Omega-6:Omega-3 PUFA Ratio, Pain, Functioning, and Distress in Adults with Knee Pain

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Abstract

Objectives—Osteoarthritis (OA) is associated with inflammation, chronic pain, functional limitations, and psychosocial distress. High Omega-3 (*n-3*) polyunsaturated fatty acids (PUFAs) are associated with lower levels of inflammatory mediators, anti-nociception, and adaptive cognitive/emotional functioning. High Omega-6 (*n-6*) PUFAs are associated with inflammation, nociception, and psychological distress. While findings related to *n*-3 supplementation in knee OA

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are mixed, consideration of the *n*-6:*n*-3 ratio and additional outcome measures may provide improved understanding of the potential relevance of these fatty acids in OA. Based on recommended and typical ranges of the *n*-6:*n*-3 ratio, we hypothesized that in adults with knee pain, those with a high *n*-6:*n*-3 ratio would have greater pain/functional limitations, experimental pain sensitivity, and psychosocial distress compared to those with a low *n*-6:*n*-3 ratio.

Methods—A cross-sectional investigation of clinical and experimental pain and physical and psychosocial functioning was completed in 167 adults ages 45–85 meeting knee OA screening criteria. Blood samples were collected and the plasma *n*-6:*n*-3 PUFA ratio determined. Quartile splits were computed and low (n=42) and high (n=41) ratio groups were compared.

Results—The high ratio group reported greater pain and functional limitations, (all p's<0.04), mechanical temporal summation (hand and knee, p<0.05), and perceived stress (p=0.008) but not depressive symptoms.

Discussion—In adults with knee pain, a high *n*-6:*n*-3 ratio is associated with greater clinical pain/functional limitations, experimental pain sensitivity, and psychosocial distress compared to a low ratio group. Findings support consideration of the *n*-6:*n*-3 PUFA ratio and additional clinical endpoints in future research efforts.

Keywords

Osteoarthritis; Pain; Functioning; Essential Fatty Acids; Omega-6:Omega-3 Ratio

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Introduction

Knee osteoarthritis (OA) represents a leading cause of disability worldwide, with current lifetime risk exceeding 40% and prevalence on the rise due to increasing longevity and obesity in the U.S [1]. Knee OA is associated with inflammation, chronic pain, and psychosocial distress [2, 3]. Evidence suggests that in addition to joint-specific inflammation, systemic inflammation is also involved in the pathogenesis of OA [4]. Due to cardio-intestinal risks, standard anti-inflammatory treatments such as NSAIDs have significant limitations in addition to minimal effectiveness, a common issue with other pain medications [5]. Hence, alternative safe and effective approaches to reduce inflammation and pain and improve function are needed [5].

Omega-3 (*n*-3) poly unsaturated fatty acids (PUFA) represents a group of essential fatty acids. Commonly recognized n-3 PUFAs include alpha linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), there is also docosapentaenoic acid (DPA) in addition to a few others [6]. Higher levels of *n*-3 PUFAs are associated with lower levels of inflammation and pain in conditions such as rheumatoid arthritis, inflammatory bowel disease, dysmenorrhea, and neuropathy [7–9]. Additionally, recent findings indicate that SPMs, specialized pro-resolving mediators, compounds derived from essential PUFAs that serve as anti-inflammatory mediators, promoting regeneration, recovery, resolution of inflammatory process and return to homeostasis across numerous systems [7]. A recent clinical trial comparing low versus high dose *n*-3 PUFAs (fish oil supplementation) in adults with symptomatic knee osteoarthritis demonstrated reduced clinical pain and increased functioning, at year one for both groups, but at year two the low

dose group showed the greatest benefit. Additionally, there were no significant changes in cartilage volume loss between the two groups [10].

Beyond affecting the OA joint, systemic inflammatory processes can directly influence pain processing peripherally and centrally, contributing toward central sensitization [11, 12]. Importantly, *n*-3 PUFAs have peripheral and central anti-inflammatory and anti-nociceptive properties [6–9, 13]. The central anti-inflammatory properties are reflected by the ability of *n*-3 PUFAs to increase neuroplasticity and neurogenesis and in their positive association with cognition and emotional functioning [6, 8, 14–16]. Omega-3 PUFAs are believed to confer neuroprotective effects and reduced neuroinflammation via metabolism to eicosanoids and docosanoids [6, 14].

