyzed with a GC/FID gas

chromatograph (Agilent 6890) equipped with a 30-m DBFFAP capillary column. Fatty acids are identified through comparison with a fatty acid methyl ester mixture (GLC-462) and reported as individual fatty acids. Two composite fatty acid indices (Harris 2009; Stark 2008) [39,40] are also calculated. The n-6 in HUFA score is equal to the proportion of n-6 fatty acids in total highly unsaturated fatty acids (HUFA). The n-3 Index is equal to the sum of erythrocyte n-3 EPA and DHA as a percent of total fatty acids.

- **Dietary Intake Assessment.** Nutrient intake data is collected during each phase of the study via two telephone administered 24-h dietary recalls on non-consecutive days – one weekend day and one weekday. Calls are conducted by trained research staff of the UNC Nutrition and Obesity Research Center's Diet and Physical Activity Core (DK56350) using the standard multiple pass interviewing method. Analysis is performed using Nutrient Data System for Research (NDSR) software developed by the Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN, Food and Nutrient Database. Integration of 24-h dietary recalls and the NDSR software generates estimates of daily consumption of 155 nutrients and other variables (www.ncc.umn.edu/products/database.html). The food and nutrient dataset includes approximately 18,000 foods. A total of six 24-h dietary recall assessments take place during the 26-week study period. Pre-intervention nutrient intakes are calculated from 24-h dietary recalls performed during the 4–6 weeks of the baseline period. The same procedure is performed in the final 6 weeks of the 16-week active intervention period, allowing for comparison of pre- and post-intervention nutrient consumption. Finally, the same procedure (2 more non-consecutive 24-h dietary recalls) is repeated in the final 6 weeks of the 6-week post-active intervention period. The goal of the final recalls is to determine whether participants are able to continue the diets without provision of the food. The NDSR database is updated with information derived from study recipes and the NIAAA nutrient analyses of study-provided foods.
- **Diet Acceptability Form (Acceptability of Experimental Diets):** This instrument has been developed for this study to assess satisfaction and acceptability regarding the prescribed diet and will be evaluated for its psychometric properties. An appropriate instrument with known psychometric properties with which to assess systematically the acceptability of specific diets could not be identified in the literature. The Diet Acceptability Form consists of a series of questions (5 simple questions plus open-ended query), assessing participant's satisfaction with their assigned diet. The instrument is not designed to assess aspects of the diet that the participants found unsatisfactory, but rather, is intended as a metric to estimate the likelihood that the participant would be willing and able to adhere to the diet. The format for the questions are based on the Diabetes Quality of Life Measure for the Diabetes Control and Complications Trial [41] with the response options "Strongly Agree, Agree, Disagree, or Strongly Disagree". Participants complete this online questionnaire

during four visits after randomization at weeks 4, 10, and 16 of the intervention, and at week 22 of the post-intervention.

Outcomes are listed within the Schedule of Evaluations shown in Fig. 3.

2.5. Statistical analysis

2.5.1. Sample size and randomization—The sample size was selected based on expected available funding and the resources of the Research Kitchen over 5 years. Power was calculated for the available sample size (Table 4).

The primary biochemical hypothesis is that the H3-L6 intervention (Diet A) will result in significantly higher levels of 17-Hydroxy DHA than the control L3-H6 intervention (Diet C) as measured after 16 weeks of the dietary intervention. In the previous study, 17-Hydroxy DHA increased significantly (by 0.9 lnNm, $p = 0.0001$) in the Diet A group ($n = 33$) relative to the comparator ($n=34$). Power for the present study was calculated for an overall difference (ANOVA) using an alpha of 0.05 and for between group comparisons using contrasts with a Bonferroni-corrected alpha of 0.0167. Since repeated measures are available, we also estimated the power based on the rate of change in 17-Hydroxy DHA using assumptions based on the Chronic Daily Headache trial. We expect to have ample power to test the overall difference among groups and the contrast between Diet A and the control diet. Estimates for Diet B are unknown; this study will provide important information regarding effect sizes for Diet B, enabling future research to address this question more fully.

