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Circulating polyunsaturated fatty acids, pressure pain thresholds, and nociplastic pain conditions

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Abstract

Objective: Polyunsaturated fatty acids (PUFAs) play a role in pain regulation. This study sought to determine whether free PUFAs found in red blood cells also play a role in nociceptive processing. We examined associations between circulating PUFAs and nociceptive thresholds to noxious mechanical stimuli. We also determined whether nociceptive thresholds were associated with nociplastic pain conditions.

Methods: This cross-sectional study used stored red bloods cells and data from 605 adult participants in the OPPERA-2 study of chronic overlapping pain conditions. In OPPERA-2 adults completed quantitative sensory testing in which pressure algometry measured deep muscular

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CRediT authorship contribution statement

AS conducted the analysis and drafted the manuscript. GS, RF and RO were site investigators of the parent study named OPPERA. BE, DW and PS completed the fatty acids LCMS analyses. All authors were involved in the interpretation of data, critically revised and edited the manuscript. All authors approved the final version and take responsibility for data integrity and accuracy of the data analysis.

Declaration of Competing Interest

None

tissue sensitivity at six anatomical sites. Standardized protocols classified adults for presence or absence of five nociplastic pain conditions: temporomandibular disorder, headache, low back pain, irritable bowel syndrome and fibromyalgia. Liquid chromatography tandem mass spectroscopy quantified erythrocyte PUFAs. We conducted three sets of analyses. First, a multivariable linear regression model assessed the association between n-6/n-3 PUFA ratio and the number of overlapping nociplastic pain conditions. Second, a series of 36 multivariable linear regression models assessed covariate-adjusted associations between PUFAs and nociceptive thresholds at each of six anatomical sites. Third, a series of 30 multivariable linear regression models assessed covariate-adjusted associations between nociceptive thresholds at six anatomical sites and each of five pain conditions.

Results: In multiple linear regression, each unit increase in n-6/n-3 PUFA ratio was associated with more pain conditions ($\beta = 0.30$, 95% confidence limits: 0.07, 0.53, $p = 0.012$). Omega-6 linoleic acid and arachidonic acid were negatively associated with lower nociceptive thresholds at three and at five, respectively, anatomical sites. In contrast, omega-3 alpha-linolenic acid, eicosapentaenoic acid, docosahexaenoic acid and the n-6/n-3 PUFA ratio were not associated with nociceptive thresholds at any site. Pain cases had significantly lower nociceptive thresholds than non-case controls at all anatomical sites.

Conclusion: A higher n-6/n-3 PUFA ratio was associated with more pain conditions. Omega-6 PUFAs may promote a generalized upregulation of nociceptive processing.

Keywords

Epidemiology; Experimental pain sensitivity; Quantitative sensory testing; Polyunsaturated fatty acids; Chronic overlapping pain conditions; Human

1. Introduction

Linoleic acid (LA) and arachidonic acid (AA) are omega-6 (n-6) polyunsaturated fatty acids (PUFAs) that metabolize through cyclooxygenase (COX), lipoxygenase (LOX) or cytochrome P450 (CYP450) enzymes into several families of predominantly pronociceptive oxylipins, including prostaglandins and leukotrienes. Preclinical studies show that these oxylipins promote excitability of the peripheral somatosensory system, increasing risk of hyperalgesia (exaggerated pain response to noxious stimulus), allodynia (pain from an innocuous stimulus) and persistent pain states. [1,2]

Countering these pronociceptive effects are the antinociceptive effects of enzymatically oxidized lipids metabolized from omega-3 (n-3) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These n-3 oxylipins include specialized pro-resolving mediators (SPMs) knowns as resolvins protectins, and maresins. SPMs derived from DHA include D-resolvins (RvD), maresins and protectins; whereas SPMs derived from EPA include E-resolvins (RvE). In preclinical experiments, Boyd et al. [3] demonstrated the opposing effects of n-6 and n-3 oxylipins on nociceptive thresholds. They first showed that mice fed an n-6 PUFA enriched diet for 8 weeks developed persistent hypersensitivity to mechanical stimulation, displaying increased mechanical-evoked activity in C- and A-nerve fibers. Then, when these diets were switched to n-3 PUFA enriched intakes,

the nociceptive hypersensitivities completely reversed. In other rodent studies, n-3 PUFA-derived resolvins RvE1 and RvD1 at very low doses achieved analgesic efficacy on inflammatory hypersensitivity through both peripheral and central actions, [4], and both oxylipins showed better analgesic properties than anti-inflammatory agents. [5]

Our understanding of the mechanistic role of PUFAs in modulating nociceptive signaling draws extensively on preclinical models. Yet, animal models lack a good analog for chronic pain that is etiologically distinct from neuropathic pain (e.g., diabetic neuropathy or radicular pain) or nociceptive pain (resulting from disease or injury to somatic structures). In clinical terms, these pain conditions are labelled idiopathic because there is an absence of clinically apparent pathological or traumatic cause for the pain. In mechanistic terms, the conditions are also nociplastic, meaning that the pain occurs through altered nociception without nociceptor activation or nerve injury. [6,7] Not only are these pain conditions common in the population, but their high degree of co-occurrence has led to the descriptor *chronic overlapping pain conditions*. [8] Other features of these conditions are the absence of an identifiable pain stimulus, little evidence of systemic inflammation, a predominance in women, and comorbid central nervous system-derived symptoms such as sleep disturbance and mood problems. Common examples include fibromyalgia, headache, and temporomandibular disorder. The absence of a good animal model for these chronic pain conditions limits the translational value of preclinical studies of pain to humans with chronic pain. [9]

Chronic pain is not merely pain persisting beyond the normal time for healing. People with chronic nociplastic pain experience functional and anatomic alterations to neural pathways that modulate pain. [10, 11] One feature of chronic pain is a heightened sensitivity to noxious stimuli, quantifiable as lowered nociceptive thresholds. [12] In rodent models, sensitivity to noxious stimuli relies on behavioral responses, such as paw withdrawal. However, nociceptive thresholds can be directly assessed in human subjects with quantitative sensory testing protocols and self-reported sensory responses. These protocols deliver standardized modalities of nociception through noxious thermal or mechanical stimuli and then quantify self-reported sensory experience.