While *n*-3 PUFAs are essential in maintaining optimal health outcomes, omega-6 (*n*-6) PUFAs are equally important. Omega-6 PUFAs are comprised of arachidonic acid (AA), linoleic acid (LA), docosapentaenoic acid (DPA) among others [6]. Omega-6 PUFAs contribute to a number of necessary pro-inflammatory functions including the generation of prostaglandins which are derived from AA. Prostaglandins are vital to the inflammatory process and associated with the two cyclooxygenase isoforms (COX-1 and COX-2) by which their biosynthesis is blocked by non-steroidal anti-inflammatory drugs [17]. High levels of *n-6* PUFAs are associated with inflammation, chronic pain, and cognitive impairment and psychological distress [9, 18]. Consideration of the *n*-6 and *n-3* PUFAs balance appears to have clinical and functional relevance. A recommended *n*-6:*n*-3 PUFA ratio ranges from 2:1 to 5:1 [6, 19, 20]. Unfortunately, *n*-6 PUFA intake is excessive in the Western diet resulting in significantly higher ratios in the range of 15:1 or greater [6, 13, 20]. A high *n-6:n-3* PUFA ratio was associated with inflammation and depression in older adults [21]. Additionally, an intervention targeting increasing *n*-3 PUFAs and decreasing *n*-6 PUFAs demonstrated improved quality of life and functioning, as well as decreased distress and headache frequency and severity in individuals with chronic headaches [22, 23]. Evaluating the *n*-6:*n*-3 PUFA ratio provides information regarding metabolism, absorption, and relative balance and may help elucidate the role of these fatty acids in OA-related pain and function [13, 20]. We extend previous findings by investigating the associations between the *n*-6:*n*-3 PUFA ratio and clinical pain, experimental pain sensitivity, and physical and psychosocial functioning in middle-aged and older adults with knee pain related to or at risk for OA. Based on recommended and typical *n*-6:*n*-3 PUFA ranges [6, 20], we evaluated a low ratio group compared to a high ratio group and hypothesized: in individuals with knee pain, those with a high *n*-6:*n*-3 PUFA ratio would have greater 1) clinical pain and functional limitations, 2) experimental pain sensitivity, and 3) psychosocial distress compared to those with a low *n*-6:*n*-3 PUFA ratio.

Materials and Methods

Study Design

Participants were recruited for a cross-sectional study from the communities surrounding the University of Florida (UF) and the University of Alabama at Birmingham (UAB) from Jan 2010 to Dec 2012. Participants involved in the current investigation are from a larger study

entitled *Understanding Pain and Limitations in Osteoarthritic Disease* (UPLOAD). All procedures were reviewed and approved by the UF and UAB Institutional Review Boards.

Participants

A total of 167 individuals, ages 45–85 who screened positive for symptomatic knee OA were included in the investigation [24]. A description of the screening, inclusion, and exclusion criteria can be found in Table 1 and are previously reported [25, 26]. Participants completed a health assessment session and a quantitative sensory testing session on separate days. The health assessment included a review of health history, current treatment, physical exam, and knee radiographs. The measures and procedures described below are limited to those included in the current analysis.

Clinical Pain and Functioning Measures

Participants completed self-report measures of clinical pain and functional limitations including the Western Ontario and McMaster Universities Index of Osteoarthritis (WOMAC) [27] and the Graded Chronic Pain Scale (GCPS) [28]. Physical function was assessed with the Short Physical Performance Battery (SPPB) [29, 30].

Experimental Pain Measures

The current analysis evaluated pressure and punctate mechanical quantitative sensory testing procedures. Both have demonstrated robust differences between individuals with versus without OA-related knee pain. Additionally, mechanical temporal summation provides a measure of central sensitization, a key question in this investigation [25, 31]. Pressure pain sensitivity was assessed on the most symptomatic knee (i.e., index knee) and the ipsilateral forearm with a handheld digital pressure algometer (Algomed, MEDOC, Ramat Yishai, Israel). The algometer was applied at a constant rate of 30 kilopascals (kPa) per second until the participant pressed a button when the sensation "first became painful." The amount of pressure (kPa) that first produced a painful sensation was recorded.

Punctate stimuli and temporal summation of pain were evaluated with a nylon monofilament (Touchtest Sensory Evaluator 6.65), calibrated to bend at 300g of pressure. Testing sites order was randomized and included the patella of the index knee and the dorsum of the ipsilateral hand. Participants were instructed to provide a verbal rating of pain following a single contact (i.e., 1st Trial) and following a series of 10 contacts (i.e., 10th trial), which were provided at a rate of 1 contact per second. Verbal ratings were on scale of 0 (no pain sensation) to 100 (the most intense pain sensation imaginable). The procedure was completed twice at each site. For each site, verbal ratings for the single and multiple contacts were averaged, and a change score (e.g., rating at 10th trial – rating at 1st trial) was calculated as an index of temporal summation.

Psychosocial Measures

Depressive symptoms were assessed with the Center for Epidemiologic Studies Depression scale, CES-D which measures symptoms of depression over the past week [32]. Perceived stress was measured with the Perceived Stress Scale, PSS [33]. Trait positive and negative

affect was assessed with the Positive and Negative Affect Schedule, PANAS [34]. Optimism was measured with the Life Orientation Test-Revised, LOT-R [35].

Omega-6 and Omega-3 Fatty Acid Analysis

A blood sample was collected at the conclusion of the second study session and placed on ice. It was centrifuged at 4°C for 10 minutes at 3000 rpm. Plasma was pipetted (980 μl) into a 1.5 mL cryovial and 10 μl of 5 mmol Butylated hydroxytoluene (BHT) and 10 μl 5 mmol Diethylenetriaminepentaacetic acid (DTPA) were added for a final volume of 1 mL. The sample was mixed gently by inversion and placed in a -80°C freezer.