Power analysis is also provided for the primary clinical outcome, the Headache Impact Test (HIT-6). Relative to the control diet, the HIT-6 in Diet A is hypothesized to show a significant reduction post-intervention. In our previous diet trial in participants with CDH, the HIT-6 for the Diet A group improved by a mean of 7.5 points (in an ITT analysis assuming no change in scores of participants who dropped after randomization) compared with a drop of 2.0 points in the comparator diet ($p = 0.01$, based on ANCOVA model controlling for baseline). Power for the present study was calculated for an overall difference (ANOVA) using an alpha of 0.05 and for between group comparisons using contrasts with a Bonferroni-corrected alpha of 0.0167.

Based on our previous research experience we estimate the drop-out rate to be 20–30% prior to randomization. With this drop-out rate, we need to recruit at least 191 participants into the baseline phase. Our drop-out rate in the intervention phase, post randomization, is estimated at 15–20%. If participants drop out early in the intervention period, new participants will be enrolled, potentially increasing the effective sample size for intention-to-treat (ITT) analysis. The analysis will retain all randomized participants for ITT analysis. To reduce selection bias, missing data post-randomization will be imputed using multiple imputation procedures. Participants who violate protocol (e.g., consume fatty fish while on Diet C) will be retained in their randomized group for analysis. In addition to the ITT analysis, we will complete an exploratory per protocol analysis, removing participants from the analysis who withdraw from the study or are not compliant with the protocol.

In this randomized study, only exploratory analysis of subgroups is planned, taking effect modification into consideration; sample size is not adjusted for interaction effects.

2.6. Definition of populations

We are defining our intention-to-treat population as all participants who are randomized. We will retain randomized participants in the groups to which they were randomized. For a per protocol analysis, we will analyze data from participants who attend all study visits and complete at least 80% of their diary data.

2.7. Interim analyses and stopping rules

We are not planning any pre-specified interim analyses for any reason. The study may be suspended by our PI or the Data and Safety Monitoring Board (DSMB) if a severe adverse event occurs that is deemed to be secondary to one of the dietary interventions. The DSMB may also suspend or stop the study or a study arm if reported adverse events are increasing, either across all intervention arms or in a particular study arm.

2.8. Outcomes

Outcomes include biochemical measures (analyzed at NIH/NIA) and self-reported clinical outcomes. All self-report measures are entered into an electronic database directly by participants. The study coordinator verifies complete data entry after each study visit.

2.9. Data analyses

This study is a three-arm intervention trial with a baseline measurement and repeated measures of the outcome variable over time. A descriptive analysis will be conducted to report the observed outcome differences in treatment groups and to check outliers in the data. Regression analyses will be performed to examine all stated specific aims. Because of the multiple arms in the design of the study, we will first assess a global test of no difference in the outcome in the three arms and, if found significant, will conduct pair-wise group comparisons with a Bonferroni-adjusted level of significance. All models will include an indicator variable for study recruitment site.

2.9.1. Primary biochemical outcome—Change in plasma 17-hydroxy DHA—this analysis will utilize the baseline and 16-week measure of 17-hydroxy DHA. We will transform the variable as needed to achieve normality. We will construct an analysis of covariance model with the 16-week ln(17-hydroxy DHA) as the outcome and indicator variables for the two treatment arms (β_1 and β_2) with the baseline ln(17-hydroxy DHA) as a control variable. Study site (0 for Site 1, 1 for Site 2) will be added as an additional control variable. First, we will test the hypothesis that $\beta_1 = \beta_2 = 0$ with a partial F test. If this test is statistically significant at the $\alpha = 0.05$ level, we will test the pairwise comparisons, using Bonferroni corrections.

2.9.2. Primary clinical outcome—Change in HIT-6—this analysis will also use the baseline and 16-week measures and a similar statistical procedure. The HIT-6 is usually normally distributed.

