Krill Oil for Pain and Physical Function in Older Adults

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Protocol

1. **Title:** Krill Oil for Pain and Physical Function in Older Adults

   Short Title: Krill Oil for Pain in Elders (KOPE)

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3. Abstract:

Mobility is a critical factor in the maintenance of independence and quality of life of older adults. Chronic musculoskeletal pain contributes to mobility disability disproportionately among older adults. Current treatments for pain and functional decline are often ineffective and add to heightened risks of polypharmacy in older adults. As such, nutritional interventions can play a significant role in promoting health and longevity, managing pain, and enhancing physical function in older adults. Omega (ω)-3 polyunsaturated fatty acids (PUFAs) are essential nutrients that are well recognized for their anti-inflammatory and cardioprotective benefits, as well as their analgesic and anti-nociceptive properties. Most American adults do not meet the recommendations for ω-3 intakes, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), found primarily in seafood. Due to competing pathways, an elevated ω-6 to ω-3 ratio contributes to an overproduction of pro-inflammatory eicosanoids and the development of chronic diseases. A high ω-6:ω-3 ratio is associated with higher chronic pain prevalence and increased pain severity. Additionally, ω-3 PUFAs may play a role in the preservation of muscle and physical function in older adults. Low levels of ω-3s in blood are associated with reduced muscle strength, slower gait speed, and mobility disability among older adults.

Considered largely safe and cost-effective, ω-3 supplementation may be crucial to increasing the intake of these essential nutrients and achieving optimal levels among older adults. Although the use of EPA and DHA has been incorporated into several guidelines, a scarcity of data has prevented the development of strong recommendations on the use of ω-3 supplementation for the maintenance of physical function in older adults, particularly those with chronic musculoskeletal pain. Krill oil has been recently proposed as an advantageous alternative to traditional fish oil supplements, due to a greater bioavailability of EPA and DHA and additional bioactive compounds. The goal of this pilot study is to assess the feasibility of a 3-month randomized controlled trial to determine the effectiveness of krill oil supplementation on pain and physical function in older adults with chronic musculoskeletal pain. We will enroll 40 older adults (≥60 years) who will be randomly assigned to 4 g krill oil (1,288 mg/d EPA+DHA, 0.45 mg astaxanthin,

320 mg choline) daily or matched placebo (mixed lipids without EPA and DHA). We will determine the impact of krill oil supplementation on the omega-3 index (%EPA+DHA in erythrocytes), the ω-6/ω-3 ratio, and inflammatory biomarkers in blood, and obtain preliminary evidence of its impact on pain and physical function in older adults. The findings of this pilot will inform a future fully-powered randomized controlled trial by assessing the feasibility and acceptability of krill oil supplementation among older U.S. adults with chronic musculoskeletal pain.

4. Background:

Mobility limitations are experienced by about a third of U.S. older adults,[1] affecting their independence and quality of life.[2] Considered a key hallmark of functional aging,[3] mobility limitations are indicative of physical decline, predicting disability and mortality.[4,5] Chronic musculoskeletal pain, similarly prevalent among older adults,[6] contributes significantly to mobility limitations,[7,8] functional decline, and disability.[9-11] As such, the intersection of chronic pain and physical function represents an important target for interventions. However, pain management is particularly challenging and often ineffective in older adults,[12] as are interventions to prevent or delay functional decline.[13,14] With increased risks of polypharmacy in older adults, there is a need for non-pharmacological approaches for pain and physical function in older adults.[12,15]

Considered generally safe and cost-effective, supplementation with omega (ω)-3 polyunsaturated fatty acids (PUFAs) has been proposed for various age-related conditions, including chronic pain, cognitive health, and functional decline.[16-18] Despite being essential nutrients, ω-3s are inadequately consumed by most older U.S. adults.[19] In particular, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are recognized for their anti-inflammatory properties and potential health benefits in aging.[17] Omega-3s could also reduce neuropathic and nociceptive pain by modulating peripheral and central sensitization.[20] In contrast, dietary ω-6 PUFAs are abundant and contribute to an overproduction of pro-inflammatory eicosanoids.[21] An elevated ω-6:ω-3 ratio is associated with higher chronic pain prevalence and increased pain severity.[22,23] Moreover, low serum ω-3 is associated with reduced muscle strength,[24] slower gait speed,[25] and mobility disability[26] among older adults.

Of the various sources of ω-3s, krill oil has been recently proposed as an advantageous alternative to traditional fish oil supplements.[27] Krill oil offers greater bioavailability of EPA+DHA[28,29] and is a naturally occurring source of additional bioactive compounds, such as astaxanthin, a potent anti-inflammatory/antioxidant.[27] Krill oil supplementation has been shown to reduce self-reported pain intensity, mostly in middle-aged adults and samples that do not adequately represent the older U.S. population.[30-34] To our knowledge, only one study has examined the effects of krill oil on functional outcomes in older adults.[35] Noteworthy, this study found significant improvements in both muscle strength and function in generally healthy older adults.[35] However, no study to date has examined the effects of krill oil specifically on older U.S. adults with chronic musculoskeletal pain.