Sample Preparation

Samples were prepared by aliquoting 100 μL of sample or calibrator into a microcentrifuge tube and adding 10 μL of internal standard (10 μg/mL DHAd5, 3 μg/mL EPAd5, 10μg/mL AAd8, 5μg/mL ALAd14, 20μg/mL LAd4 prepared in 3:1 acetonitrile:methanol with 100 g/L Butylated hydroxyl toluene, BHT). Next, 300 μL of ethanol containing 100 g/L BHT was added to each vial, rapidly mixed using a vortex, then cooled at 4C for 10 min followed by centrifugation at 20,000 rcf for 10 min to pellet the protein. The supernatant was transferred to a clean microcentrifuge tube and dried down for 1 hour using a vacuum dryer. The dried sample was dissolved in 50 μL of 1:1 acetonitrile:methanol containing 0.1% acetic acid, mixed, centrifuged at 20,000 rcf for 5 min and transferred to a 96 well plate. The injection volume for LC-MS/MS analysis was 5 μL. All labeled internal standards and unlabeled standards were obtained from Cayman Chemical (Ann Arbor, MI). All solvents used were of LC/MS grade and obtained from FisherScientific (Fairlawn, NJ).

LC-MS/MS Analysis

Analysis was performed by liquid chromatography tandem mass spectrometry (LC-MS/MS) using an Accela 1250 UHPLC, Accela Open autosampler and TSQ Quantum Access (ThermoScientific, San Jose, CA). The mass spectrometer was operated in negative heated electrospray (HESI) with 3000 V spray voltage, 50C HESI probe temperature, 50 arb sheath gas, 20 arbitrary units auxiliary gas, 300C capillary temperature, and position D for distance of the HESI probe. Selected reaction monitoring (SRM) was used for detection of DHA, EPA, and AA while single ion monitoring (SIM) was used for ALA and LA. DPA *n-6* and DPA *n-3* among other PUFAs were not quantified as they were not part of the LC-MS/MS assay and were not validated. All parameters for SRM and SIM are described in Table 1. Separation was achieved with gradient elution on a Thermo Hypersil aQ column (100 mm × 2.1mm, 1.7 μm, ThermoScientific) at a flow rate of 300 μL/min and column temperature of 40C. Mobile phase A was 1mM Ammonium acetate, mobile phase B was 0.1% acetic acid in acetonitrile, and mobile phase C was 0.1% acetic acid in methanol. The gradient started at 25% A, 65% B and 10% C from 0–0.25 min, increased to 90% B and 10% C from 0.25–2 min, held constant from 2–4 min, returned to initial conditions from 4–4.5 min and equilibrated from 4.5–6.5 min. Percent totals were obtained for *n*-6 and *n*-3 and then the ratio was computed. See the Supplemental Table for the response factors for LC-MS/MS.

Data Analysis

Quartile splits were completed to create a low ratio group and high ratio group that reflected recommended and typical *n*-6:*n*-3 PUFA ratio ranges [6, 19, 20]. Descriptive analyses for continuous and categorical variables were conducted and group differences in those variables were tested using either ANOVA F-test or Chi-square test. Linear regression models were used to conduct model based tests for comparing the low versus high ratio group difference adjusting for age, ethnicity, gender and site variables. The model-based means of outcomes in the two groups are computed with the 95% confidence interval and ANCOVA F-test and p-value for testing the group difference with covariates in the model. Since the SPPB scales are limited in range and non-normally distributed, non-parametric analyses were also completed.

Smoking status, exercise, and body mass index (BMI), were explored as additional covariates in the model by 1) completing a partial correlation analyses with *n*-6:*n*-3 PUFA ratio controlling for primary covariates (age, sex, ethnicity, and study site), if significant, then 2) partial correlations were completed with outcome variables controlling for primary covariates (age, sex, ethnicity, and study site). The only variable that showed a significant relationship with *n*-6:*n*-3 PUFA ratio was smoking status (BMI r = .214, p = 0.060; exercise r = −.203, p = 0.074; and smoking status r = .241, p = 0.034). Smoking status and BMI were also significantly correlated (r = −.257, p = 0.023). A partial correlation was then completed for smoking status and outcome variables with primary covariates in the model. Smoking status was significantly correlated with the PANAS negative affect score (r = .262, p = 0.023) and included in a secondary analysis of this outcome measure. Additional analyses were also completed stratifying by ethnicity to better understand possible ethnic group differences. The data analysis was conducted using SAS 9.4.

Results

Quartile Grouping: n-6:n-3 Ratio

Table 2 displays the unadjusted and adjusted means for each individual *n*-6 and *n*-3 PUFA by the low and high *n*-6:*n*-3 PUFA ratio groups. The *n*-6:*n*-3 PUFA ratio for the low and high groups based on the quartile splits (25th% and 75th%) were in the recommended (5) and typical (>5) ranges respectively.

Demographic and Biobehavioral Factors

Of the 167 participants in the study, 53% were African American, 47% were non-Hispanic white adults, 29% were men and 71% were women. Demographic characteristics of the low ratio (n=42) and high ratio (n=41) groups are presented in Table 3. The high ratio group included a greater proportion of African Americans and smokers and had a greater body mass index (BMI) compared to the low ratio group (all p's < 0.01). A greater proportion of individuals in the low ratio group reported supplementing with some form of *n*-3 PUFA product (n=9) compared to the high ratio group (n=2). No differences were observed for age, sex, employment, marital status, exercise frequency, and Kellgren Lawrence scores [36].

Clinical Pain and Functioning

As noted in Table 4, the high ratio group had higher scores (greater clinical pain and functional limitations) for the WOMAC total score (p = 0.011) and associated subscales (all p's<0.04), and slightly lower physical functioning scores on the SPPB chair stand (p = 0.001) and total (p = 0.008) scores indicated by non-parametric analysis. Group differences were not indicated for the GCPS pain intensity and interference score.