2.9.3. Longitudinal model for primary outcomes—Trajectory of change for plasma 17-hydroxy DHA—we expect to be able to transform the data successfully to enable us to maintain the variables as continuous. In this linear mixed model, the measurement of each of the fatty acid derivatives at specific times will be the dependent variable. Fixed effects will include the independent variables, the allocated diets (treatments), time, the treatment* time variables and the recruitment site. Random effects will include participants. Significant regression coefficients of the interaction terms will indicate that the rate of change in the fatty acid derivatives differ by treatment assignment. We will use the SAS procedure MIXED (or GLIMMIX) to estimate the model and test hypotheses (SAS Institute, Inc., Cary NC). A test of global hypothesis of no treatment effect will be the joint hypothesis that the regression coefficients of the two treatment*time coefficients are zero. If this global test is rejected, we will conduct pair-wise comparisons with Bonferroni adjustments. Model fitting, including choice of covariance structure, will depend on comparisons of the Aikake Information Criteria. A similar approach will be applied to the long-itudinal HIT-6 analysis.

2.9.4. Longitudinal models for diary data—Trajectory of change in headache hours per day and severe head ache hours per day over the 16 weeks of the intervention—we will extract this information from the diary as summary measures. These measures are available for a baseline phase of 4–6 weeks, and an intervention phase of 16 weeks, and a post-intervention phase of 6 weeks (varies among individuals). We will treat the outcome measure as a repeated count variable (number of headache hours). Because the outcome measures for an individual are correlated, we will fit Poisson regression models using a GEE approach. The treatment indicators, time, and time*treatment will be used as independent variables. The significance of interaction variables will determine whether the change in headache hours depends on the diet group. It is expected that there will be a large number of zero headache hours in the data. To take this into account, we will include an over-dispersion parameter in the model and assess the fit of alternative distributions (zero-inflated Poisson, negative binomial zero-inflated Poisson) and alternative models (subject-specific random effects model). If the severe headache hours' model does not demonstrate adequate fit parameters, we will model the probability of a severe headache day, defined as a day with at least 8 h of mild headache or any hour of a moderate or severe headache.

In the analysis, we anticipate using a linear (or quadratic) spline variable with cut points at the day of randomization and the day of the last intervention visit. This will allow us to model the slope of Headache Hours per day over the three time periods of the study—baseline, intervention, and post-intervention. We will also create graphical displays of mean headache hours per day and severe headache hours per day by intervention group to facilitate visualization of the results. We anticipate using a Loess graphical smoothing procedure for this display.

2.9.5. Secondary clinical outcomes—Pre- to post-intervention and trajectory of change assessments of secondary clinical outcomes (e.g., MIDAS, PROMIS measures, total pain score, cost of care) will be similar to the primary clinical outcome.

2.9.6. Secondary biochemical outcomes—Pre- to post-intervention and trajectory of change for n-6 and n-3 free precursor fatty acids, lipid mediator derivatives and pathway markers. Analysis will be similar to Outcome 1 and Outcome 3.

2.9.7. Process measures—1) erythrocyte EPA, DHA, LA, and AA collected at the randomization visit and at 4, 10, 16, and 22 weeks after randomization; and 2) two 24-h dietary recalls by telephone in the baseline phase, between weeks 10 and 16 post-randomization, and between weeks 16 and 22 post-intervention. We will calculate mean and median values for erythrocyte EPA, DHA, LA, and AA by intervention group at each time point. Similarly, we will calculate mean and median intakes of dietary EPA, DHA, LA, and AA from the 24-h dietary recall using established methods (nonlinear mixed effects model with Box Cox transformation) to assess usual intakes [42,43].

2.9.8. Subgroup analyses—To assess effect modification in the above analyses, we will add interaction terms for age and race to the models. For example, for the primary biochemical outcome, we will test the contribution of the interaction between treatment and sex and treatment and race. The importance of interaction terms will be tested by removing the variables and running a likelihood ratio test. If the interaction terms add significantly to the analysis, we will present stratified results.

2.9.9. Exploratory analyses—In this analysis, we hypothesize that changes in the RBC fatty acids (Fig. 4, **Pathway 6**) and their lipid mediator derivatives (Fig. 4, **Pathway 4**) will result in significant improvements in headache-specific quality-of-life and headache frequency and duration. In the first analysis, the HIT-6 will be the outcome with each (log-transformed) RBC fatty PUFA as independent variables in a linear regression model. We will also test a model with combined EPA/DHA and LA to assess the impact of LA in the model while controlling for changes in n-3 fatty acids. A similar approach will test the influence of the fatty acid derivatives on the HIT-6 outcome. Analysis of headache frequency/duration (headache hours per day) will employ a longitudinal Poisson regression model as described in the Outcome 5 analysis section. Available data will also allow us to test for mediation effects on the HIT-6 outcome. We can test the linear regression of dietary EPA + DHA intakes on the HIT-6 score and add RBC PUFAs to the model. If significant mediation is present, we should see a marked decrease in the effect estimates associated with EPA + DHA intakes. A similar analysis will test mediation of fatty acid derivatives on RBC PUFA effects on the HIT-6.