PUFA levels are generally measured in plasma, however, it has been shown that plasma PUFA levels are only stable for hours while the levels in red blood cells are around 120 days. [13]This shows a more reflective measurement on PUFA uptake in the body over a sustained period of time. PUFAs circulate through the body and are often incorporated into membrane phospholipids, cellular glycerolipids, or esterified into other types of lipids (such as triglycerides). Membrane bound PUFAs can also be released as free fatty acids via diacylglyceride and monoacylglyceride lipases. [14] Generally bound and circulating PUFAs are measured, however, recent studies have focused more on the biological effects of circulating PUFAs. [15–17]

The overall objective of this study was to examine the association of circulating levels of n-6 and n-3 PUFAs with nociceptive thresholds to mechanical stimuli in humans. We compared nociceptive thresholds in adults with and without the following nociplastic pain conditions: painful temporomandibular disorder, headache, low back pain, irritable bowel syndrome,

and fibromyalgia. Based on our earlier research, we hypothesized that a higher ratio of n-6 to n-3 PUFAs was positively associated with the number of pain conditions that people experienced.

If supported, we then aimed to determine whether higher concentrations of circulating n-6 PUFAs were associated with lower pain thresholds, irrespective of pain status, and, conversely, whether higher concentrations of circulating n-3 PUFAs were associated with higher pain thresholds. Finally, we evaluated whether pain thresholds were lower in people with these pain conditions than in people without pain conditions.

2. Materials and methods

2.1. Study design, setting, participants

This study used cross-sectional data from OPPERA-II, the second phase of the Orofacial Pain Prospective Evaluation and Research Assessment (OPPERA) study. [18] In its first phase, OPPERA recruited 1008 TMD cases and 3258 TMD-free controls to identify risk factors for TMD. [19,20] Cases and controls were a community-based sample of volunteers aged 18 – 44 years from communities near academic health centers in Baltimore, MD; Buffalo, NY; Chapel Hill, NC; and Gainesville, FL between 2006 and 2013. The second phase of OPPERA (OPPERA-II), conducted between 2014 and 2016, entailed follow-up of 543 OPPERA participants along with an additional 127 adults aged 18–74 years with recent-onset TMD who were recruited from the same four communities. One objective of OPPERA-II was to study the overlap of TMD with other idiopathic pain conditions. All participants underwent clinical examinations and completed standardized questionnaires and a venous non-fasting blood draw. This current study used data and biospecimens from OPPERA-II participants. The study was reviewed and approved by the UNC Office of Human Research Ethics (study 13–2232). All study participants verbally agreed to a screening interview done by telephone and provided informed, signed consent for all other study procedures.

2.2. Pressure algometry protocol

The pressure pain threshold (PPT) represents the minimal amount of pressure evoked by the blunt mechanical pressure stimulus changes from feeling “pressure” to feeling “painful”. A low PPT indicates greater pain sensitivity. Pain sensitivity was assessed by PPTs at different anatomical locations using a pressure algometer (Somedic; Hörby, Sweden). Six anatomical sites were tested, bilaterally, in the following order: (1) the center of the temporalis muscle, (2) the center of the masseter muscle, (3) overlying the temporomandibular joint, (4) the center of the trapezius muscle, (5) overlying the lateral epicondyle and (6) the center of the anterior tibialis muscle.

Full details of the protocol are described elsewhere. [21] In summary, the protocol involved the examiner increasing pressure at a steady rate (30 kPa/second), until the participant indicated first pain sensation by pressing a button. If no pain sensation was recorded when the stimulus reached 600 kPa, a value of 600 was used as the threshold value. A minimum of two trials were administered per site, with an interstimulus interval of 2 – 3 s. If the

values derived from those trials were not within 20 kPa of one another, additional trials were administered until either (1) two trials were within 20 kPa (not necessarily sequential), or (2) a total of five trials was administered. In the former case, the value midway between the two values was recorded as the PPT for that site; in the latter case, the median of the five values was used. In a reliability exercise, two examiners assessed PPTs at each body site in 16 volunteers. The overall intraclass correlation coefficient was 0.91, ranging from 0.87 to 0.94.

2.3. Chronic nociplastic pain conditions

A set of nociplastic pain conditions labelled chronic overlapping pain conditions comprise 10 disorders with shared neurobiological vulnerabilities that often co-occur in the same people. [8] OPPERA participants were classified according to the presence or absence of five of these: painful temporomandibular disorder, irritable bowel syndrome, fibromyalgia, low back pain, and headache.

2.3.1. Classification of temporomandibular disorder—Temporomandibular disorder was classified by examiners using Diagnostic Criteria for Temporomandibular Disorder. [22] In brief, trained examiners assessed presence of pain reported in the cheeks, jaw muscles, temples, or jaw joints to classify presence or absence of temporomandibular disorder. To be classified as cases, participants had to have all four of the following findings: a) history of orofacial pain in examiner-verified locations of masseter, temporalis, submandibular or temporomandibular joint area; b) evoked pain in the same muscles and/or temporomandibular joint(s) following palpation of those structures or jaw maneuver; c) reported familiarity of evoked pain to facial pain symptoms during the preceding 30 days; and d) pain that was modified by jaw function (i.e., chewing, opening the mouth, or jaw habits).

2.3.2. Classification of headache—Headache was classified using responses to a questionnaire designed for OPPERA that asked about symptoms of tension-type headache and migraine during the preceding 12 months. Participants who experienced more than one type of headache recorded responses separately for up to three different types of headache. Questions about tension-type headache were from the International Classification of Headache Disorders, third edition (ICHD-3). [23] Symptoms of migraine were based on questions used in the ID-Migraine questionnaire. [24] Migraine was classified when participants reported headache(s) on one or more day per month and at least two of three symptoms accompanying the headache: nausea; sensitivity to light; or being kept from everyday activities. For this analysis, headache was classified for any subject who reported symptoms consistent with probable tension-type headache or migraine, and who had experienced such headache(s) in the preceding 3 months.