4.1. Chronic pain impairs physical function in older adults: Chronic musculoskeletal pain is the greatest cause of disability worldwide,[36] and older adults are especially vulnerable to chronic pain and painful conditions.[37] Over a third of older U.S. adults suffer from chronic pain (i.e., pain that persists for >3 months).[38] Furthermore, older adults are highly susceptible to the detrimental impacts of chronic pain, including functional impairments and disability, as well as chronic diseases and psychiatric conditions that can be exacerbated by chronic pain.[9,37] Alarmingly, the prevalence of chronic pain has been on the rise.[39] As the number and proportion of older adults continue to grow to comprise nearly a quarter of the U.S. population by 2060,[40] the chronic pain burden in this population represents a significant challenge to public health and health systems.

4.2. Omega-3 fatty acids are essential nutrients that must be obtained through the diet since humans cannot synthesize them. More specifically, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are conditionally essential since they can be synthesized from dietary alpha-linolenic acid (ALA); however, the conversion is limited in humans.[41] ALA is found mainly in plants and EPA/DHA are found mainly in seafood. The majority of older adults in the U.S. do not meet the recommendations for ω-3 PUFA intake and less than 2% have EPA+DHA blood concentrations associated with low cardiovascular disease risk.[19,42]

4.3. Omega-3s modulate pain: ω-3 PUFAs are recognized for their anti-inflammatory and anti-oxidative properties, leading to increased interest in their role and utilization in inflammatory and musculoskeletal conditions.[17,18] EPA and DHA generate prostaglandins, leukotrienes, and D- and E-series resolvins, which have potent anti-inflammatory effects through cyclooxygenase (COX) and lipoxygenase (LOX) activity (**Fig. 2**). Additionally, ω-3 fatty acids exert analgesic and anti-nociceptive activity, influencing pain sensitivity via peripheral and central nerve signaling.[43] As such, ω-3 PUFAs can have a positive impact on joint pain associated with conditions such as arthritis. Indeed, a recent meta-analysis of nine randomized controlled trials (N=2,070) found that ω-3 supplementation resulted in significant improvements in osteoarthritis pain and joint function compared to placebo, with no serious adverse events.[44]

4.4. The ω-6/ω-3 ratio influences inflammatory pathways: Both ω-6 and ω-3 PUFAs are essential nutrients with beneficial properties as compared to saturated fats. In contrast to ω-3 PUFAs, ω-6s contribute to the formation of pro-inflammatory lipid mediators necessary as part of a healthy inflammatory response. Yet, whereas an ω-6:ω-3 ratio of 4:1 to 1:1 is considered optimal for human health, the typical Western diet has resulted in a ratio of 15:1 or greater.[45] Due to a competing pathway of long-chain PUFA synthesis enzymes (**Fig. 1**), an imbalance of ω-6 to ω-3 PUFAs results in the overproduction of pro-inflammatory signaling

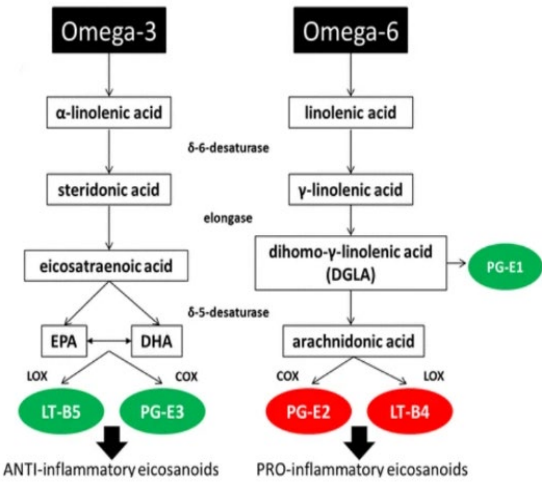


Figure 1. PUFA metabolism, Curr Surg Rep 4,28(2016)

molecules. Given the abundance of ω-6 PUFAs in the Western diet and that these possess health benefits as compared to saturated fats,[46] supplementation with ω-3 PUFAs is now thought to be essential to achieve an adequate ω-6:ω-3 ratio.[45]

4.5. Omega-3s may preserve physical function: ω-3 supplementation may be beneficial for preserving age-related loss of muscle mass, strength, and physical function.[16,17] Although not fully elucidated, potential mechanisms for these beneficial effects include anti-catabolic and anabolic properties, improved insulin sensitivity, preservation of mitochondrial function, and neuroprotective effects.[47] Recent meta-analyses of ω-3 supplementation trials have shown improvements in lower-body strength and function in older adults.[47,48]

4.6. Krill oil is considered a superior source of ω-3s as compared to fish oil and is a naturally occurring source of additional bioactive compounds that may enhance its benefits.[27] Krill (*Ephausia superba*) are small marine crustaceans. While krill oil contains similar amounts of EPA+DHA as other common fish oils, ω-3 PUFAs in krill oil are mainly found in the form of **phospholipids**, whereas in fish oil they are mainly found as triglycerides.[27] Because phospholipids are components of cell membranes, this distinction is thought to increase the bioavailability of ω-3 PUFAs in krill oil.[29,49] Indeed, in healthy volunteers, comparable rises in plasma ω-3 levels were achieved with krill oil and fish oil supplementation, despite a nearly 30% lower EPA+DHA content in krill oil.[50] When similar EPA+DHA contents are provided, krill oil demonstrates a higher 72-hour bioavailability compared to fish oil.[28]