3. Results

3.1. Progress on study

Current enrollment for the study (as of March 31, 2017) has totaled 214 participants. To enroll that number, the study team has contacted 1151 potential participants. Participants are primarily recruited from neurology clinics in Chapel Hill, Raleigh, and Durham area as well as from the UNC community. Stakeholder engagement with the clinics have been critical to maintain patient referral for the study. Study team members check in or visit each clinic site every other week to keep referring providers and clinic personnel engaged in the project. In

addition to provider engagement, study flyers and brochures are posted within the clinic areas to engage patients as well as around the UNC community. To keep recruitment steady, recruitment emails are sent through the university's mass emailing system every 2–4 months.

Of the 214 screened and enrolled to date, 141 have been randomized. We anticipate having our target number randomized by December 2017.

Most adverse events and unanticipated problems have been unrelated to the study intervention. One participant who had prior gastric bypass surgery was unable to stay on the study diet and had to withdraw from the study, which should be taken into consideration as a possible exclusion criterion for diets such as the ones tested in this study. Also, participants with gallstones or gall bladder removal may have difficulties adjusting to the diet; therefore, future studies may want to consider these as exclusion criteria or carefully screen participants, as they may have issues with fatty diets as well.

4. Discussion

Episodic migraine is a debilitating chronic pain condition afflicting 12% of American adults. Current conventional treatments rely on medications that provide limited or transient relief, target symptoms rather than the underlying causes of pain, and are associated with significant side effects and costs. It is therefore important to investigate non-pharmacologic approaches to conventional headache treatments.

Omega-6 (n-6) and omega-3 (n-3) fatty acids regulate multiple pain-related biochemical pathways. Controlled clinical trials investigating pain modulation in response to dietary changes while exploring relevant mechanisms of action in humans are lacking. This protocol utilizes an innovative design and hypotheses to examine clinical efficacy and underlying mechanisms of a promising dietary manipulation with the distinct potential for high impact in terms of ameliorating a chronic, disabling pain disorder.

