KENNESAW STATE UNIVERSITY

Informed Consent Agreement

Docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and their effects on adults ages 25-50 years: a 16-week dietary supplement intervention study

IRB #: 20-472

Research team primary contacts

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Introduction

You are invited to participate in a research study conducted by Brent Uken and Dr. Trisha VanDusseldorp of Kennesaw State University (KSU). Before you decide to participate in this study, you should read this Informed Consent Agreement (ICA) and ask questions about anything that you do not understand.

Study description

Omega-3 long-chain polyunsaturated fatty acids (LCPUFA) are of great medical and public interest with extensive scientific evidence of health benefits, including anti-inflammatory and cardioprotective effects. Specifically, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are the omega-3 LCPUFA that are primarily responsible for these effects. DHA and EPA are commonly obtained via the diet by consuming cold water, oily fish such as salmon, tuna, mackerel, and sardines. DHA and EPA may also be obtained via oral supplementation, typically in the form of softgels (also commonly referred to as “fish oil supplements”). The majority of research to date has examined the effects of DHA and EPA in combination, rather than isolating their singular impact.

Our study is designed to explore the effects of DHA and EPA on [i] cardiometabolic health markers (e.g., metabolic panel, lipid panel, body composition, blood pressure, resting energy expenditure); [ii] inflammation and oxidative metabolism (e.g., oxidative stress); [iii] mitochondrial function; [iv] heart rate activity (e.g., heart rate variability, resting heart rate, and heart rate recovery); [v] exercise economy (e.g., oxygen consumption, lactate production and clearance, and exercise energy expenditure); and [vi] cognitive function (e.g., mood state and rating of perceived exertion).

Study duration

The study will be 16 weeks in duration.

Procedures and time requirements

During the study, you will perform the following activities:

- Consume 4 grams of an omega-3 fish oil supplement or a placebo (daily)
- Continue with your normal diet and exercise routine:
    - Record food and beverage consumption (daily)
    - Record exercise activities (daily)
- Visit the KSU Exercise Science Physiology Lab on three separate occasions:
    - Visit 1 (V1): week 1, day 1 (duration = 3.5 hours)
    - Visit 2 (V2): week 8, on or about day 56 (duration = 3.0 hours)
    - Visit 3 (V3): week 16, on or about day 112 (duration = 3.0 hours)
- The KSU Exercise Science Physiology Lab (ESPL):
    - Located on the KSU Kennesaw main campus
    - Address: 520 Parliament Garden Way NW, Kennesaw GA 30144
    - Prillaman Hall Room 1102
- The procedures are essentially the same for all three visits:
    - Welcome and orientation
    - Collection of height, weight, and body composition data
    - Collection of resting metabolic rate and heart rate data
    - Collection of urine and blood samples
    - Performance of an exercise test on a treadmill
    - Distribution of 8 weeks of either supplement or placebo (V1 and V2)
    - Provide instruction for your upcoming responsibilities and answer questions you may have about the study

Supplement and placebo

- This is a “blind” study, which means that neither you nor the research team will know to which group you have been assigned (DHA supplement, EPA supplement, or placebo).
- Supplements will be either 4 grams (4g) of DHA per day or 4 grams of EPA per day. Those assigned a placebo will be consuming 4 grams (4g) of an alternative, non-omega-3 oil (e.g., corn or olive oil) per day.
- Placebo softgels will be the same size, shape, and texture as the DHA and EPA softgels. All softgels will contain 1 gram of either fish oil (DHA or EPA) or alternative oil (placebo, e.g., corn or olive oil).
- For each 8-week block, you will be provided with a 60-day supply of softgels (4 softgels per day x 60 days = 240 softgels).
- For best results (to mitigate any potential digestive side effects and improve absorption into your body), we recommend that you:
    - Split the dose (2 softgels with a morning or mid-day meal, and 2 softgels with an evening meal)
    - Do not consume the softgels on an empty stomach
    - Consume the softgels with a meal that includes foods that contain fat
    - Drink 8 ounces of water or another beverage when you consume the softgels
- Because the study is measuring chronic DHA and EPA intake, it is essential that you consume the entire daily dose (4g) every 24 hours.