4.7. Additional bioactive compounds in krill: Krill oil also naturally contains **astaxanthin**, a reddish-orange xanthophyll carotenoid with powerful anti-inflammatory/antioxidant properties. As astaxanthin is primarily found in seaweeds and seafood, it is poorly consumed in the Western diet. Astaxanthin is considered a geroprotector,[51] as it may confer several aging benefits beyond its antioxidant properties, such as by crossing the blood-brain barrier and interacting with molecular pathways that promote neurogenesis.[52,53] Growing evidence from *in vitro* and *in vivo* studies suggest potential benefits of astaxanthin on several age-related conditions,[54] including sarcopenia.[55] Rodent models also suggest that astaxanthin may modulate inflammatory and neuropathic pain.[56,57] Krill is also a natural source of phosphatidylcholine. **Choline** is an essential nutrient that is involved in several biological processes relevant to aging, such as neurotransmission, cell signaling, lipid metabolism, and homocysteine regulation.[58] Choline is consumed at suboptimal levels by the vast majority of U.S. older adults, with fewer than 3% of adults aged ≥71 years consuming over the adequate intake level.[59,60] These levels of consumption have been associated with liver disease, muscle damage, and neurological disorders.[58] Although much of the focus around choline has centered on brain health and cognitive function,[61,62] choline is also essential for skeletal muscle. Choline modulates fat and protein metabolism in muscle, decreasing fat synthesis and promoting muscle growth and function, as well as modulating inflammation, apoptosis, and autophagy.[63] Moreover, animal models demonstrate analgesic and anti-nociceptive actions of choline via activation of α-7 nicotinic acetylcholine receptors and inhibition of inflammatory pathways (i.e., HMGB1, TLR4, and NF-κB).[64,65]

4.8. Krill oil may reduce pain in older adults. To date, five studies have investigated the effectiveness of krill oil supplementation on pain and function, mostly in middle-aged and non-U.S. samples.[30-34] Krill oil supplementation ranged from 0.3-4 grams for a duration of 1-6 months. Of note, two studies used a krill oil-based product with added astaxanthin and hyaluronic acid.[30,31] All studies found statistically significant reductions in self-reported pain intensity and functional interference. In rodent models, krill oil has outperformed fish oil in reducing clinical measures of arthritis,[66] as well as inflammatory markers (IL-6 and TNF-α) and nociceptive pain (i.e., pain that arises from actual or potential damage to non-neural tissue).[67]

4.9. Krill oil may improve physical function in older adults. Of the studies mentioned above, only one included an objective assessment of physical function. No changes in a 6-minute walk were seen after 8 weeks of krill oil supplementation among mostly middle-aged adults.[30] However, in a recent randomized controlled trial of generally healthy older adults (≥65 y), 4 g/d krill oil supplementation for 6 months resulted in significant improvements in muscle function and size relative to placebo. Specifically, krill oil supplementation resulted in increased knee extensor maximal torque, grip strength, and vastus lateralis muscle thickness.[35]

Despite the promising results, studies of krill oil supplementation on targeted samples of older adults with chronic musculoskeletal pain and mobility limitations with objective measures of physical function are needed.

5. Specific Aims:

This pilot study will assess the feasibility of a 3-month double-blinded, randomized, placebo-controlled trial to determine the effectiveness of krill oil supplementation on pain and physical function in older adults with chronic musculoskeletal pain. We will enroll 40 older adults (≥60 years) who will be randomly assigned (1:1) to 3 months of krill oil supplementation or a matching placebo control. According to NIH guidelines for pilot studies, the proposed study will primarily assess the feasibility and acceptability of the approach.[68] Due to small sample sizes, pilot studies cannot adequately provide evidence of safety, tolerability, or effect sizes.[68] As such, the following aims will be accomplished:

Specific Aim 1: Determine the feasibility and acceptability of a double-blinded, randomized, placebo-controlled trial to investigate the effectiveness of krill oil supplementation on chronic pain and physical function in older adults with chronic musculoskeletal pain. Feasibility will be determined as (1) meeting at least 80% of the recruitment goal, (2) no more than 20% attrition, and (3) treatment acceptance and adherence (≥70% consumption of prescribed doses and increased blood ω-3 index [%EPA+DHA in erythrocytes]).

Specific Aim 2: Evaluate the impact of krill oil supplementation on purported physiological changes. Specifically, we will assess the impact of krill oil supplementation on the blood profile of essential fatty acids (i.e., ω-3 index [%EPA+DHA in erythrocytes] and the ω-6:ω-3 ratio) and inflammatory biomarkers.

Specific Aim 3: Obtain preliminary evidence of the effectiveness of krill oil supplementation to reduce pain and improve physical function in older adults with chronic musculoskeletal pain.

Table 1. Objectives and Endpoints

Objectives	Endpoints	Justification for Endpoints
Primary		
Feasibility	• ≥80% recruitment • <20% attrition • ≥70% adherence	The pilot will serve as a small-scale study to inform the feasibility of future large-scale trials.
Secondary		
Blood profile of essential fatty acids	• Omega-3 index ≥8% • n-6:n-3 ratio ≥4:1	The purported physiological change of krill oil supplementation is an increased omega-3 index and reduced n-6:n-3 ratio.
Tertiary/Exploratory		
Pain and physical function	• 20% pain reduction (0-10 scale) • 1-pt improvement in SPPB • 50-m improvement in 6MWT	Pain and physical function are the primary endpoints of a future fully powered clinical trial.