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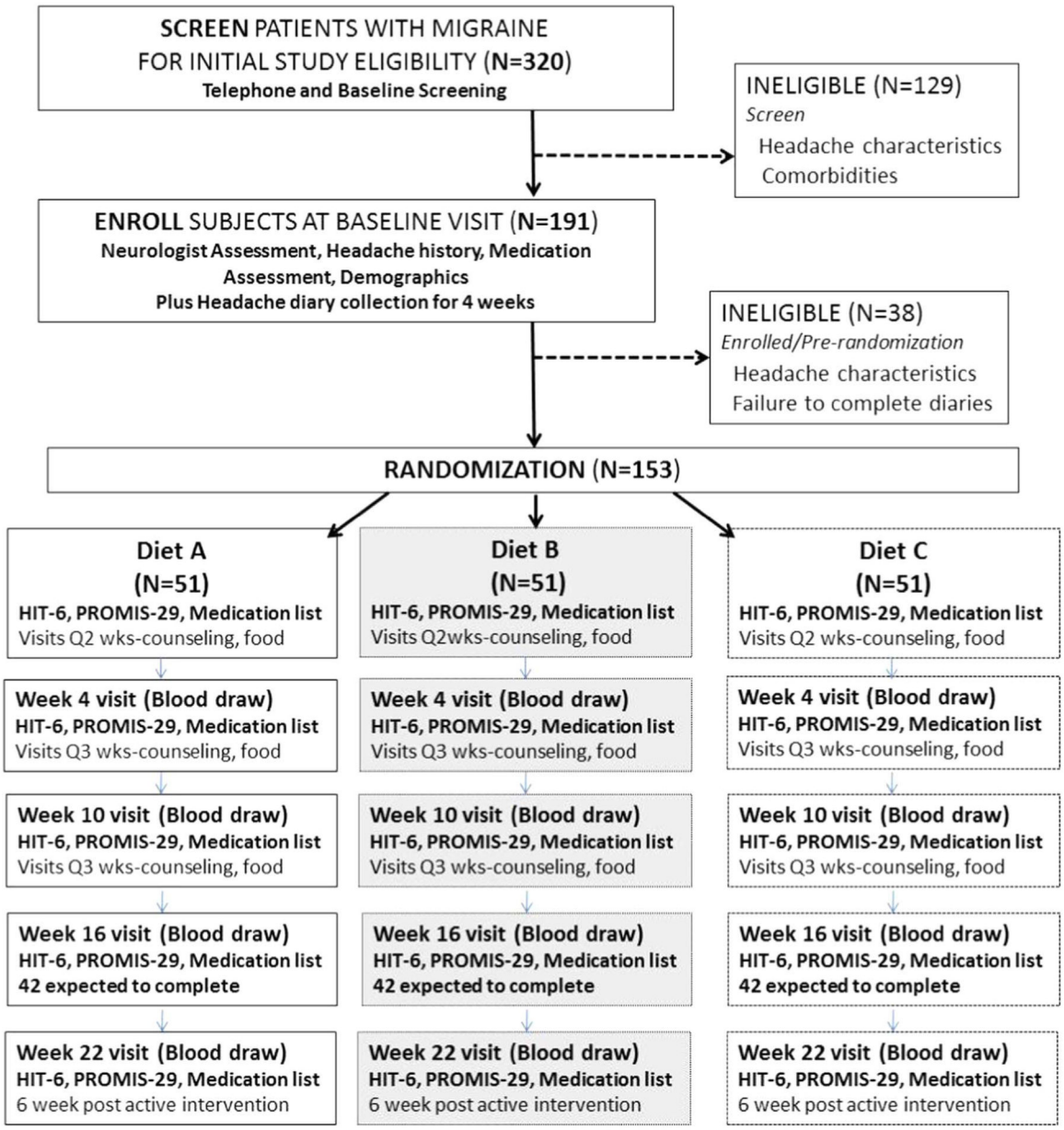

Fig. 1.
Study flow diagram.

Assessment	Screening: Visit 1 Week -4	Between Visit 1 & Visit 2	End of BL Visit 2 (W0)	Visit 3 (W2)	Visit 4 (W4)	Visit 5 (W7)	Visit 6 (W10)	Visit 7 (W13)	Between Visit 6 & Visit 8	Visit 8 (W16)	Between Visit 8 & Visit 9	Visit 9 FINAL (W 22)
Informed Consent Review	X											
Demographics	X											
Enrollment	X											
Medical History	X											
Physical Examination	X											
Inclusion/Exclusion Criteria	X											
Blood for biochemical outcomes			X		X		X			X		X
Dietary Counseling			X	X	X	X	X	X		X		
Questionnaires	X		X		X		X			X		X
Blood pressure	X		X		X		X			X		X
Weight	X		X	X	X	X	X	X		X		X
Food Pick Up			X	X	X	X	X	X				
Randomization			X									
Diary Review (& weekly)		X	X	X	X	X	X	X		X		X
Adverse Events (& weekly)			X	X	X	X	X	X		X		X
24-hr dietary recall telephone assessment (attempts to complete 2 calls)		X							X		X	
Post-participation Interview												X

Fig. 2.
Schedule of evaluations.