- **If you miss a day of taking your softgels, please contact the Primary Investigator as soon as practical to discuss alternatives for you to remain in the study.**
- We will help you remain compliant with your softgel intake by [i] reviewing your entries into the Cronometer platform; [ii] checking in with you via text, email, or phone call; and [iii] conducting an end-of-period softgel count.
- You do not need to, nor should you modify any existing supplement or medication protocols (e.g., prescriptions) during the study.

Safety, quality of data collection, and physical contact

I understand that my safety and the quality of the data collected during a research study are primary concerns. Consequently, while performing certain procedures, a research team member may touch my body to align, secure, or position it (examples include positioning your body for a DEXA scan, placing EKG electrodes on your torso, and positioning your arm during venipuncture). I understand and acknowledge these circumstances and expressly consent to the physical contact required in these/similar instances and for these/similar reasons. If I am uncomfortable with any physical contact with a research team member or his/her verbal instruction, regardless of the reason, I understand that I have a duty to inform him/her immediately of my unwillingness to participate in the subject procedure. I agree that I will discuss the situation with the research assistant to identify a reasonable, mutually acceptable alternative in such an instance. If no alternative is available, I understand that a lack of physical contact will preclude my ability to complete one or more procedures. I also understand that the inability to proceed with one or more procedures will lead to the termination of my participation in this research study.

Visit procedures

<u>Anthropometric data, including body composition</u>

- For all of the measures that comprise this part of your visit, you will remove your shoes, socks, jewelry (e.g., rings, earrings, and body piercings), and other loose items (e.g., belts). It will be most convenient for you and the test administrator if you remove these items before leaving for KSU (and leave them at home).
- We will measure your height, weight, and waist circumference.
- Your body composition will be assessed using dual-energy x-ray absorptiometry (DEXA) and bioelectrical impedance analysis (BIA).
    - DEXA is used to measure your bone mineral density and body composition using low dose radiation. The amount of radiation to which you will be exposed during this test is extremely small (the radiation levels used during a DEXA scan are [i] lower than the natural background radiation that you are exposed to each day and [ii] significantly less than a chest x-ray).
    - BIA measures the opposition to the flow of a low voltage electric current through body tissue. The amount of resistance provides an estimate of total body water, and that estimate is used to calculate your body fat percentage.
- You will review and complete the DEXA Consent Form before completing the DEXA scan.
    - If you are pregnant or believe that you are pregnant, you will not be allowed to complete a DEXA scan out of an abundance of caution.

    - If there is a possibility that you are pregnant, you have the option to complete a urine-based pregnancy test.
- During the DEXA scan, you will lie face-up on the scanning platform with your arms and legs slightly away from your torso. You will be asked to remain silent and motionless for approximately 10-15 minutes while the machine performs the scan. The procedure is painless.
- The BIA body composition assessment requires you to stand on a platform while holding a small cylindrical handle in each hand; the procedure is less than 5 minutes and is painless.

Urine samples

- You will provide a urine sample during the initial portion of each visit. It will be collected in a standard, sterile, screw-top container and is the same procedure that is used in physician offices.
- The restroom used for urine collection is located inside the ESPL and will provide the necessary privacy for collection.
- The urine that we collect will be analyzed solely for research-related purposes (e.g., mitochondrial function). We will not perform any clinical diagnoses.