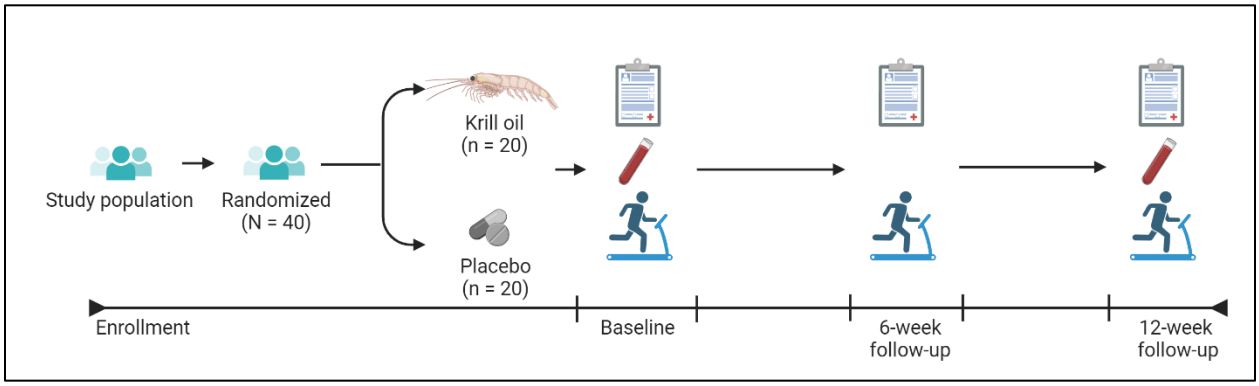
6. Research Plan:

6.1. Preliminary evidence: A high plasma ω-6:ω-3 PUFA ratio was associated with increased pain and functional limitations in middle-aged and older adults with chronic knee osteoarthritis.[22] The study consisted of a cross-sectional analysis of 167 participants recruited from the communities surrounding the University of Florida (UF) and the University of Alabama at Birmingham (UAB) as part of the Understanding Pain and Limitations in Osteoarthritic Disease (UPLOAD) cohort. Participants were categorized as having a low or high plasma ω-6:ω-3 ratio based on quartile splits (25th% and 75th%). Those with a high ratio reported greater pain, stiffness, and functional limitations (all P<0.04), and demonstrated lower physical functioning on the Short Physical Performance Battery (P=0.001). On experimental pain measures, those with a high ratio demonstrated higher punctate pain sensitivity at the knee and hand after 10 mechanical taps.

6.2. Experimental Design: This pilot will serve as a small-scale study to inform the feasibility of future large-scale trials. Feasibility is measured by metrics that indicate the ability to conduct a large-scale study, such as recruitment of the target population, retention of participants, and adherence to the intervention.[68] This study will utilize a double-blind, randomized, placebo-controlled, repeated measures design to determine the effectiveness of krill oil supplementation on pain and physical function among older adults with chronic musculoskeletal pain (**Fig. 3**). Specifically, the design will determine whether 3 months of supplementation with krill oil will (1) reduce pain intensity and interference, (2) improve physical function, (3) increase plasma ω-3 index (%EPA+DHA in erythrocytes) and reduce ω-6:ω-3 ratio, and (4) reduce inflammatory markers. Eligible participants will be randomized on a 1:1 ratio to either krill oil (*n*=20) or placebo (*n*=20). Participants will be instructed to maintain their current dietary habits, including and

especially their consumption of ω-3 (e.g., fatty fish) and ω-6 PUFAs (e.g., vegetable oils), as well as their current physical activity levels.

Figure 2. KOPE Study Design



6.3. Investigational products: We will use a commercially available krill oil product (SuperbaBOOST, Aker BioMarine Antarctic US LLC; Metuchen, NJ, USA) which contains 1 g krill oil per capsule (*E. superba* oil; 193 mg EPA, 96 mg DHA, of which 73% of EPA and DHA is bound to phospholipids, 0.11 mg astaxanthin, and 79 mg choline). Participants will be randomly assigned to consume 4 capsules/day of either krill oil (1,288 mg/d EPA+DHA, 0.45 mg astaxanthin, 320 mg choline) or placebo on one occasion with a meal. Placebo capsules will be matched to the krill oil capsules in appearance, odor, and taste, containing 1 g mixed vegetable oil (olive oil, corn oil, palm oil, and medium-chain triglycerides) comprising 31% SFAs, 46% MUFAs, and 22% PUFAs, with no EPA or DHA. This product and dosage have been previously used and found to be well tolerated in older adults.[32,35]