2.3.3. Classification of low back pain—Low back pain was classified using responses to screening questions designed for face-to-face interviews and paper or online questionnaires recommended for studies of back pain prevalence by Dionne and colleagues. [25] Participants were classified with low back pain if they reported pain that occurred in the lower back (as indicated with a shaded manikin drawing) during the preceding 3 months that

was bad enough to limit usual activities or change their routine for more than one day and that was not related to fever or menstruation.

2.3.4. Classification of irritable bowel syndrome—Irritable bowel syndrome was classified using the ROME III diagnostic criteria. [26] These criteria require that an individual must have at least 12 weeks of abdominal discomfort or pain, which need not be consecutive, in the preceding 12 months. In addition, that pain must recur on average at least 1 day/week in the last 3 months and be associated with two or more of: pain that increases or decreases with defecation; a change in frequency of stool; or a change in form (appearance) of stool.

2.3.5. Classification of fibromyalgia—Fibromyalgia was classified based on findings from examinations and questionnaires, consistent with the 1990 American College of Rheumatology criteria. [27] Subjects were classified with fibromyalgia when ≥ 11 of 18 body sites were tender to algometer-delivered pressure of up to 4.0 kg/cm2 and when the tenderness occurred in both the axial skeleton and in at least one set of opposing diagonal quadrants of the body. Also, fibromyalgia cases had to report a history of pain lasting for at least 1 day per month in the preceding 3 months.

2.4. Covariates

Covariates were selected based on prior knowledge of associations with fatty acids, PPT, or pain states. OPPERA study site was included because recruitment communities differed on key characteristics. Sex was included because of sex differences in the biosynthesis of ALA to EPA and DHA [28] and because women show lower pressure pain thresholds than men. [29] Age in years was included because pressure pain thresholds decrease with age. [30] Because of racial differences in PUFA metabolism, pressure pain detection thresholds [30] and pain prevalence, we distinguished between categories of white and African American participants, and pooled other groups because of their low enrollment numbers. Socioeconomic indicators varied little in OPPERA's study population, so socioeconomic variables were not included. Body mass index (BMI) was included because high levels of n-6 may increase risk for obesity, and because obesity is strongly associated with an imbalanced n-6/n-3 PUFA ratio and several of the selected pain conditions. Standardized equipment measured weight and height during clinical examinations. BMI was calculated by dividing weight in kilograms by the square of height in meters.

2.5. Blood sample

At the OPPERA-II study visit, a non-fasting 20 ml sample of circulating blood was obtained by venipuncture and collected into tubes containing EDTA that were promptly centrifuged for 10 min at 4 °C. After removing the supernatant plasma, erythrocytes were washed with sodium perborate, vortexed and again centrifuged. After removing the sodium perborate supernatant, erythrocytes were aliquoted into 400 uL cryotubes prior to storage at −80 °C.

2.6. Sample preparation and LC-MS/MS analysis

Red blood cells were obtained and stored at −80 °C until extraction. To 150 μL of red blood cells, 1 mL of 90:10 methanol to water was added. Samples were vortexed then centrifuged

at 20,000 rcf for 10 min. The supernatant was dried down and resuspended in 150 μL of 90:10 methanol to water containing deuterated internal standards (50 ng/mL). Two quality control standards with known concentrations (20 and 500 ng/mL) as well as the deuterated internal standard mixture were analyzed at twice per batch of 96 samples to ensure accuracy and reproducibility. The calibration curve was analyzed twice per batch of 96 samples and averaged to include error across the analysis, using standard deviation as error bars.

Analysis was conducted using a Waters Acquity Ultra-Performance Liquid Chromatography system tandem to a ThermoScientific TSQ Vantage. Separations were performed on a 150 mm x 2.1 mm BEH C18 with a flow rate of 0.25 mL/min and an injection volume of 10 μL. Initial mobile phase composition was 65% A (water with 30 mM ammonium formate) and 35% B (80% acetonitrile 20% methanol). A linear decrease was performed to 45% A at 2 min with a hold for 1 min followed by another decrease to 20% A at 7 min. Another slight decrease was performed to 5% A at 8 min and lastly at 10 min to 0% A. The gradient was returned to 65% A at 13 min and held for 3 min for a total of 16 min. Single reaction monitoring (SRM) was performed in negative mode. The peak width was set to 0.7 Da with a scan time of 0.05 s per transition. The transitions are provided - in the accompanying Data-in-Brief article. The source conditions were as follows: spray voltage 3200 V, sheath gas 50 units, auxiliary gas 15 units, and capillary temp 270 °C. Analytes with their class, limit of detection (LOD), and limit of quantitation (LOQ). These also appear in the Data-in-Brief article.

We quantified linoleic acid (LA; 18:2n-6) and α-linolenic acid (ALA; 18:3n-3). As mammals are unable to synthesize these 18-carbon chain PUFAs, all sources of LA and ALA are exogenous. We also quantified exogenous and endogenous long-chain arachidonic acid (AA; 20:4n-6), eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3). From these long-chain PUFAs, i.e., those with 20 or 22-carbon atoms, we calculated the ratio of the n-6/n-3 PUFAs, as AA/(EPA+DHA).

2.7. Statistical analysis and power calculation

Observations were omitted from analysis for 28 participants with unreported race/ethnicity status, BMI or smoking status. Also omitted were 7 biospecimens that produced no signal for LA, AA, EPA and DHA and 2 biospecimens with levels of LA or DHA below the limit of quantitation. For the 150 ALA biospecimens with values below the laboratory limit of detection (0.97 ng/mL), we assigned a value of 0.05, chosen as an approximate midpoint between zero and 0.97 ng/mL, on the assumption that ALA was present in samples but only at very low levels. This produced a dataset for this analysis of 605 participants with no missing values.