Blood samples

- A member of the research team who is trained and experienced with blood collection will perform each procedure. This test administrator will collect blood via a vein in your arm (also referred to as "venipuncture") and by finger stick.
- The blood we collect each visit will be the equivalent of approximately 5 tablespoons. The total amount of blood we will collect from you across all three visits is approximately 210 ml, significantly less than the amount of blood collected during a single blood donation (approximately 475 ml).
- You have the option to complete the procedure while sitting or lying down.
- The venipuncture procedure will last approximately 15 minutes, which includes cleaning and disinfecting the puncture site, collecting the blood, and placing a gauze pad on the site once the needle is removed. You will also be provided with a bandage to cover the puncture site.
- Blood collected via finger stick will be minimal (approximately 10-15 drips on a single blood spot collection card at the beginning of your visit, and 1-2 drops per test strip before, during, and after the exercise procedure). Depending on the number of stages you complete in the exercise treadmill procedure, the total number of measurements via finger stick is estimated to range from 10-12.
- The blood we collected will be analyzed solely for research-related purposes (e.g., inflammation and oxidative stress). We will not perform any clinical diagnoses.

Resting metabolic rate and heart rate variability

- Because measures of both resting metabolic rate (RMR) and heart rate variability (HRV) require a stable, resting heart rate when you are in a relaxed state, we will assess both RMR and HRV in a quiet, dimly lit room.
- You will lie face-up on the RMR platform. A ventilated hood of clear plastic will be placed over your head. The hood is used to collect and analyze the air that you exhale.

- To promote the accuracy of these measurements, you will be asked to avoid talking, moving, and sleeping for the duration of the test. The test is painless and will last approximately 20 minutes.
- You will not need to control your breathing during the test (you should continue to breathe spontaneously ("normally" and without conscious effort).
- We will measure your HRV by attaching three electrocardiogram (ECG) leads on your upper body (two near your shoulders and one near your hip). These leads connect to a computer that will record your heart's electrical impulses for the duration of the measurement period. The procedure is painless.
- If you are uncomfortable at any point during the procedure, you have the right to terminate the test.

<u>Treadmill exercise protocol</u>

- The treadmill exercise protocol will be the basis for assessing running economy and associated measures, such as peak and maximal aerobic capacity (also known as VO_{2peak} and VO_{2max}), rating of perceived exertion (RPE), and heart rate (e.g., heart rate recovery).
- You will wear two pieces of equipment during this test:
    - Heart rate chest strap
    - Two-way breathing mask (you will breathe normally while wearing the mask; the purpose of the mask is to collect the air you exhale so that it can be analyzed by the computer to which the mask is connected)
- You will wear both pieces of equipment for the entire duration of the measurement period, including during the recovery periods. At the end of the measurement period, a test administrator will remove the mask (we ask that you do not remove the mask yourself).
- Blood lactate and glucose levels will be measured before you begin your warm-up (finger stick blood collection).
- You will warm-up for 5 minutes at a pace that is comfortable for you. You may warm up by walking or jogging.
- The starting incline (grade) on the treadmill will be 1%.
- The test will take place in multiple stages that are progressively more challenging. We will ask you to complete as many stages as possible (i.e., to keep running until you reach a maximum RPE of 20). When you have reached exhaustion, you will terminate the test. **You may also terminate the trial at any time for any reason, including, but not limited to, chest pain, light-headedness, dizziness, and difficulty breathing).**
- The duration of each stage will be 3 minutes.
- The incline and speed for each stage are:
    - Stage 1: incline = 1%; speed = 6 mph
    - Stage 2: incline = 1%; speed = 7 mph
    - Stage 3: incline = 1%; speed = 8 mph
    - Stage 4: incline = 3%; speed = 8 mph
    - Stage 5: incline = 5%; speed = 8 mph
    - Stage 6: incline = 7%; speed = 8 mph
- There will be a 1.5 minute recovery period in-between stages to allow the test administrator to collect a blood lactate sample from you via finger stick.

- For your safety and the safety of the test administrator:
    - The speed of the treadmill will be reduced to 0 mph at the end of each 3-minute stage.
    - Once your blood glucose and lactate levels are measured, the test administrator will bandage your finger (approximately 60 seconds).
    - You will step back on the treadmill and resume a walking pace (3 mph) for approximately 20 seconds.
    - During the final 10 seconds of the recovery period, the speed of the treadmill will be increased to the level required for the next stage of the test.
- At the end of each stage of the test, you will be asked to rate your perceived level of exertion (RPE) using the "Borg scale." The Borg scale ranges from 6-20, with a "6" meaning no exertion and a "20" meaning maximal exertion.