6.4. Participants: The target population of this study will be community-dwelling older adults with chronic musculoskeletal pain and moderate mobility limitations. As shown in **Table 2**, individuals will be eligible based on the following inclusion criteria: (1) age 60 years or older, (2) moderate pain (≥4 out of 10) of the hip, knees, or lower back for more than 3 months, (3) demonstrate mobility limitations, and (4) are able and willing to give informed consent. Due to the antiplatelet properties of ω-3s, we will exclude anyone with a known coagulation or bleeding disorder, as well as those with a standing regimen of anticoagulants or full-dose aspirin. That said, current evidence does not support an increased risk of bleeding with ω-3 supplementation with or without the use of anticoagulants/antiplatelet drugs.[69] Currently, the U.S. Food and Drug Administration (FDA) considers ω-3 supplementation up to 5 g/day of EPA+DHA to be safe.[70] In an abundance of caution, we will exclude those with a seafood allergy. Additionally, we will exclude those who have supplemented with ω-3 PUFAs in the last 3 months. To ensure enrollment of older adults with low intakes of ω-3 PUFAs, we will exclude those who report consuming fatty fish over 2 times per week. The current *Dietary Guidelines for Americans* recommends an intake of at least 8 ounces (i.e., 2 servings) of seafood per week providing ~250 mg/day of EPA+DHA. Lastly, we will exclude anyone with regular use of opioids or high-dose non-steroidal anti-inflammatory drugs within 30 days of randomization.

Table 2. Inclusion and Exclusion Criteria

Inclusion
• Provision of signed and dated informed consent form • Stated willingness to comply with all study procedures and availability for the duration of the study • Male or female, aged ≥60 years • Exhibiting chronic musculoskeletal pain of the hip, knees, or lower back (>3 months) • Average pain ≥4 on a 0–10 numeric rating scale • Exhibiting moderate mobility limitations (Short Physical Performance Battery score 4-10) • Ability to take oral supplement and be willing to adhere to the supplementation regimen • Agreement to adhere to Lifestyle Considerations (see section 6.4.d) throughout study duration
Exclusion
• Any known coagulation or bleeding disorders • Standing regimen of anticoagulants or full-dose aspirin • Regular use of opioids or high-dose NSAIDs • Taking medication known to affect muscle (e.g. steroids) • Taking selective serotonin reuptake inhibitors (SSRIs) • Omega-3 supplementation within the past 3 months • High consumption of fatty fish (>2 servings/week) • Habitual supplementation with other complementary medicines/supplements that may affect the study results, including St. John's Wort • Known allergy to seafood • Clinically significant conditions: uncontrolled diabetes, severe cardiovascular disease, seizure disorders, uncontrolled hypertension (>150/90mmhg at baseline), cancer or cancer that has been in remission <5 years • History of atrial fibrillation or atrial flutter • Dementia • History of smoking, alcohol abuse, or illicit drug use • Ambulatory impairments which would limit the ability to perform physical function tests • Treatment with another investigational drug or other intervention within 3 months • Planning a surgical procedure during the study period • Planning to permanently leave the area during the study period

6.4.a. Recruitment: Our team has substantial experience recruiting older adults with chronic pain and moderate functional impairments.[22,71,72] Recruitment efforts will be aided by the UF Older Americans Independence Center's Clinical Research Core. Participants will be recruited from the areas surrounding the University of Florida in Gainesville/Alachua County, Florida. There are >284,000 older adults (≥65y) residing in Alachua County, FL. If needed, recruitment will be expanded to neighboring North/Central Florida regions.

6.4.b. Screening: Screening for eligibility will consist of a two-step process, starting with a phone screening to assess basic eligibility criteria. If found eligible via phone screen, interested individuals will be invited to an in-person screening visit. The screening visit will collect demographic characteristics (e.g., age, race/ethnicity, education, income), anthropometric measures, as well as a comprehensive pain history and physical function testing (SPPB) to assess functional status.

6.4.c. Randomization and blinding: The trial will use a double-blinded design, so that participants, investigators, and assessors will be blinded to group assignments. Randomization for this study will be conducted using REDCap (Research Electronic Data

Capture), a secure, web-based application designed to support data capture for research studies. REDCap provides automated randomization to ensure unbiased allocation of participants to study groups and audit trails to maintain a detailed log of all randomization activities for transparency and reproducibility. The randomization module in REDCap will be configured to use the random block method, ensuring balanced allocation across groups.

6.4.d. Lifestyle considerations: During this study, participants are asked to (1) maintain their current dietary habits, especially their consumption of ω-3 (e.g., fatty fish) and ω-6 PUFAs (e.g., vegetable oils), (2) maintain current physical activity levels, and (3) not participate in other interventional research studies.

6.5. Assessments (Table 3):

6.5.a. Safety.

- Vitals. Blood pressure, pulse, and temperature will be obtained at the beginning of every study visit.

  Blood chemistry. Blood samples will be collected and used for comprehensive metabolic panel (CMP), complete blood count (CBC), lipid panel, and high sensitivity C-reactive protein (hs-CRP) at baseline and subsequent in-person visits. In case the initial tests are inconclusive or if further analysis is required, the participants may be asked to return for an additional blood draw.
- Adverse events. Participants will be queried about adverse events at every follow-up, including quarterly phone calls and follow-up visits.

- Medical history will be evaluated at screening and updates will be obtained at every visit thereafter.

6.5.b. Adherence to supplementation protocol. Remaining capsules will be counted at every follow-up. Adherence will be quantified as the percentage of capsules taken out of those dispensed.

6.5.c. Common Data Elements (CDEs). Key sociodemographic characteristics will be obtained at the Screening Visit (e.g., age, sex, race and ethnicity, income).