Statistical analyses were conducted in Stata/SE 14.2 (Stata Corporation, College Station, TX). Distributions of PUFAs were assessed for normality using the Shapiro-Wilk test, and given the large sample size, were analyzed using parametric tests. Prior to multivariable modeling, pressure pain thresholds and PUFA concentrations were standardized to z-scores. This allowed a comparison of the relative magnitude of the effects of a one standard deviation change in the independent variable on the outcome of interest. The ratio of n-6

to n3 long-chain PUFAs, calculated as AA divided by the sum of DHA and EPA, was not standardized.

We performed three sets of multiple linear regression analyses. In the first set, the sole dependent variable was the number of chronic overlapping pain conditions that a participant had at the time of the study. The model was run six times; each time with a different PUFA as the main predictor, and finally with the n-6/n-3 PUFA ratio as the main predictor variable. In all three sets of linear regression models analyses, models adjusted for OPPERA study site, sex, age in years, race/ethnicity categories, BMI as a continuous variable, and smoking status categories. For this first aim, the critical threshold for statistical significance was set at $P < 0.008$ to protect against type I error, representing a Bonferroni correction given that six models were used to evaluate the aim.

The second set of analyses comprised 36 separate multiple linear regression models in which the dependent variable was PPT at one of six anatomical sites. We ran each of these six models six times, each time with a different standardized PUFA measure as the main predictor, beginning with LA, and then, in a separate model, AA, followed by ALA, EPA, DHA and finally the non-standardized n-6/n-3 PUFA ratio. These analyses tested the hypotheses that: (a) as n-6 PUFA concentrations increase, PPTs decrease; and that (b) as n-3 PUFA concentrations increase, PPTs increase. For this aim, the critical threshold for statistical significance was set at $P < 0.0013$ to protect against type I error, representing a Bonferroni correction given that 36 models were used to evaluate the aim.

The third set of multiple linear regression analyses comprised 30 separate multiple linear regression models in which, like the second set, the dependent variable was PPT at one of the six anatomical sites. We ran each of these six models five times, each time with a different pain condition as the main predictor variable. These analyses tested the hypothesis that pressure pain thresholds are lower in pain cases than controls. For this aim, the critical threshold for statistical significance was set at $P < 0.0016$ to protect against type I error, representing a Bonferroni correction given that 30 models were used to evaluate the aim.

Statistical power was calculated for the fixed sample size of 605 OPPERA-II participants with stored blood samples and non-missing values for the variables analyzed in this paper. Using the SAS v9.4 power procedure for tests of Pearson correlation between two variables and specifying power of 0.80 and type I error as noted above each aim's critical P-value, we determined that the minimum detectable correlation coefficients were 0.142 for the first aim (type I error = 0.008), 0.165 for the third aim (type I error = 0.0013), and 0.162 for the third aim (type I error = 0.0016). These correlations are equivalent to standardized beta coefficient for the association between PUFAs ratio and the dependent variables for each aim.

3. Results

3.1. Characteristics of study participants

Women comprised two thirds of the study population of 605 participants. Approximately equal proportions of participants were younger and older than 40 years. Headache was the most common pain condition, with prevalence of 41.7%, followed by temporomandibular

disorder (35.7%), irritable bowel syndrome (25.6%), low back pain (21.9%) and fibromyalgia (7.8%). Overall, 200 participants (33.1%) had none of these five pain conditions, while 29.3% had one condition, 18.4% had two conditions, 12.2% had three, 5.5% had four, and 1.7% had all five pain conditions.

Circulating PUFA concentrations were dominated by n-6 LA, reflecting its abundance in the modern Western diet. The mean concentrations of n-6 LA and AA were 958 and 70 ng/mL respectively, while the mean concentrations of n-3 ALA, DHA and EPA were 63, 86, and 16 ng/mL, respectively. When PUFAs were examined according to participant characteristics (Table 1), higher mean concentrations of both n-6 and n-3 PUFAs were associated with male sex, older age, non-white race, higher body mass index, and being a cigarette smoker.

3.2. Pressure pain thresholds according to study participant characteristics

Mean PPT values were lower in the head region than at other body sites (Table 2), indicative of greater pain sensitivity in the head region. Men had higher PPTs than women at all tested body sites. PPTs did not differ significantly on the bases of age, race, body mass index or smoking status.

3.3. Ratio of n-6/n-3 PUFAs and number of concurrent chronic overlapping pain conditions

Supporting the study hypothesis, we found that a higher n-6/n-3 PUFA ratio was positively associated with the number of pain conditions (Table 3). Each unit increase in n-6/n-3 PUFA ratio was associated with a 0.3-point increase (95% CL: 0.07, 0.53) in number of concurrent pain conditions per participant, on average, adjusted for covariates.

3.4. PUFAs and pressure pain thresholds at six anatomical sites

The direction and strength of relationships between standardized PUFAs and standardized PPTs are depicted in forest plots (Fig 1). Each plot reports covariate-adjusted regression coefficients with 95% CI from multiple regression models. Fig. 1A plots the standardized mean change in PPT per standard deviation increase in n-6 LA concentration. All point estimates appear on the left side of the null value (0.0), indicating that PPTs decrease with increasing LA concentration. At the conventional threshold of statistical significance ($P < 0.05$), effects were statistically significant at the lateral epicondyle, temporomandibular joint and trapezius sites, but were non-significant at the $P < 0.0013$ threshold with Bonferonni correction. The same direction of effect was observed for n-6 AA (Fig 1B). For each standard deviation increase in AA concentration, mean PPTs decreased, and effects were statistically significant at all anatomical sites expect at the anterior tibialis. The relationships between n-3 PUFAs and PPTs were much weaker. If n-3 PUFAs raise pain thresholds, as hypothesized, estimates would be positive values. However, for n-3 ALA, estimates were negative (Fig 1C). Associations of ALA, EPA (Fig 1D) and DHA (Fig 1E) were statistically non-significant at all PPT sites. In addition, the relationship between n-6/n-3 PUFA ratio and PPTs were non-significant.