Cronometer platform overview and instruction

- To run appropriate statistical analyses, we need to collect your daily food and beverage intake and your daily exercise activities. We ask that you do not consciously alter either, but instead maintain your regular dietary and training regimens.
- We ask you to track these items (food and beverage intake and exercise activities) using an online application. The application we are using, Cronometer (www.cronometer.com), is also used by numerous professional athletes and Olympians.
- We will provide instruction on how to use the platform. You also will have access to Cronometer's online help (support tutorials and videos) and phone-based support if you encounter any technical, account, or other issues.

Wrap-up and next steps

- Each visit will conclude with a brief discussion concerning the visit and the procedures performed. You are encouraged to ask questions and articulate any concerns you have about the study or the procedures.
- The lead test administrator or Primary Investigator will also discuss your upcoming responsibilities, confirm your understanding of supplement ingestion and daily recording requirements, as well as the mode and timing of reminders that may be sent to you in-between visits to the ESPL.
- At the conclusion of V1 and V2, you will be given an 8-week supply of either one of the supplements or placebo. As described in Section 2(a), the composition of the supplement or placebo will be blinded to both you and the research team.

Pre-visit preparation

To ensure accurate measurements, as well as your safety, we ask that you strictly adhere to the following pre-visit preparation guidelines:

- No food or drink (except for water) for a minimum of 8 hours prior to the visit (this includes the softgels that are part of this study, but this does **not** pertain to any prescription medication you take on a regular basis)
- No alcohol for a minimum of 24 hours prior to the visit

- No change in stimulant consumption or prescription medication for a minimum of 12 hours prior to the visit (you may consume black coffee on the morning of a visit, but **only if that is a daily occurrence**)
- No calcium supplements for a minimum of 24 hours prior to the visit
- No exercise for a minimum of 24 hours prior to the visit
- No medical procedures involving a barium examination or injection with a contrast material for a computed tomography (CT) scan or radioisotope scan for a minimum of 14 days prior to the visit
- Wear athletic clothes and footwear that are conducive to exercise
- Leave all non-essential accessories (e.g., jewelry) at home
- Bring food and beverage for consumption after completion of the treadmill graded exercise procedure
- Bring your smartphone
- You are welcome to bring a change of clothes and toiletries for use after testing. You also have the option to use the shower facilities in the ESPL.

Risks and discomforts

We believe that the risks associated with this study are minimal. Based on similar research studies, we summarize the most common risks and discomforts below:

- Omega-3 supplements have been produced and consumed for decades and have minimal side-effects, the most common of which are "gastrointestinal distress" (indigestion) and "fish burps." Both of these side effects can be mitigated by:
    - Storing the supplements in your freezer
    - Consuming these supplements with meals and with foods that contain fat (i.e., do not consume them on an empty stomach)
    - Consuming these supplements with 8 ounces of water or other beverage
- Numerous studies spanning multiple decades have been completed, including several with doses of omega-3 LCPUFA exceeding 5 grams per day, with no reported serious adverse events.
- A DEXA scan is a painless procedure that exposes you to low-dose radiation. The radiation levels used are lower than those experienced in normal daily living (atmospheric exposure).
- Blood drawn via fingertip stick is relatively painless but may result in momentary discomfort at the site of the lancet insertion (near your fingertip).
- Blood collected via venipuncture usually results in momentary pain during needle insertion and when the needle is removed (when pressure is applied to facilitate blood clotting).
- As with any procedure where the skin is punctured, there is a risk of infection. This risk will be minimized by employing standard methods that include cleaning and disinfecting the insertion site. Additionally, following the procedure and immediately upon the removal of the needle, a sterile gauze pad will be placed on the site of insertion.
- It is possible for bruising to occur at the site of needle insertion.
- Some individuals have become dizzy, light-headed, or have fainted as a result of venipuncture. Consequently, we only draw blood from individuals who are seated or lying down to mitigate the risk of any of these events from occurring.