Experimental Pain Sensitivity

While pressure pain thresholds (PPTh) were similar between the two groups (all p's > 0.10), a significant group difference emerged for punctate sensitivity at the knee and hand (Table 4). Specifically, participants in the high ratio PUFA group reported greater pain intensity following a series of ten mechanical taps and a greater change score (10th tap – 1st tap), reflecting mechanical temporal summation (Figures 1A & B).

Psychosocial Functioning

The high ratio group had higher scores for perceived stress (PSS) and negative affect (PANAS), but not for depressive symptoms (CES-D), optimism (LOTR), or positive affect (PANAS), Table 4. Secondary analysis of PANAS Negative Affect was completed with smoking status (current smoker – yes/no) as a covariate in the model, although group differences were no longer significant, the trend continued to show lower scores in the low ratio (mean = 13.2) compared to the high ratio group (mean = 15.2, [p = 0.086]).

Additional Analyses

As the low and high n-6:n-3 PUFA ratio groups differed by ethnicity, the n-6:n-3 PUFA ratio and selected measures are presented in Table 5 stratified by ethnic group. Although interpretation is limited due to small sample sizes, the low and high ratio group differences were similar across ethnic groups, but differences in the WOMAC and PSS appeared somewhat larger for the African American group compared to the non-Hispanic white group.

Discussion

Our findings suggest that in adults with knee pain, a low plasma n-6:n-3 PUFA ratio is associated with lower levels of knee pain symptoms, improved physical functioning, lower levels of temporal summation of mechanical pain, and less psychosocial distress compared to those with a high plasma n-6:n-3 PUFA ratio. This is the first study to investigate the relationship of n-6:n-3 PUFA ratio with an expanded array of outcome variables including experimental pain sensitivity and psychosocial functioning in individuals screened for OA-related knee pain. Findings encourage the consideration of the n-6:n-3 PUFA ratio and additional clinical endpoints in future research and when evaluating applicability to clinical care.

n-6:n-3 Ratio

Our high ratio group had an average n-6:n-3 PUFA ratio of 9.92, almost twice the recommended ratio of less than 5 [6, 17, 20]. The low ratio group had an average n-6:n-3

PUFA ratio of 5.08, near the top end of the recommended ratio level. There is evidence that diet [6] and supplementation with n-3 PUFAs can increase n-3 PUFA levels and lower the n-6:n-3 PUFA ratio [13, 15]. Although characterized by significant variability in product type and frequency of consumption, we found that more individuals from the low ratio group used some form of n-3 PUFA supplementation, 21%, compared to 5% in the high ratio group. Additionally, a person with an n-6:n-3 ratio below 5 is likely eating different foods compared to someone with a high ratio and also likely living a healthier lifestyle, further investigations exploring these relationships are needed [19,20].

Clinical Pain

Hill and colleagues recently reported that knee OA patients receiving low dose n-3 PUFA supplementation showed greater decreases in WOMAC pain and function scores over a two-year period compared to patients randomized to high dose supplementation [10]. Although cross-sectional, our findings suggest that individuals with higher n-3 PUFAs relative to n-6 PUFAs endorsed lower levels of pain, stiffness, and functional limitations (WOMAC). Additionally, we extend previous findings by including an objective measure of functioning, the SPPB, which has been associated with clinical pain [25, 37]. One of the challenges with the SPPB in our sample is the limited range and non-normal distribution of scores, which dictated use of a non-parametric analysis. The analysis revealed poorer performance on the SPPB chair stand and total scores in the high versus low ratio group. However, the non-parametric approach prohibits controlling for possible confounding variables.

Experimental Pain

Prior studies of n-3 PUFA levels and n-3 PUFA supplementation in knee OA have focused on the peripheral anti-inflammatory benefits specific to joint health [10, 38]. Less investigated are the benefits of n-3 PUFAs in protecting peripheral and central nervous system functioning, including potential influences on nociceptive processing. Early preclinical and clinical findings suggest fatty acids play a role in the onset and suppression of pain by way of peripheral and central mechanistic influences [9]. We add to this developing body of literature by demonstrating that individuals with a high n-6:n-3 PUFA ratio show greater mechanical temporal summation, a measure of central sensitization found to be predictive of OA-related clinical pain [25].

Psychosocial

The relationships between low levels of n-3 PUFAs and high levels of n-6 PUFAs on cognitive and emotional functioning have been reported across populations and conditions [15, 16, 21]. Chronic pain is associated with increased negative affect, depression, anxiety, and psychosocial distress [39, 40]. Supplementation of n-3 PUFAs in medical students resulted in decreased anxiety, improved performance and lower n-6:n-3 PUFA ratios [15], and increasing n-3 PUFAs and decreasing n-6 PUFAs was associated with improved function in adults with chronic headaches [22]. Although a cross-sectional study, we found that a high n-6:n-3 PUFA ratio is associated with greater levels of perceived stress and a trend for greater negative affect among individuals with knee pain.