3.5. Pressure pain thresholds and chronic overlapping pain conditions

Forest plots also depict the direction and strength of relationships between PPTs and pain conditions (Fig 2). Each plot shows covariate-adjusted mean differences (95% CI) in PPTs at the six anatomical sites. Temporomandibular cases (Fig 2A) had significantly lower PPT values than non-cases at all anatomical sites. Cases likewise had lower pain thresholds than non-cases at all sites for irritable bowel syndrome (Fig 2D) and fibromyalgia (Fig 2E).

PPTs were significantly lower for cases than controls (all $P < 0.0016$) at four of the six sites for headache (Fig 2B) and at five of the six sites for low back pain (Fig 2C).

4. Discussion and conclusions

In this community-based study, adults with a higher n-6/n-3 PUFA ratio exhibited a higher number of concurrent pain conditions, on average, suggesting that this nutritional imbalance may increase susceptibility to additional pain conditions. We also found that adults with higher circulating levels of pronociceptive n-6 LA and n-6 AA had greater sensitivity to mechanical pressure stimuli at multiple body regions, irrespective of their clinical pain status. Expressed simply, at higher n-6 PUFA levels, less pressure was required to produce pain in all adults. Counter to our expectation, higher levels of n-3 ALA, EPA and DHA, and a lower ratio of n-6 to n-3 PUFAs were not associated with pain inhibition.

When stratified by clinical pain status, cases showed greater responsivity than non-cases to the same level of deep tissue stimulus in muscles and joints, even at anatomical sites unaffected by clinical pain, and greater reduction in pain threshold. Headache cases had lower pressure pain thresholds than non-cases at four of the six anatomical sites. Low back pain cases had lower pain thresholds at five of the six sites. Most pronounced were effects in people with temporomandibular disorder, irritable bowel syndrome, or fibromyalgia, for whom pressure pain thresholds were significantly lower than for non-cases at all six anatomical sites. Since pain hypersensitivity was observed at various anatomical sites, some of which were remote from localized pain conditions, these effects are more likely to reflect alterations in pain processing than tissue specific pathology.

The Western pattern diet is characterized by excessive consumption of n-6 PUFAs and low n-3 PUFA intake, and these characteristics were apparent in our study. Several clinical studies show that n-6 PUFAs and their lipid metabolites are associated with irritable bowel syndrome, [31] dysmenorrhea, [32] migraine, [33] headache severity, [34] and achilles tendinopathy. [35] Dietary n-6 LA and AA are incorporated into membrane phospholipids, accumulate in lumbar dorsal root ganglia, and are metabolized into pronociceptive oxylipins. AA is the precursor to COX-derived prostaglandin E2 that plays a prominent role in inflammation and a mediator of clinical pain. In fact, by inhibiting COX, the enzyme that mediates metabolism of prostaglandin E2 from AA, aspirin and non-steroidal anti-inflammatory agents relieve inflammation and pain. Of course, some AA-derived oxylipins such as lipoxins are also produced that resolve inflammation, which underscores the complexity of AA metabolism.

Given the demonstrated analgesic efficacy of n-3 PUFAs in preclinical and clinical studies, [36–39], we expected to find that higher levels of n-3 ALA, EPA and DHA, and a lower n-6/n-3 PUFA ratio, would be associated with higher nociceptive thresholds. Instead, we found all those associations were null. One explanation is that n-3 PUFAs levels were too low in this study to modify pain thresholds. Both n-6 LA and n-3 ALA are competitively metabolized by the same set of desaturation, elongation, and oxygenase enzymes. High levels of n-6 LA limit the capacity of n-3 ALA to generate adequate quantities of n-3 oxylipins. For example, Taha and colleagues demonstrated that increasing dietary n-6 LA in rats not only increased tissue levels of pronociceptive oxylipins, but it also reduced n-3 derived oxylipins. [40] Finding antinociceptive effects of n-3 oxylipins is more likely in randomized clinical trials where participants are administered targeted diets or supplements than in an observational study such as this. In addition, it is possible that n-3 ALA, EPA, and DHA are lower in other lipid storage depots, which we did not examine in this study.

Valdes and colleagues [41] demonstrated that the compound 17-hydroxy-docosahexaenoic acid (17-HDHA), which is derivative of n-3 DHA and the precursor to D-series resolvins, was associated with increased heat pain thresholds in 250 healthy volunteers. That same study compared 62 knee osteoarthritis cases with 52 healthy controls and found that circulating 17-HDHA was negatively associated with osteoarthritis pain intensity.

Of note, however, the D-series resolvins were not associated with thermal pain sensitivity or pain intensity in the healthy volunteers or the osteoarthritis cases, [41] suggesting that 17-HDHA was directly involved in pain.

This study was not an isolated report of antinociceptive effects of 17-HDHA in humans. In a non-randomized, open-label clinical trial, 44 adults with moderate or severe pain were administered a marine lipid supplement standardized to 17-HDHA and 18-HEPE for four weeks. In addition to favorable psychological and sleep quality outcomes, study participants experienced reductions in pain intensity and in pain interference [42] Strongest evidence for the efficacy of 17-HDHA comes from a randomized controlled trial in which 182 adults with migraine were randomized for 16 weeks to a control diet or to one of two dietary interventions that either increased n-3 PUFA intake alone, or increased n-3 PUFA intake and simultaneously decreased n-6 PUFA intake. Compared to the control diet, both intervention diets achieved greater increases in circulating 17-HDHA levels, which was the primary biochemical endpoint, and both intervention groups achieved greater reductions in pain outcomes. [39]

Quantitative sensory testing employs a variety of psychophysical test modalities with standardized protocols to understand somatosensory function under normal and pathological conditions. This study examined mechanical pressure pain thresholds because our studies have shown these to discriminate more consistently between pain cases and non-cases than other modalities of threshold and tolerance to painful stimuli including mechanical cutaneous pinprick pain sensitivity, heat pain ratings, heat pain aftersensations and tolerance, and heat pain temporal summation. [21,43,44]

There are several strengths to this study. Our findings suggest that omega-6 PUFAs may promote a generalized upregulation of nociceptive processing. As an ancillary study to OPPERA, our study capitalized on the expertise, data and biospecimens of the parent study. We determined pain status by clinical examination, or if examinations were not possible, by standardized protocols. We quantified circulating PUFAs with liquid chromatography tandem mass spectrometry that quantifies both exogenous and endogenous sources of long-chain PUFAs. Unlike plasma, PUFAs measured in erythrocytes are insensitive to fasting status and reflect dietary PUFA intake over the preceding 120 days. [45,46] Examiners at each study site underwent comprehensive training in measuring pressure pain thresholds and they exhibited excellent inter-examiner reliability, with intraclass correlation coefficients ranging from 0.87 to 0.94.