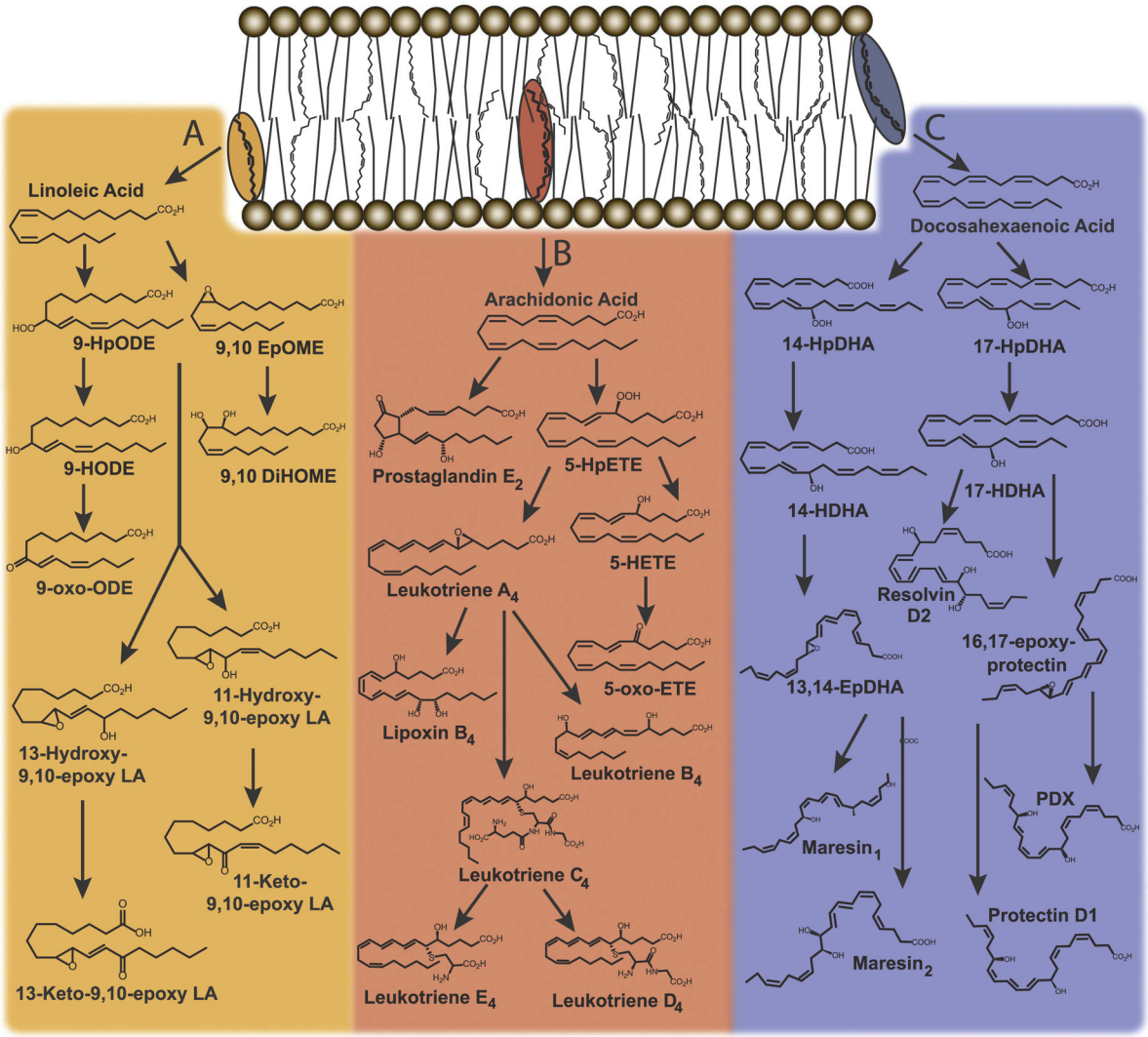

Fig. 3. Biosynthetic pathways converting dietary and membrane n-6 and n-3 fatty acids to lipid mediators of inflammation and pain.

(A) LA converted by lipoxygenases to 9-HpODE can reduced by glutathione peroxidases to 9-HODE, or converted via hydroperoxide isomerization to generate hydroxy-epoxide and keto-epoxide LA derivatives. LA can be converted to 9,10-EpOME and 9,10-DiHOME by the actions of cytochrome P450 epoxygenases and soluble epoxide hydrolase, respectively. HODEs, EpOMEs, and hydroxy-epoxide LA derivatives modulate pain responses in preclinical models. (B) Arachidonic acid can be converted by cyclooxygenases to generate prostanoids including PGE2, or by lipoxygenases to form leukotrienes and lipoxins. Prostanoids evoke pain in human trials; leukotrienes and lipoxins modulate pain responses in preclinical models. (C) Docosahexaenoic acid can be converted by 12-lipoxygenase to generate maresins, or by 15-lipoxygenase to generate resolvins and protectins. These specialized pro-resolving mediators have anti-nociceptive properties in pre-clinical models. Abbreviations; LA, linoleic acid, HpODE, 9-hydroperoxy-octadecadienoate; HODE, hydroxy-octadecadienoate; oxo-ODE, oxo-octadecadienoate; EpOME, epoxy-

octadecenoate; DiHOME, dihydroxy-octadecenoate; PGE2 prostaglandin E2; DHA, docosahexaenoic acid; HpDHA, hydroperoxy—DHA; HDHA, hydroxy-DHA.