Additional Considerations

Health disparities in OA-related knee pain, functional limitations, and environmental/social stress have been consistently reported, with African Americans experiencing higher levels of pain, functional limitations, and environmental/social stress compared to non-Hispanic whites [41]. Our findings highlight some potentially important opportunities. Although the low and high ratio group differed by ethnicity, with more African Americans in the higher ratio group and more non-Hispanic whites in the low ratio group, the mean n-6:n-3 PUFA ratio in each group did not significantly differ by ethnicity. Interpretation of post hoc analyses warrants caution given that the sample sizes are small, however, the pattern of findings was similar across ethnic groups, though n-6:n-3 PUFA ratio group differences for WOMAC and PSS appeared larger for African Americans, i.e., lower levels of pain and perceived stress for the low ratio group. Importantly, targeting a low n-6:n-3 PUFA ratio may have benefits regardless of ethnicity.

Limitations and Future Directions

Since the study was cross-sectional, to permit causal interpretations, a prospective intervention study is needed with a similar array of comprehensive measures including clinical, functional, experimental pain, and psychosocial. As this was an initial investigation of the n-6:n-3 PUFA ratio, future investigations will benefit from inclusion of additional individual PUFAs such as DPA n-6 and DPA n-3. The incorporation of other functional measures such as walking speed, which has significant predictive utility in healthy aging and functioning, and well-validated quality of life measures would further enhance our understanding of the contributions of the n-6:n-3 PUFA ratio in individuals with OA-related knee pain. While excluding individuals taking frequent opioids, the current investigation did not include an evaluation of NSAIDs, other pain medications, and/or psychotropics. Future investigations would benefit from inclusion of pain medications in the analyses to determine the influence on n-3 PUFAs, n-6 PUFAs and overall PUFA ratio and the comprehensive array of pain and functioning measures in order to improve understanding of potential clinical applicability. Additionally, deciphering the relationships between BMI, smoking status, and the n-6:n-3 PUFA ratio warrant further investigations. While we controlled for some obvious confounders, the n-6:n-3 PUFA ratio may represent a proxy for other health-related variables that were not measured or included in the model, such as diet and other lifestyle factors. Further research is needed to better understand the relationship between diet, lifestyle behaviors and the n-6:n-3 PUFA ratio. Finally, replication of findings in studies with larger sample sizes, varying OA severity, and balanced gender groups will improve the interpretability of the relevance of the n-6:n-3 PUFA ratio to pain and functioning associated with OA-related knee pain. In general, a greater proportion of women do experience knee OA pain, however, a greater representation of men will allow for the investigation of possible sex differences.

Conclusions

Our findings provide evidence that a lower n-6:n-3 PUFA ratio is associated with clinical, nociceptive, physical, and psychosocial functioning in individuals with knee pain. The results of the current study highlight the importance of 1) considering the n-6:n-3 PUFA