6.5.d. Anthropometrics. Height and weight will be obtained and used to calculate body mass index (kg/m^2). Height will only be measured at baseline. Weight and waist circumference will be measured at every study visit.

Table 3. Schedule of Activities

Procedures	Screening	Enrollment/ Baseline	Q1 Calls	Midpoint	Q3 Calls	Final
Visit #	1	2	Phone	3	Phone	4
Week(s)	-4 to 1	1	1-4 +/- 1	6 +/- 1	9 +/- 1	12 +/- 1
Duration	1 hr	2-3 hrs	15 mins	1.5-2 hrs	15 mins	2-3 hrs
Informed consent	X					
Randomization	X					
Capsule count & dispensation		X		X		X
Health assessment						
Medical history/update	X	X		X		X
Adverse events			X	X	X	X
Vital signs	X	X		X		X
Anthropometrics						
Height	X					
Weight	X	X		X		X
Waist circumference		X		X		X
Physical Performance						
Short Physical Performance Battery (SPPB)	X			X		X
Handgrip strength		X				X
6-minute walk test (6MWT)		X				X
Whole blood collection		X		X		X
Dried blood spot (OmegaQuant)		X		X		X
CMP, CBC (safety), lipids, hs-CRP		X		X		X
Inflammatory markers (IL-6 and TNF-α)		X				X
Questionnaires						
Common Data Elements (CDEs)	X					
Pain history	X					
Michigan Body Map	X					X
Graded Chronic Pain Scale-Revised (GCPS-R)	X	X		X		X
Western Ontario and McMaster Universities Arthritis Index (WOMAC)		X		X		X
PainDETECT		X				X
Patient Global Impression of Change (PGIC)			X	X	X	X
Medicine Acceptability Questionnaire (MAQ)				X		X
Pepper Assessment Tool for Disability (PAT-D)		X		X		X
Fear Avoidance Beliefs Questionnaire (FABQ)		X				X
Pain Self-Efficacy Questionnaire (PSEQ)		X				X
EuroQol 5 Dimension 5 Level (EQ-5D-5L)		X				X
Brief Pittsburg Sleep Quality Index (B-PSQI)		X		X		X
Perceived Stress Scale (PSS)		X				X
Geriatric Depression Scale-Short Form (GDS-S)		X				X
Diet assessment (DHQ-III)		X				X
Montreal Cognitive Assessment (MoCA)	X			X		X
NIH Toolbox		X				X

6.5.e. Biological specimen collection and laboratory evaluations. Whole blood samples will be collected to assess the following:

- Omegas 3 & 6 profile: Analysis of essential fatty acids will be conducted at baseline, 6 weeks, and 12 weeks with dried blood spots, as described elsewhere.[73] Gas chromatography will be used to quantify 24 fatty acids, including 7 ω-6s and 4 ω-3s, from which the ω-3 index and the ω-6/ω-3 ratio can be calculated. The CV for the dried blood spot ω-3 index is <5%.[73]

The omega-3 index, the percent of EPA+DHA of total fatty acids in erythrocytes, has been proposed as a more useful and clinically relevant biomarker of ω-3 status.[74] Although the ω-6:ω-3 ratio has shown utility in chronic disease,[21] it is limited by several theoretical and practical difficulties.[75] Evidence suggests that much of the associations seen with the ω-6:ω-3 ratio may be largely explained by the ω-3 index.[75] Moreover, the ω-3 index is highly responsive to changes in EPA and DHA intake, making it a more useful marker of intake.[75]

- Inflammatory markers: We will measure hs-CRP at baseline and 6- and 12-week follow-ups. We will also measure IL-6 and TNF-α from blood samples collected at baseline and 12-week follow-up.

6.5.f. Pain.

- A *Pain History* will be obtained, including an assessment of painful bodily locations/distribution (with the *Michigan Body Map*),[76] etiology of pain (if identifiable), duration, character and quality of pain, and aggravating and alleviating factors.

- *The Graded Chronic Pain Scale (GCPS)* is a 7-item questionnaire that measures pain intensity and interference over the last 1, 3, or 6 months. Scores can be used to classify participants on a Chronic Pain Grade: pain-free (grade 1), low disability and low intensity (grade 2), low disability, high intensity (grade 3), high disability, moderately limiting (grade 4), and high disability, severely limiting (grade 5).[77]

- *The Western Ontario and McMaster Universities Arthritis Index (WOMAC)* is a well-validated and widely utilized measure of pain symptoms and physical disability. It is comprised of 24 items to evaluate three domains: pain, stiffness, and physical function. Responses can be summated for domain-specific scores, as well as a global score.[78]

- *The PainDETECT* will be used to determine the presence of neuropathic pain.[79]

- *Movement-evoked pain (MEP)* refers to the pain during activity, which is a primary driver of musculoskeletal pain.[80,81] MEP will be assessed immediately after each physical task using a 101-point numerical rating scale, with 0 corresponding to “no pain” and 100 to “the most intense pain imaginable.”[82]

6.5.g. Disability. *The Pepper Assessment Tool for Disability (PAT-D)* is a validated, 23-item instrument measuring 5 domains of function in older adults: mobility, transferring, upper extremity, activities of daily living (ADL), and instrumental activities of daily living (IADL).[83,84]

6.5.h. Physical function.