- Some participants have found that the mask worn during the treadmill procedure is uncomfortable.
- The treadmill is a common piece of exercise equipment. Although it is possible to be injured using a treadmill, injury usually occurs when the machine is misused (i.e., when a user "jumps the rails"). Because inclusion criteria comprise familiarity and experience using a treadmill, and you have represented that you meet the inclusion criteria, we believe that your risk of injury from using the treadmill is minimal.
- There is always the risk of a "cardiac event" when exercising vigorously. These risks are mitigated, to the extent possible, through the use of PAR-Q+ screening and our study inclusion criteria, which include minimum and average levels of regular exercise of at least moderate intensity. **Our ability to help mitigate these risks are dependent on the accuracy and completeness of your responses to our questionnaires (e.g., PAR-Q+), intake forms, and representation that you meet our study inclusion criteria.**

Benefits from participation

We appreciate your consideration to volunteer for this study and the investment of time to complete the required procedures. An overarching benefit to you as a result of your willingness to participate is knowing that you are making a significant contribution to science. Well-designed and well-executed research studies are shared with a global audience of researchers and practitioners. These studies have implications for the health and well-being of millions of individuals globally.

We intend to submit our study findings for publication and use them in presentations to maximize the impact of our results. On publishing, we will provide you with a copy of the journal article if you are interested in receiving one. The published article will be evidence of your involvement in a study to which you so graciously contributed your time and effort.

The benefits of omega-3 supplementation are well-documented. If you are in either the DHA or EPA supplement group, it is highly likely that one or many of these benefits will accrue to you (assuming that you comply with the study requirements, i.e., daily supplement consumption). On the conclusion of this study, if you were included in the placebo group and would like to receive a short-term supply of omega-3 fish oil softgels, it will be our pleasure to provide them to you.

Additionally, we believe that providing you with data that we collect, at no cost to you, will be of significant benefit. We estimate the total value of these benefits (listed below) to exceed $3,000 at retail prices.

- Obtaining vital information about your health, including clinical-quality measurements of your body composition, comprehensive blood tests, VO_{2peak}, resting metabolic rate, and heart rate variability
- Interpretation of your test results and guidance on how to apply them
- Access to a leading-edge application used by professional athletes and Olympians to track diet and exercise
- Gifts after reaching each of the 4, 8, and 12-week participation milestones (you will select from items such as t-shirts, fitness towels, shaker bottles, and books)

- When you complete all 16 weeks of the study, you will also be eligible to meet with a professional coach (the Principal Investigator) for up to 60 minutes to discuss your test results and how you can use the information to help you meet your ongoing health, fitness, and athletic performance goals

Confidentiality

All data collected during your participation will be held in strict confidence. To promote confidentiality, we store all data collected using a unique identifier assigned to you. All data will be secured as appropriate (e.g., locked storage and password-protected computer files). Although we will submit the results of this study for publication, all data included in any such submission will be presented in aggregate or by statistical grouping.

No individual data or results will be shared with any party without your express written consent unless required by law. All data associated with your participation will be permanently deleted or otherwise destroyed at the end of the required holding period. By executing this ICA you authorize third parties to share your data with us for use in our research efforts and statistical analyses. An example of a third party in this context is Cronometer, the platform that you will use to track your daily dietary intake and exercise activities.