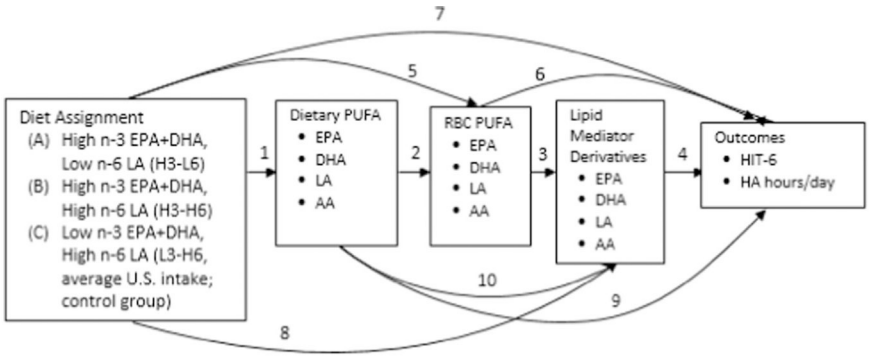

Fig. 4.

Schematic model linking dietary PUFA intakes to headache outcomes.

Table 1

Fatty acid intake targets for controlled dietary alterations in n-6 LA and n-3 EPA+DHA.

Diet group	LA	ALA	AA	EPA + DHA	Comments
	% of food energy (%E)		*mg per 2000kcal*		
ARM 1: Diet A (High n-3, Low n-6)	***1.8***[a]	0.6	150	***1500***[c]	This intervention demonstrated, marked biochemical alterations and clinical efficacy in pilot study
ARM 2: Diet B (High n-3, High n-6)	7.2[a]	0.6	150	***1500***[b,c]	Diet B vs. Diet A evaluates n-6 LA as a controlled variable[c]
ARM 3: Diet C (Low n-3, High n-6)	7.2	0.6	150	**150**[b]	Average US PUFA content (control). Diet C vs. Diet B evaluates EPA + DHA as a controlled variable

Abbreviations: LA = linoleic acid; ALA = alpha linolenic acid; AA = arachidonic acid; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid.

[a]Indicates that LA is modified as a controlled variable via isocaloric replacement of n-6 LA with oleic acid in Diet A. This is accomplished by providing Diet Groups A and B with identical interventions aside from added visible oils differing in LA and oleic acid.

[b]Indicates that EPA+DHA is modified as a controlled variable in Diet B by substituting carefully selected fatty fish for lean fish and lean poultry, with otherwise identical interventions.

[c]EPA+DHA provide an additional 10 cal per day, replaced by oleic acid in the Control Diet.

Table 2

Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
Both genders, all racial and. ethnic groups ages 18 and above with 5–20 migraines/month;	Change in hormone or birth control use within the past six months;
Meeting the 2004 International Classification of Headache Disorders (ICHD-II) criteria for migraine, with or without aura;	Pregnancy or anticipated pregnancy, breast feeding, because of the unpredictable hormonal influences on migraine during pregnancy and lactation;
Headaches present for at least 2 consecutive years;	Marked depression, anxiety or psychosis at time of enrollment;
Under the care of a physician for their headaches*;	Receiving active treatment for a major medical illness*;
**May be caking migraine prevention medications or abortive medications;*	*Well managed diabetes and thyroid disorders will not be excluded;
Able to read and communicate in English;	History of significant head trauma or surgery of the head or neck within 3 years of screening;
Have internet access through at least one device;	Cognitive impairment that prevents understanding the protocol;
Willing to be randomized to any of the study arms;	Other chronic pain syndromes requiring pain clinic management;
Able to understand study procedures;	History of food allergy with associated rash or pulmonary symptoms such as shortness of breath or wheezing;
Able to attend 7 dietary counseling visits;	Stated aversion to eating seafood;
Able to attend post-intervention visit 6 weeks after intervention end.	Regular exposure to fish oil or omega-3 fatty acids in past 2 years; Currently on a diet for the purpose of weight reduction for a period of greater than 3 weeks; Previous participation in a dietary intervention investigational study; Participation in any intervention study in past 12 months; History suggesting substance abuse within the past 5 years; Any condition that would prevent complete participation in the trial; Suspected, potentially disabling or life-threatening conditions with headache as a major feature such as: vasculitis, intra-cranial mass, history of subarachnoid hemorrhage, history of central nervous system infection within the preceding 5 years