- *The Short Physical Performance Battery (SPPB)* is a widely used geriatric assessment designed to evaluate the physical performance and mobility of older adults. It consists of a series of simple tests that assess three major components: balance, gait speed, and lower extremity strength (chair stand test). Each component is scored on a scale

from 0 to 4, with higher scores indicating better performance.[85] An SPPB of ≤10 is predictive of mobility disability[86] and all-cause mortality.[87]

- *Handgrip strength test* will be measured with a dynamometer. Handgrip strength is a measure of physical function and is suggested as a biomarker of aging.[88] Maximal grip strength for each hand will be measured using a handheld dynamometer (Jamar, Lafayette Instruments, Lafayette, IN).

- *The 6-minute walk test (6MWT)* is a simple, inexpensive, sub-maximal exercise test commonly used to assess functional capacity and endurance in older adults.[89] Participants are instructed to cover as much distance as possible in 6 minutes along a rectangular pathway. Normative data for 6MWT performance by age in older adults is available for comparison.[90]

6.5.i. Dietary intake. Dietary intake data will be collected using the *Diet History Questionnaire (DHQ), version III* (2018). The DHQ-III is a freely available web-based, self-administered food frequency questionnaire consisting of 135 food and beverage items and 26 dietary supplement questions regarding the type, frequency, and serving sizes of foods, beverages, and dietary supplements consumed within the previous month.[91]

6.5.j. Cognitive function.

- *The Montreal Cognitive Assessment (MoCA)* is a brief, 10-minute measure of global cognitive function and a screening tool for mild-to-severe cognitive impairment. The test assesses cognitive domains, such as attention, memory, orientation, language, conceptual thinking, and planning.[92] Different versions of the MoCA will be used at each visit to minimize retest effects.

- *The NIH Toolbox Cognition Battery (NIHTB-CB)* is comprised of 7 tests that measure 8 abilities within 6 major cognitive domains: *executive function, episodic memory, language, processing speed, working memory,* and *attention*. Composite scores will be calculated for (1) fluid, (2) crystallized, and (3) global cognitive function. The toolbox takes approximately 30 minutes to complete and is administered with a tablet, facilitating administration and reducing data management burden.[93,94]

6.5.k. Other.

- Acceptance of the intervention will be assessed with the *Patient Global Impression of Change (PGIC)*[95] and the *Medicine Acceptability Questionnaire (MAQ).*[96]

- Mood/affective factors will be assessed with the *Fear Avoidance Beliefs Questionnaire (FABQ)*,[97] *Pain Self-Efficacy Questionnaire (PSEQ),*[98] *Perceived Stress Scale (PSS)*,[99] and the *Geriatric Depression Scale-Short Form (GES-S).* [100]

- The *Brief Pittsburg Sleep Quality Index (B-PSQI)* will be used to assess sleep quality.[101]

- Quality of life (QoL) will be assessed with the *EuroQol 5 Dimension 5 Level (EQ-5D-5L).*[102]

6.5.l. Sample size and statistical approach: This pilot study is designed as a small-scale test to guide the design and implementation of large-scale studies to evaluate the effectiveness of krill oil supplementation among older adults with chronic musculoskeletal pain. We will enroll 40 older adults who will be randomly assigned (1:1) to either krill oil (*n*=20) or placebo (*n*=20) for a 3-month trial duration. Approximately 16 participants per group after a 20% loss to follow-up is expected to provide sufficient data to indicate the feasibility of a larger study and to provide descriptive estimates of effects. **Aim 1.** Feasibility and acceptability will be determined as (1) meeting at least 80% of the recruitment goal (*n*=32), (2) no more than 20% attrition, and (3) treatment acceptance and adherence (≥70% consumption of prescribed doses and increased blood ω-3 index). **Aim 2.** Mean changes in ω-3 index and ω-6:ω-3 ratio will be evaluated with paired-samples T-tests for within-group difference and independent-samples T-tests for between-group difference. An intention-to-treat approach will be used for between-group analyses. While there is currently no guidance on what may constitute a minimal clinically meaningful change in plasma ω-3 index, current evidence supports that an ω-3 index ≥8% may be protective against cardiovascular disease whereas an index of ≤4% indicates increased risk.[103] Similarly, an ω-6:ω-3 ratio of 4:1 or lower is considered optimal for health.[45] Baseline and 3-month levels of the ω-3 index and the ω-6:ω-3 ratio will be compared against these benchmarks. **Aim 3.** Due to the repeated-measures design, we will use mixed-effects linear regression models to evaluate changes in pain intensity and functional interference (WOMAC), as well as changes in objective measures of physical function (SPPB, 6MWT). Fixed effects will be specified for the treatment group, time (in weeks), and a group*time interaction effect. An independence covariance matrix structure will be assumed with a random intercept for each participant included. A 20% pain reduction on a 0-10 numeric rating scale is considered a clinically significant pain reduction.[104] A 1-point change in the SPPB and a 50-m increase in the 6MWT are considered clinically meaningful changes for older adults.[105]

6.6. Adverse events:

6.6.a. Definition of adverse events (AEs): Adverse event means any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research. Adverse events encompass both physical and psychological harms.