ratio in addition to evaluating n-6 and/or n-3 PUFA measures independently, and 2) including a comprehensive range of clinical, functional, and psychosocial outcome measures in future investigations. First, the *n*-6:*n*-3 PUFA ratio provides an easy to understand metric reflecting the *n*-6 and *n*-3 PUFA balance. Second, evaluating a broad array of clinical and functional outcome measures will enhance our understanding of the role of PUFAs specific to pain and promote the identification of possible treatments for individuals with or at risk for symptomatic knee osteoarthritis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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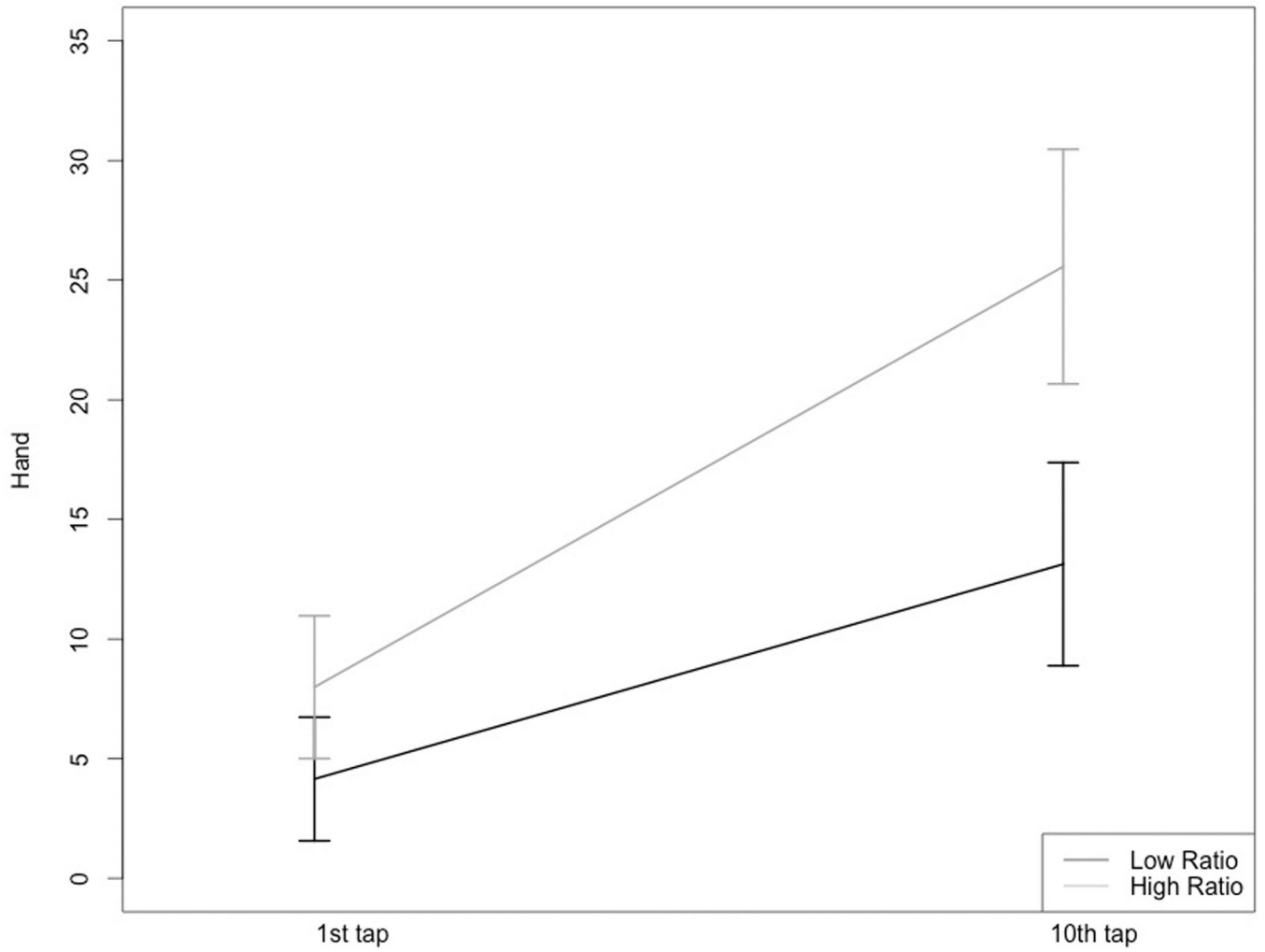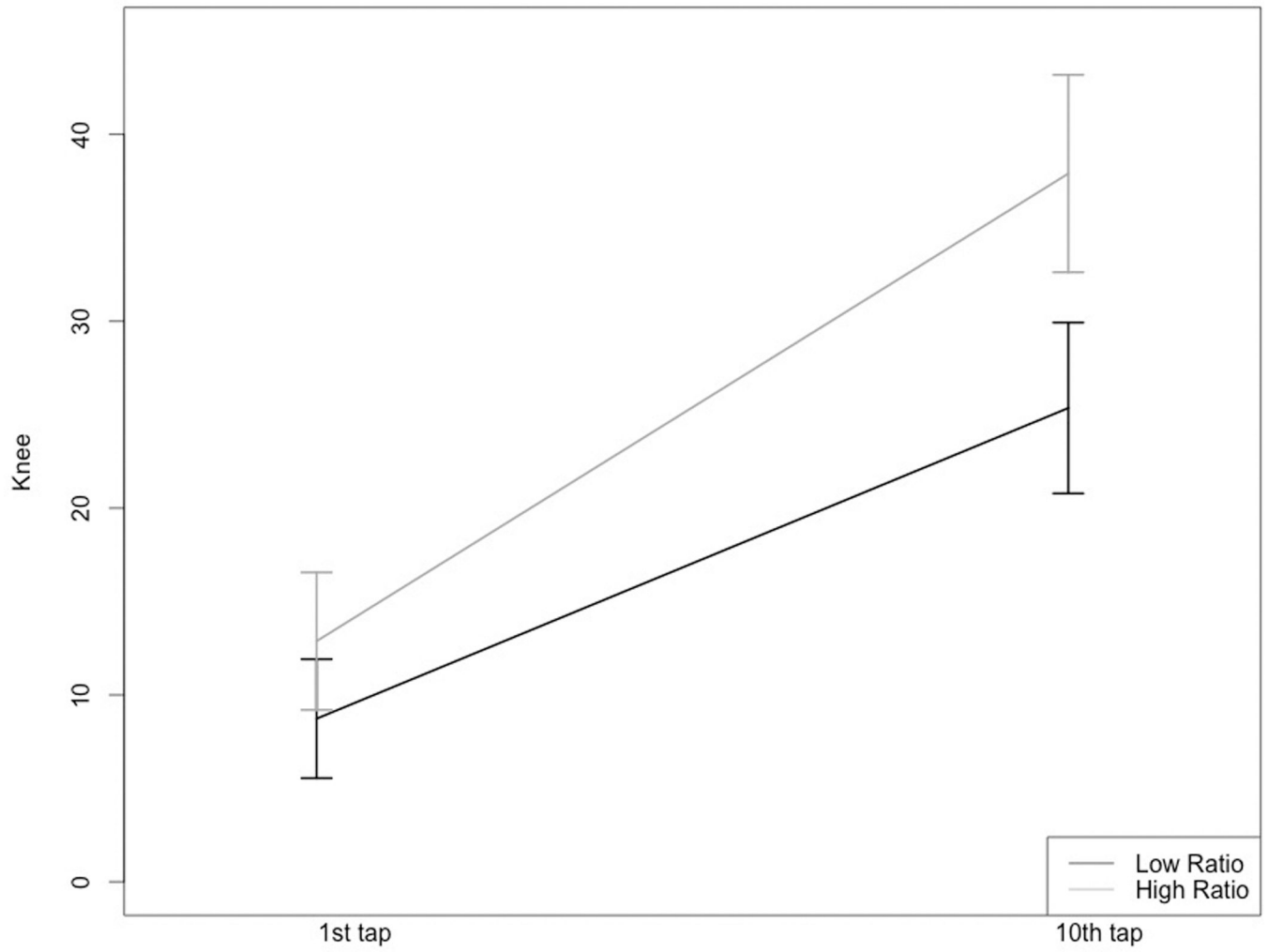
Figures 1A & 1B.