There were limitations too. Apart from the inability of cross-sectional data to determine bioactivity or causality, our analysis was restricted to the n-3 and n-6 precursor PUFAs, rather than their oxylipins derivatives. We were unable to quantify in a sufficient number of samples oxidized derivatives of n-6 LA including epoxyoctadecaenoic acid (EpOMEs) and hydroxyoctadecadienoic acids (HODEs) or oxidized derivatives from n-6 AA such as prostanoids, epoxyeicosatrienoic acids (EETs) and mid-chain hydroxyeicosatetraenoic acids (HETEs). Neither were we able to quantify the n-3 resolvin precursor 17-hydroxydocosahexaenoic acid (17-HDHA) or the EPA-metabolite 18-hydroxyeicosapentaenoic acid (18-HEPE) from most samples. Future work assessing the role of oxylipins in nociplastic pain conditions should investigate possible noninflammatory pathways, since unlike nociceptive pain, inflammation does not appear to play a major mechanistic or etiologic role in these common pain conditions. [7]

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Abbreviations

ALA	alpha-linolenic acid
ARA	arachidonic acid
DHA	docosahexaenoic acid
EPA	eicosapentaenoic acid
LA	linoleic acid
OPPERA	Orofacial Pain Prospective Evaluation and Risk Assessment
PUFA	polyunsaturated fatty acid
PPT	pressure pain threshold

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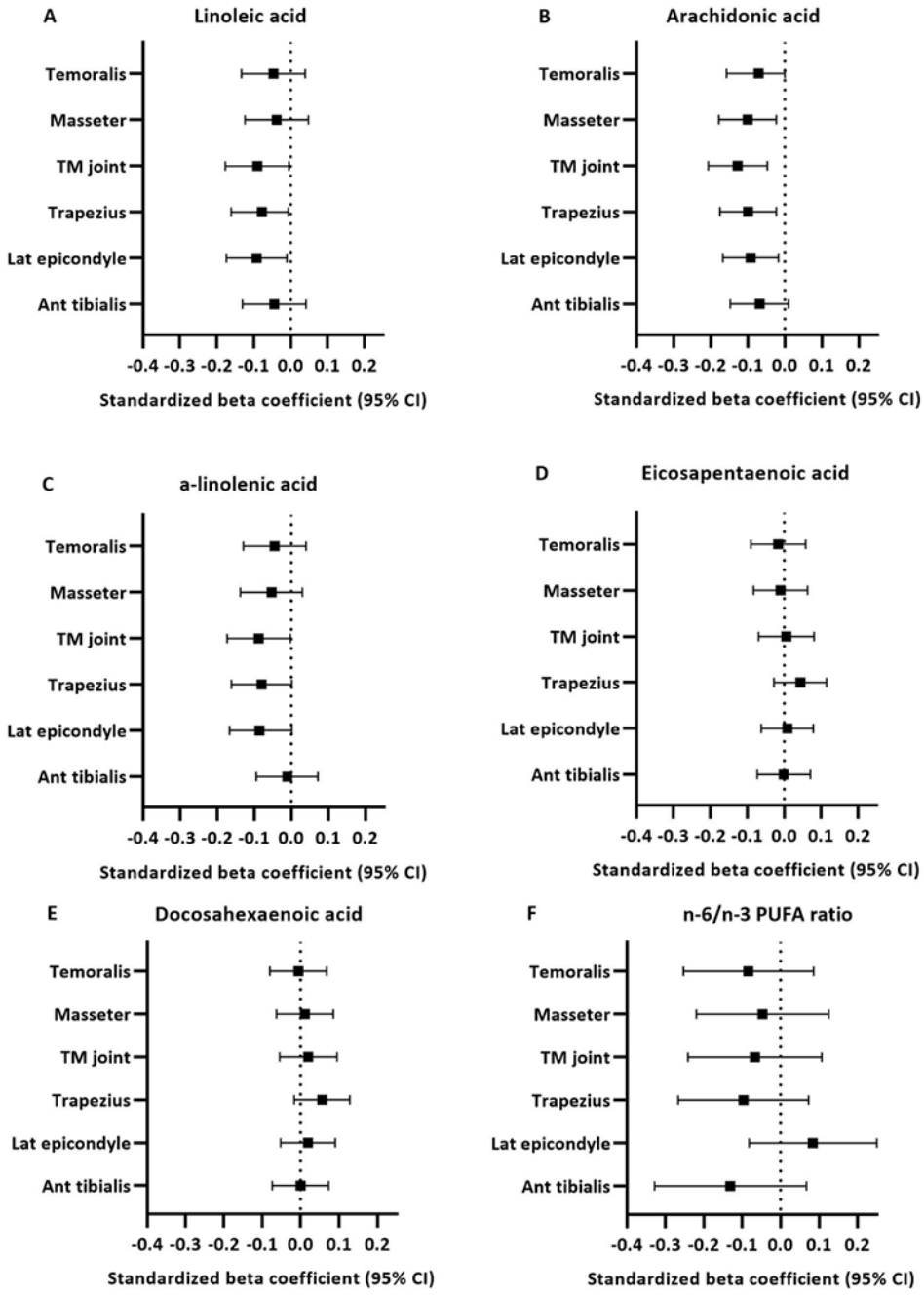

Fig. 1.