Criteria for participation

Research studies yield the most impactful results when there is homogeneity (sameness) among participants. Consequently, research teams seek to ensure this "sameness" across the participant group by stating the requirements for inclusion and exclusion. By signing this ICA, you are confirming that you meet all of the criteria listed below:

<u>Inclusion criteria</u>

- Adults ages 25-50 years (male or female)
- Females: pre-menopausal, neither pregnant nor lactating, nor planning to become pregnant during the 16-week study duration
- Regular participation in moderate to vigorous exercise for a minimum of 120 minutes per week, with an average of 180-240 minutes per week (you will be expected to maintain this level of activity for the 16-week duration of the study)
- Familiarity and experience jogging or running on a treadmill
- Ownership of, and familiarity with how to access and use smartphone applications (required to facilitate field data collection using the Cronometer app)

<u>Exclusion criteria</u>

- Unwillingness or inability to commit to the daily requirements comprising the study protocol
- Significant weight loss or weight gain within 30 days of V1 to the Kennesaw State University Exercise Science Physiology Lab
- Exercise activity of fewer than 120 minutes per week

- Upcoming travel, vacations, or significant life events that would preclude completion of daily protocol requirements
- Consumption of omega-3 supplements within the past 4 months
- Regular consumption of oily fish (e.g., salmon) defined as more than once per week
- Difficulty swallowing pills, softgels, or capsules
- Blood or bleeding disorders
- Unwillingness or inability to participate in the collection of blood either via venipuncture or finger stick
- Prior episodes of fainting during blood collection
- Unsatisfactory completion of the PAR-Q+ ("unsatisfactory completion" is defined as one or more incomplete responses or the identification of an unacceptable number of risk factors)
- Injury or illness that prevents you from maintaining your diet and exercise regimen

Expectations and responsibilities of participation

Because your compliance with the study protocol is of paramount importance, our expectations, including communication protocol and use of the Cronometer app, will be discussed in detail for your acknowledgment and acceptance. If you are unwilling to agree to periodic check-ins, unwilling to use the Cronometer app, or in any way unable to complete the required tasks (including visits to the ESPL), we will have to exclude your data.

This type of research study relies on full and consistent participant engagement and compliance with detailed protocols. Without full commitment and compliance, study results will be compromised and conclusions we attempt to draw from the research will be fundamentally flawed. Consequently, we ask that **if you decide to participate that you engage fully, comply with detailed instructions, and participate for the duration of the study**.

We intend to help you complete the required procedures by providing assistance as and if needed. This assistance will include answering your questions during the study and communicating with you in various forms, such as text, phone call, and email. These messages will be responsive (answering your questions) and proactive (providing you with reminders).

Communication

Compliance with daily protocol requirements is essential for successful data collection and corresponding statistical analyses. Consequently, the members of the research team will be in regular contact with you. Forms of communication will include email, text messaging, and telephone calls. The primary purpose of our communication with you is to remind you of upcoming visits (including pre-visit responsibilities) and to facilitate compliance with study protocols.

Signed consent

I have reviewed this Informed Consent Agreement in its entirety. I understand, acknowledge, and agree to the scope and procedures described herein, and I explicitly consent to participate in this research study.

I confirm that [i] I meet all of the study inclusion and exclusion criteria and [ii] I will complete, for the duration of this study, all questionnaires, forms, and interview questions, both accurate and in their entirety. I understand and acknowledge that my failure to understand and ask questions about test procedures may compromise study results, as well as place me at risk of serious injury and possibly death. Therefore, I agree to ask questions about any item contained in this Informed Consent Agreement that I do not understand to help ensure my safety and the safety of the research team members.

I understand that my participation is voluntary and that I am not under any pressure or undue influence to participate. I further understand that I may withdraw from the study and correspondingly withdraw my consent at any time without penalty.

_____

Name of Participant (print)

_____ _____

Signature of Participant Date

_____

Name of Investigator (print)

_____ _____

Signature of Investigator Date

Research at Kennesaw State University that involves human participants is carried out under the oversight of an Institutional Review Board (IRB).

Questions or problems regarding these activities should be addressed to the Institutional Review Board, Kennesaw State University, 585 Cobb Avenue, KH3417, Kennesaw, GA 30144-5591. The Kennesaw State University IRB may also be reached at (470) 578-7721.