Mechanical temporal summation at the hand and knee for the low and high *n*-6:*n*-3 PUFA ratio groups

Low ratio group, n=42; High ratio group, n=41

Table 1

Inclusion/exclusion criteria

Exclusion Criteria

- Prosthetic knee replacement or non-arthroscopic surgery to the symptomatic knee

- Serious medical conditions (e.g., uncontrolled hypertension greater than 150/95, heart failure, history of acute myocardial infarction)

- Peripheral neuropathy

- Systemic rheumatologic disorders (e.g., rheumatoid arthritis, systemic lupus erythematosus, fibromyalgia)

- Daily opioid use

- Mini Mental Status Exam (MMSE) score 22

- Excessive anxiety regarding protocol procedures (e.g., intravenous catheter insertion, experimental pain procedures

- Psychiatric hospitalization within the preceding year

General Inclusion Criteria

- Between the ages of 45 and 85 years of age

- Self-identified as either African American or non-Hispanic white

Inclusion Criteria for Knee OA Pain

- Positive response to one of four knee osteoarthritis screening questions [24].

    1. During past 4 weeks, have you had knee pain on most days?

    2. During past 4 weeks, have you had knee pain while climbing down stairs or walking down slopes?

    3. During past 4 weeks, have you had swelling in one or both knees?

    4. Do you have knee OA (if you do, was the diagnosis made by a rheumatologist or general practitioner?); Or

- Reporting of pain in the index knee over the past 48 hours[a] and/or prior 6 months[b]

[a] Western Ontario and McMaster Universities Index of Osteoarthritis (WOMAC) and the

[b] Graded Chronic Pain Scale (GCPS)

Table 2

Unadjusted/Adjusted means for each PUFA for low & high *n*-6:*n*-3 PUFA ratio groups

	Low Ratio n = 42			High Ratio n = 41			Group Difference	
	Mean	95% CI		Mean	95% CI		F-value	p-value
		Lower Bound	Upper Bound		Lower Bound	Upper Bound		
Omega-3								
DHA%	*6*	*5.14*	*6.67*	*2*	*1.09*	*2.64*	*54.48*	*<.0001*
	6.31	5.32	7.30	2.13	0.99	3.27	48.76	<.0001
ALA%	*10*	*9.34*	*11.33*	*7*	*6.14*	*8.16*	*20.14*	*<.0001*
	9.67	8.38	10.95	6.66	5.18	8.14	14.91	0.0002
EPA%	*1*	*1.04*	*1.38*	*0*	*0.15*	*0.50*	*49.99*	*<.0001*
	1.19	0.96	1.41	0.36	0.09	0.62	36.99	<.0001
Sum%	*17*	*16.35*	*18.56*	*9*	*8.23*	*10.46*	*105.43*	*<.0001*
	17.17	15.73	18.61	9.14	7.48	10.80	84.45	<.0001
Omega-6								
AA%	*8*	*6.58*	*9.40*	*4*	*2.71*	*5.56*	*14.7*	*0.0002*
	9.76	8.13	11.39	5.85	3.97	7.73	15.66	0.0002
LA%	*75*	*72.63*	*76.48*	*87*	*84.58*	*88.47*	*75.53*	*<.0001*
	73.07	70.72	75.42	85.01	82.30	87.72	70.20	<.0001
Sum%	*83*	*81.45*	*83.65*	*91*	*89.54*	*91.77*	*105.38*	*<.0001*
	82.84	81.40	84.28	90.86	89.20	92.52	84.43	<.0001
n-6:n-3 Ratio								
n-6:*n*-3 ratio	*5*	*4.65*	*5.38*	*10*	*9.47*	*10.21*	*337.73*	*<.0001*
	5.08	4.61	5.56	9.92	9.37	10.47	280.96	<.0001

Adjusted (*italics*) and unadjusted (**bold**), controlling for ethnicity, age, sex, and study site yielded similar findings.

Table 3

Demographic/bio-behavioral measures by low and high *n-6:n-3* PUFA ratio groups

Demographic Measures	Low Ratio n = 42	High Ratio n = 41	Statistics	P-value
Age	59.3 (7.5)	56.8 (6.9)	t=1.56	0.123
Race			$\chi^2 = 7.69$	0.006
African Americans	40.5%	70.7%		
Non-Hispanic Whites	59.5%	29.3%		
Gender			$\chi^2 = 1.56$	0.212
Female	73.8%	61.0%		
Male	26.2%	39.0%		
Employment			$\chi^2 = 1.04$	0.309
Employed	47.6%	36.6%		
Not employed	52.4%	63.4%		
Marital Status			$\chi^2 = 1.52$	0.218
Married	50.0%	36.6%		
Not married	50.0%	63.4%		
BMI [kg/m2 (sd)]	29.1 (6.0)	34.4 (9.4)	t=-3.04	0.003
Smoking			$\chi^2 = 12.72$	0.002
Non-smoker	61.9%	51.2%		
Former smoker	33.3%	14.6%		
Current smoker	4.8%	34.1%		
Exercise frequency			$\chi^2 = 4.35$	0.114
<1 time per week	19.0%	40.0%		
1 to 3 times per week	54.8%	40.0%		
>= 4 times per week	26.2%	20.0%		
Omega-3 supplement	21.0%	5.0%	$X^2 = 3.61$	0.046