6.6.b. Definition of serious adverse events (SAEs): An adverse event or suspected adverse reaction is considered "serious" if, in the view of the investigators, it meets any of the following criteria:

- Results in death

- Is life-threatening (places the subject at immediate risk of death from the event as it occurred)
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in a persistent or significant disability/incapacity
- Results in a congenital anomaly/birth defect
- Any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition
  Examples of such events include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

6.6.c. Classification of an adverse event

6.6.c.1. Severity of event: The following guidelines will be used to describe the severity of AEs

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".

6.6.c.2. Relationship to study intervention: All AEs must have their relationship to the study intervention assessed by the Safety Monitor who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs

or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.

- **Potentially Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.
- **Unlikely to be related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).
- **Not Related** – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

6.6.c.3. Time period and frequency for event assessment and follow-up: The occurrence of an AE or SAE may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

The Safety Monitor will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization**.**

6.6.d. Reporting of AEs/SAEs: Study progress and safety will be reviewed bi-weekly or more frequently by the principal investigator. Progress reports, including AEs will be provided to an Independent Monitoring Committee for bi-annual reviews. The Independent Monitoring Committee for this study will consist of an established board that reviews all studies conducted within the University of Florida's Pepper Center during bi-annual conference calls. This board is comprised of (1) Stephen Kritchevsky, Ph.D., Chair, epidemiologist, (2) Jing Cheng, Ph.D., biostatistician, and (3) Jack Guralnik, M.D., physician/clinical researcher. An annual report will be compiled and will include a list and summary of AEs, and whether adverse event rates are consistent with pre-study assumptions.

The PI will immediately report to the sponsor any SAE, whether or not considered study intervention related, including those listed in the protocol, and must include an assessment of whether there is a reasonable possibility that the study intervention caused the event. All SAEs will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the participant is stable.

The study sponsor will be responsible for notifying the Food and Drug Administration (FDA) of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the sponsor's initial receipt of the information. In addition, the sponsor must notify FDA and all participating investigators in an Investigational New Drug (IND) safety report of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting.

6.6.e. Reporting events to participants: In the case that adverse events are identified from blood chemistry tests, participants will be immediately notified by the investigators.

6.7. Participant discontinuation/withdrawal from the study: Participants are free to withdraw from participation in the study at any time upon request. The investigators may discontinue or withdraw a participant from the study for the following reasons:

- Significant study intervention non-compliance
- If any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- Disease progression which requires discontinuation of the study intervention
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation
- Participant unable to receive krill oil for 12 weeks

Subjects who sign the informed consent form and are randomized but do not receive the study intervention may be replaced. Subjects who sign the informed consent form, and are randomized and receive the study intervention, and subsequently withdraw, or are withdrawn or discontinued from the study, will not be replaced.

7. Possible Discomforts and Risks:

Due to its antiplatelet properties, ω-3 supplementation may increase the risk of bleeding at high doses when combined with antiplatelet/anticoagulant medications. Commonly reported side effects of ω-3 supplements are usually mild and include unpleasant taste, bad breath, heartburn, nausea, gastrointestinal discomfort, diarrhea, headache, and odoriferous sweat.

The risks associated with the placebo, a mixture of vegetable oils, are similar to those for krill oil, and include upper respiratory tract infections, joint and back pain, gastrointestinal

disorders, and headaches. There is also a potential risk for worsening of cholesterol with the mixed vegetable oil placebo.

There are potential risks associated with the questionnaires, physical function tests, and cognitive tests administered in this study. Questionnaires and cognitive function tests pose a minimal risk of mental fatigue, embarrassment, discomfort, and/or frustration. Physical function tests have an inherent risk of physical discomfort, pain, and/or injury to the participants.

Blood draws carry minimal risks, including pain and/or discomfort, bruising and swelling, excessive bleeding, lightheadedness, and rarely, infection or nerve damage at the puncture site.

8. Possible Benefits:

Benefit to participants: Based on prior evidence, we believe that participants will directly benefit from krill oil supplementation in significant ways, including an improved blood profile of ω-3 and ω-6 fatty acids, downregulated inflammation, reduced pain, and improvements in physical function. Ultimately, these beneficial changes from krill oil will support their health and independence. Participants in the control group may also benefit from this study, as findings from the study may lead to advancements in therapies for chronic musculoskeletal pain. Furthermore, upon study completion, all participants will be able to obtain the information that has been collected throughout the study, including reports of their ω-3 index, ω-6:ω-3 ratio, inflammatory biomarkers, and dietary analysis. This information is not routinely assessed in clinical practice or easily obtained and will help inform the participants about their health. As such, we believe that the potential benefits to participants outweigh the relatively minor risks of the study.

Public health benefits: Results from this research have the potential to be widely beneficial to society, with important implications for public health and geriatric medicine. Chronic musculoskeletal pain contributes to loss of independence and quality of life, as well as shorter lifespan in older adults. Current approaches for pain management and functional decline in older adults have limited effectiveness and add to the increased risks of polypharmacy in this population. Non-pharmacological approaches, particularly nutritional interventions, are considered desirable candidates for primary or adjuvant treatment strategies to manage pain and maintain physical function in older adults with chronic musculoskeletal pain. This research will contribute to the understanding of the use of krill oil supplementation as a therapeutic approach for pain management and the maintenance of physical function in older adults with chronic musculoskeletal pain.

9. Conflict of Interest:

None.

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