Data points are the covariate-adjusted standardized mean change in pressure pain threshold (PPT) per standard deviation increase in circulating concentration of five PUFAs: (A) n-6 linoleic acid; (B) n-6 arachidonic acid; (C) n-3 a-linolenic acid; (D) n-3 eicosapentaenoic acid; and (E) n-3 docosahexaenoic acid. Plot (F) depicts the unstandardized n-6/n-3 PUFA ratio expressed as AA/(EPA+DHA). Change was estimated in a linear regression model where the dependent variable was the standardized PPT at one of six anatomical locations and the predictor variable was a standardized measure of PUFA concentration. Models

adjusted for OPPERA study site, and participant sex, age, race/ethnicity, body mass index and smoking status. Estimates that cross the null value of zero are not statistically significant. Following is a screenshot of thumbnails of Fig. 1A through F to illustrate the preferred presentation for publication. For full-sized TIF images, please see attached files.

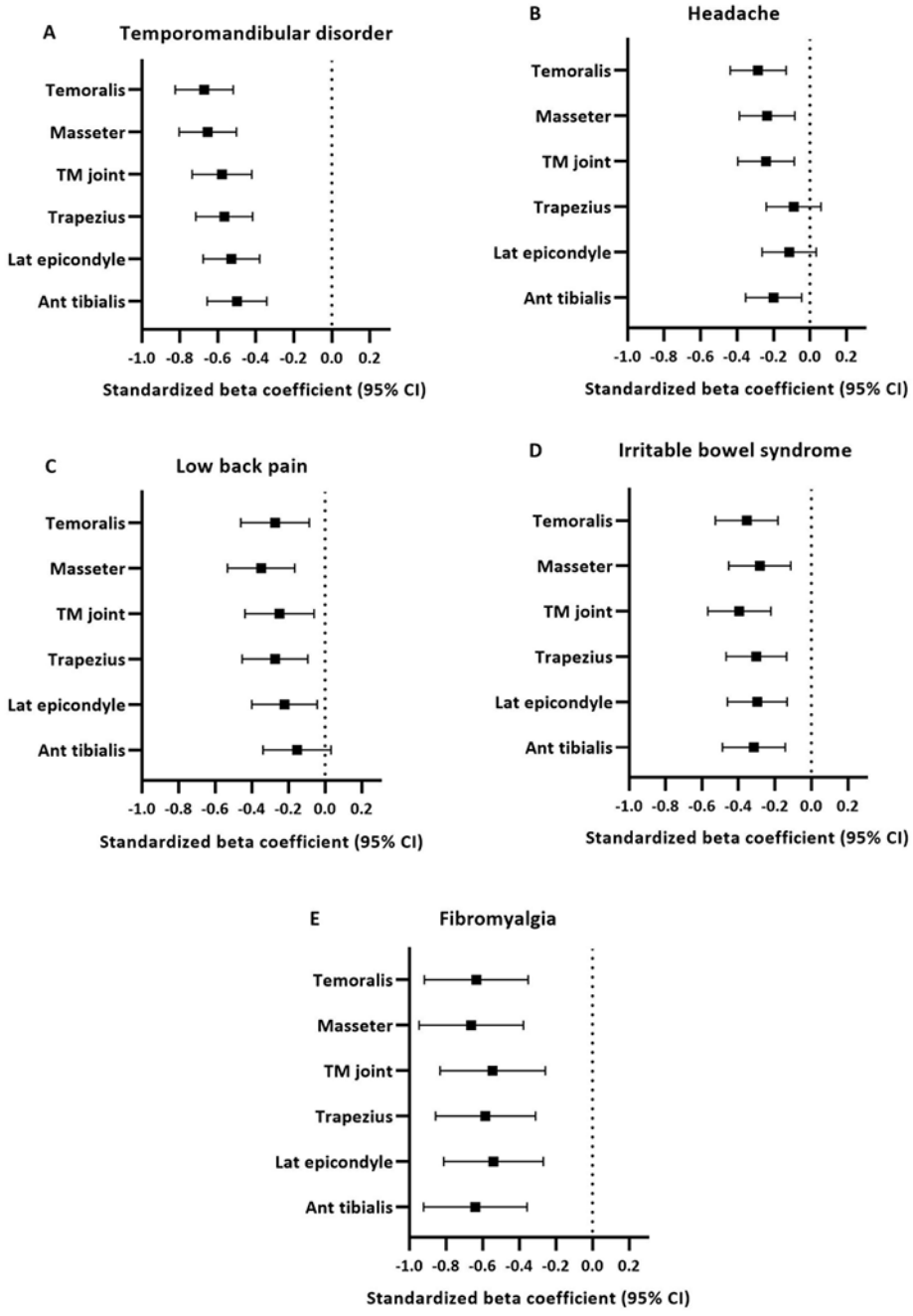

Fig. 2.

Data points are covariate-adjusted standardized mean differences (95% confidence intervals (CI)) in pressure pain thresholds (PPTs) at six anatomical sites where PPTs were measured between cases and controls of five pain conditions: (A) temporomandibular disorder; (B) headache; (C) low back pain; (D) irritable bowel syndrome; and (E) fibromyalgia. Mean differences were estimated in a linear regression model where the dependent variable was PPT and the predictor variable was binary pain case status. Models adjusted for OPPERA study site, and participant sex, age, race/ethnicity, body mass index and smoking status. All

mean differences are statistically significant except those that crossed the null value of zero. Values to the left of the null value are interpreted as PPTs being lower in cases than controls. Following is a screenshot of thumbnails of Figs. 2A through 1E to illustrate the preferred presentation for publication. For full-sized TIF images, please see attached files.