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Table 3

Laboratory analysis of precursor fatty acids and their oxylipin derivatives.

Analyte	Formula
LA	18:2 n-6
AA	20:4 n-6
DTA	22:4 n-6
DPA n-6	22:5 n-6
ALA	18:3 n-3
EPA	20:5 n-3
DPA (n-3)	22:5 n-3
DHA	22:6 n-3
EPA + DHA	20:5n-3 + 22:6n-3
Palmitic acid.	16:0
Oleic acid.	18:1 n-9
	Precursor
9-HODE	LA
13-HODE	LA
9-oxo-ODE	LA
13-oxo-ODE	LA
9,10-EpOME	LA
12,13-EpOME	LA
13-hydroxy-9,10-epoxy octadecadienoic acid	LA
11-hydroxy-9,10-epoxy octadecadienoic acid	LA
9-hydroxy-12,13-epoxy octadecadienoic acid	LA
11-hydroxy-12,13-epoxy octadecadienoic acid	LA
5-HETE	AA
5-oxo-ETE	AA
9-HETE	AA
12-HETE	AA
15-HETE	AA
20-HETE	AA
PGE2	AA
LTB4	AA
TXB2	AA
17-hydroxy-DHA (precursor to D-series resolvins)	DHA
18-HEPE (precursor to E-series resolvins)	EPA
Lipoxins A4	AA
D-series Resolvins	DHA
D- and E-series Resolvins	DHA, EPA
Maresins	DHA

Abbreviations: LA linoleic acid; AA arachidonic acid; DTA docosatetraenoic acid; DPA docosapentaenoic acid; ALA alpha-linolenic acid; EPA eicosapentaenoic acid; DHA docosahexaenoic acid; HODE hydroxy-octadecadienoic acid; oxoODE oxo-octadecadienoic acid; EpOME, epoxy-

octadecenoic acid; HETE hydroxy-eicosatetraenoic acid; oxo-ETE oxo-eicosatetraenoic acid; HEPE, hydroxy-eicosapentaenoic acid; PG prostaglandin; LT leukotriene; TX thromboxane.

Table 4

Power calculations with assumptions.

Variable	Mean Values pre- and post-intervention by group	Between Group Difference	Pooled SD	Contrast	Alpha	Power[a]	Power repeated Measures
Ln(17-Hydroxy DHA)	Pre: 4.1	0.7–0.9	0.7–0.9	Overall	0.05	93–99%	> 99.9%
	Diet A: 5.0–5.2	0.7–0.9	0.7–0.9	A vs. C	0.0167	93–99%	> 99.9%
	Diet B: 4.8–4.9	0.4–0.5	0.7–0.8	A vs. B	0.0167	71–95%	51–95%
	Diet C: 4.3	0.4–0.6	0.7–0.8	B vs. C	0.0167	71–95%	80–99%
HIT-6	Pre: 60.0	5.0–7.5	5.5–6.5	Overall	0.05	88- > 99%	
	Diet A: 52.5–55.0	5.0–7.5	5.5–6.5	A vs. C	0.0167	79- > 99%	99.9%
	Diet B: 55.0–56.5	3.0–4.0	5.5–6.0	A vs. B	0.0167	54–89%	99.9%
	Diet C: 58.5–60.0	3.5–4.5	5.5–6.0	B vs. C	0.0167	70–98%	78–97%

[a]Power is based on 51 randomized subjects per group. Ranges reflect the uncertainty associated with the effects of Diet B. SD from our prior migraine trial.