	Low Ratio n=42	High Ratio n=41	Statistics	p-value
Kellgren Lawrence Score			$X^2 = 4.995$	0.082

Demographic Measures	Low Ratio n = 42	High Ratio n = 41	Statistics	P-value
KL0/KL1	57.1%	56.1%		
KL2	19.0%	4.9%		
KL3/KL4	23.8%	39.0%		

Kellgren Lawrence scores determined based on reported criteria [36].

Table 4

Clinical, functional, experimental, and psychosocial measures men and confidence intervals by low and high *n*-6:*n*-3 PUFA ratio groups

		Low Ratio n = 42	95% CI Lower	95% CI Upper	High Ratio n = 41	95% CI Lower	95% CI Upper	F Value	P-value
WOMAC	Total	23.93	17.56	30.30	33.97	26.62	41.32	6.76	0.011
	Pain	5.08	3.74	6.41	6.76	5.23	8.30	4.38	0.040
	Stiffness	2.60	1.91	3.28	4.08	3.29	4.88	12.8	0.001
	Physical Function	16.26	11.48	21.03	23.12	17.60	28.64	5.61	0.020
GCPS	Pain Intensity	40.26	32.20	48.32	47.04	37.73	56.36	1.92	0.170
	Interference	31.77	20.60	42.94	39.69	26.79	52.59	1.37	0.246
SPPB	Total	10.50	9.73	11.27	10.00	9.11	10.89	1.17	0.284 *
	Chair	3.19	2.79	3.60	2.71	2.24	3.17	3.94	0.051 *
	Walking	3.52	3.16	3.89	3.66	3.24	4.09	0.4	0.527
	Balance	3.78	3.45	4.11	3.63	3.24	4.01	0.63	0.431
Experimental Pain									
Pressure	Med Knee	372.04	312.97	431.12	337.32	269.09	405.55	0.94	0.336
	Lateral-Knee	358.81	300.85	416.77	370.13	303.19	437.07	0.1	0.748
	Forearm	269.73	222.61	316.86	261.09	206.67	315.52	0.09	0.763
Punctate	1st Tap Hand	4.15	−0.99	9.30	7.99	2.04	13.93	1.51	0.223
	1st Tap Knee	8.73	2.39	15.08	12.88	5.55	20.21	1.16	0.285
	Change Hand	8.98	2.44	15.52	17.59	10.03	25.14	4.71	0.033
	Change Knee	16.63	10.16	23.09	25.04	17.57	32.51	4.6	0.035
Psychological Measures									
CES-D		6.63	3.86	9.41	7.02	3.94	10.10	0.06	0.815
PSS		11.75	9.32	14.17	15.68	12.99	18.37	7.48	0.008
PANAS	Positive	38.24	35.05	41.44	35.65	32.10	39.20	1.85	0.178
	Negative	12.16	10.24	14.07	14.65	12.52	16.77	4.75	0.033
LOTR		18.10	16.43	19.77	16.99	15.14	18.85	1.23	0.270

Adjusted for ethnicity, age, sex, and study site.

Abbreviations: Western Ontario and McMaster Universities Index of Osteoarthritis (WOMAC), Graded Chronic Pain Scale (GCPS), Short Physical Performance Battery (SPPB), Center for Epidemiologic Studies Depression (CES-D), Perceived Stress Scale (PSS), Positive and Negative Affect Scale (PANAS).

*
Mann-Whitney U Test (SPPB - Total Score, p = 0.001 and Chair Stand, p = 0.008)

Table 5

Mean and confidence interval for the low and high *n*-6:*n*-3 PUFA ratio groups by ethnicity across clinical, experimental, and psychosocial measures

	Ethnicity	Low Ratio (n = AA -17 and NHW - 25)			High Ratio (n = AA -29 and NHW -12)			Analysis	
		Mean	95% CI Lower Bound	95% CI Upper Bound	Mean	95% CI Lower Bound	95% CI Upper Bound	F Value	P-value
***n*-6:*n*-3 PUFA ratio**	AA	5.30	4.63	5.97	9.64	9.05	10.23	124.03	<.0001
	NHW	5.44	4.54	6.34	10.87	9.74	12.00	166.93	<.0001
WOMAC	AA	24.14	15.73	32.56	41.27	33.90	48.65	12.29	0.001
	NHW	26.56	14.61	38.51	29.24	14.24	44.24	0.23	0.634
Mechanical TS - Knee	AA	26.01	15.41	36.61	32.78	23.50	42.07	1.21	0.277
	NHW	2.78	−7.11	12.67	12.67	−0.19	24.63	4.19	0.049
PSS	AA	10.74	7.44	14.03	16.08	13.43	18.73	8.39	0.006
	NHW	13.02	8.04	17.99	15.21	8.96	21.45	0.89	0.352

Adjusted for age, sex, and study site.

Abbreviations: AA= African Americans, NHW = non-Hispanic whites, and TS = temporal summation