Table 1

Unadjusted erythrocyte concentrations (mean, standard error) of n-6 and n-3 PUFAs according to study participant characteristics (ng/mL).

	n,%	Linoleic acid		Arachidonic acid		α-linolenic acid		Eicosapentaenoic acid		Docosahexaenoic acid	
Total	605, 100.0	958.2	39.7	70.3	2.6	63.4	2.7	86.1	4.0	15.8	0.7
Sex											
Men	202, 33.4	1002.5	68.2	79.2	4.9	69.8	5.0	107.8	8.6	15.5	1.3
Women	403, 66.6	936.0	48.9	65.9	3.1	60.1	3.1	75.3	4.2	16.0	0.9
Age, y											
18–29	165, 27.3	830.1	73.8	58.7	3.5	57.1	5.0	61.0	5.0	12.7	1.0
30–39	169, 27.9	850.1	56.3	62.5	4.0	54.6	4.0	84.1	8.1	14.9	1.2
40–49	173, 28.6	1068.9	81.4	81.4	6.3	71.6	5.4	100.5	8.6	18.1	1.8
50–74	98, 16.2	1164.8	117.2	83.8	7.3	74.5	7.7	106.6	10.2	18.7	1.8
Race/ethnicity											
White	404, 66.8	908.1	45.2	67.5	3.2	59.9	3.0	84.6	4.8	14.9	0.8
African American	137, 22.6	1067.8	99.1	80.1	6.3	69.3	6.3	94.8	9.7	18.0	1.9
Other	64, 10.6	1039.8	119.7	67.3	6.6	72.6	9.3	77.0	10.1	16.9	2.3
Body mass index											
Normal	239, 39.5	885.0	67.4	63.4	4.5	58.0	4.6	75.5	6.2	16.2	1.3
Overweight	172, 28.4	977.5	74.0	65.8	3.7	62.9	4.8	80.3	6.6	15.5	1.3
Obese	194, 32.1	1031.4	64.2	82.8	5.0	70.4	4.3	104.4	7.9	15.7	1.1
Smoking status											
Never	425, 70.3	865.6	40.1	65.3	2.8	58.1	2.8	80.3	4.4	14.6	0.8
Former	94, 15.5	962.3	113.6	67.2	7.1	61.7	6.7	89.4	10.4	16.8	1.9
Current	86, 14.2	1411.3	144.4	98.6	9.1	91.2	10.0	111.2	13.7	20.7	2.6

World Health organisation BMI cut-points for categories of normal (18.5 – 24.9 kg/m^2), overweight (≥25 <29.9 kg/m^2), and obese (≥30 kg/m^2).

Table 2

Unadjusted pressure pain thresholds (mean, standard error) at six anatomical sites according to study participant characteristics (kPa).

	Temporalis		Masseter		Temporo-mandibular joint		Trapezius		Lateral epicondyle		Anterior tibialis	
Total	175.3	2.8	157.7	2.6	149.1	2.6	306.7	6.0	301.7	5.5	406.8	6.2
Sex												
Men	203.2	5.3	187.8	5.0	170.1	4.6	383.8	10.3	370.5	9.6	484.8	9.0
Women	161.3	3.1	142.6	2.8	138.5	3.0	268.2	6.6	267.0	5.9	367.0	7.4
Age, y												
18–29	170.1	5.2	154.9	5.1	142.7	4.6	294.3	11.3	289.7	10.4	406.6	11.6
30–39	176.2	5.3	162.2	5.2	150.8	4.7	314.1	11.2	299.9	10.3	418.7	11.3
40–49	176.6	5.6	155.3	4.9	148.2	5.0	307.2	11.0	307.6	10.0	399.1	12.4
50–74	180.5	7.0	159.1	6.3	158.7	7.1	314.2	15.9	314.5	14.0	400.8	15.3
Race/ethnicity												
White	175.2	3.4	157.4	3.1	150.2	3.0	315.3	7.4	303.8	6.6	413.8	7.5
African American	180.6	6.3	159.1	5.9	143.7	5.6	285.7	12.1	311.4	11.8	390.3	13.0
Other	165.1	8.3	156.9	8.6	153.6	9.1	297.2	18.5	268.1	16.9	398.6	20.3
Body mass index												
Normal	169.5	4.7	150.6	4.2	142.7	4.1	300.8	9.5	286.6	8.8	401.0	10.3
Overweight	184.3	5.1	165.1	4.9	152.7	4.4	327.0	11.1	310.9	10.3	427.2	11.4
Obese	174.5	5.0	159.8	4.6	153.8	4.9	295.9	10.6	312.2	9.3	396.1	10.5
Smoking status												
Never	172.6	3.3	157.5	3.0	147.4	3.0	302.9	7.0	296.8	6.5	401.3	7.4
Former	181.2	7.3	157.0	7.1	154.3	7.2	319.4	16.1	317.0	13.8	406.8	15.7
Current	182.4	8.1	159.6	8.0	151.7	7.3	311.8	16.0	309.3	14.9	435.5	17.1

Table 3

Multivariable-adjusted beta coefficients (β) with 95% confidence limits (CL) for association of n-6/n-3 long-chain PUFA ratio with the number of pain conditions per study participant.

	β	95% CI	P-value
n-6/n3 PUFA ratio	0.30	0.07, 0.53	0.012
Linoleic acid 18:2n-6 (z-score)	0.02	−0.23, 0.26	0.885
α-linolenic acid 18:3n-3 (z-score)	0.06	−0.19, 0.30	0.662
Sex			
Men	Ref		
Women	0.17	−0.06, 0.41	0.151
Age, y	0.00	−0.01, 0.01	0.583
Race/ethnicity			
White	Ref		
African American	−0.44	−0.74, −0.14	0.004
Other	0.13	−0.25, 0.50,	0.509
Body mass index	0.01	−0.01, 0.03	0.227
Smoking status			
Lifetime non-smoker	Ref		
Former	0.42	0.10, 0.75	0.011
Current	0.34	−0.01, 0.68	0.055
Intercept	0.44	−0.21, 1.09	0.180

Model also adjusts for OPPERA study sites.

Other racial/ethnic groups are Asian; Native American; Other or multiple races; Not stated. Includes Hispanic ethnicity.