1. **Study Identification**

1.1. **Unique Protocol Identification Number:**
1.2. **Brief Title:** The effects of fish oil supplementation on the brain health of collegiate football athletes.
1.2.1. **Acronym:** not required
1.3. **Official Title:** Study of Effect of Docosahexaenoic Acid (DHA) and Eicosapentaenoic acid (EPA) Supplementation on Biomarkers of Sub-Concussion Injuries in American Football.
1.4. **Secondary ID:** not required
1.5. **Study Type:** Interventional (clinical trial)

2. **Study Status**

2.1. **Record Verification Date:** Month: Year:
2.2. **Overall Recruitment Status:** Completed: The study has concluded normally; participants are no longer receiving an intervention or being examined (that is, last participant's last visit has occurred)
2.2.1. **Why Study Stopped:** NA
2.3. **Study Start date:** May 29, 2019
2.4. **Primary Completion date:** January 28, 2020
2.5. **Study Completion Date:** January 28, 2020

3. **Sponsor/Collaborators**

3.1. **Responsible Party, by Official Title:** Principle Investigator
3.1.1. Investigator Information:
- **Investigator Name:** Floyd H. Chilton, Ph.D.
- **Investigator Official Title:** Professor, Department of Nutritional Sciences; Associate; Director BIO5 Institute; Director, The Precision Wellness Initiative
- **Investigator Affiliation:** University of Arizona

3.2. **Name of Sponsor:** Center for Innovation in Brain Science, Roberta D. Brinton, Ph.D.
3.3. **Collaborators:** NA

4. **Oversight**

4.1. **Studies a U.S. FDA-regulated Drug Product:** No
4.2. **Studies a U.S. FDA-regulated Drug Product:** NA
4.3. **US FDA IND or IDE:** No
4.4. **Product Manufactured in and Exported from U.S:** Yes
4.5. **Human Subjects Review:** Yes
4.6. **Human Subjects Protection Review Board Status:** Submitted, approved

4.6.1. **Board Approval Number:** 1904553365
4.6.2. **Board Name:** Internal Review Board
4.6.3. Board Affiliation: University of Arizona
4.6.4. Board Contact: Christine Melton-Lopez
- **Phone:** (520) 626-8630
- **Extension:** NA
- **Email:** melton1@email.arizona.edu
- **Address:** POB 210409, Tucson, AZ 85721

4.7. **Data Monitoring Committee:** Yes
4.8. **FDA Regulated Intervention:** No

5. **Study Description**

5.1. **Brief Summary:** *A short description of the clinical study, including a brief statement of the clinical study's hypothesis, written in language intended for the lay public. Limit: 5000 characters.*

American football is one of the most popular sports in the U.S. Yet this sport is associated with increased risk of concussion (also known as mild traumatic brain injury, or mTBI) and subconcussive injury from repeated head impacts (RHI) due to the aggressive and highspeed nature of the game. Current protective equipment used by players are not sufficient to reduce concussion incidence and severity, nor are there any therapeutics available to prevent concussion. This study is a randomized, double-blind, placebo controlled trial, including 38 research participants, to determine whether an omega-3 polyunsaturated fatty acids (PUFA) fish oil supplement containing 3.0 grams of docosahexaenoic acid (DHA; 22:6n-3) and eicosapentaenoic acid (EPA; 20:5n-3) can reduce blood biomarkers of sub-concussion injuries compared to placebo (high-oleic safflower oil) over a course of the American football season among collegiate football athletes. Secondary outcomes include 1) determining the effect of daily DHA and EPA supplementation on brain imaging markers of sub-concussion injuries, and 2) a responder analysis to determine whether genotype differences in fatty acid desaturase 1 (FADS1), FADS2, elongation of very long chain fatty acids 2 (ELOVL2), and ELOVL5 genes affect treatment outcome of DHA. An exploratory outcome is the discovery of novel and more sensitive metabolic or lipidomic biomarkers of sub-concussion injury. The dosage of DHA/EPA used in this study is generally safe, and procedures involved, monthly blood draws, surveys, and Magnetic Resonance Imaging (MRI), pose minimal risks to participants. While this study provides no direct benefit to participants, successful outcomes of this study can benefit the society by shedding light on development of potential preventative therapeutics for sports-induced mTBI and brain injury from RHI. The risk-benefit profile is appropriate for conducting this study. Based on preclinical studies and previous clinical study results, we expect that in comparison to placebo treatment, DHA and EPA treatment throughout the course of one American football season can maintain lower levels of sub-concussion associated biomarkers,

inflammatory cytokines, and cardiovascular risk markers. We also expect participants treated with DHA and EPA to have lower brain MRI imaging markers of sub concussion injury.

5.2. **Detailed Description:** (not required) *Definition: Extended description of the protocol, including more technical information (as compared to the Brief Summary), if desired. Do not include the entire protocol; do not duplicate information recorded in other data elements, such as Eligibility Criteria or outcome measures. Limit: 32,000 characters.*

6. **Conditions and key words**

6.1. **Primary Condition Being Studied:** Sub-concussive brain injury as measured by biomarkers of axonal damage from repeated head impacts (RHI).
6.2. **Keywords:** (not-required) concussion, mTBI, repeated head impacts, football, brain, Chronic traumatic encephalopathy, Magnetic resonance imaging, Neurofilament light chain, biomarker

7. **Study design**

7.1. **Primary Purpose:** Prevention
7.2. **Study Phase:** NA
7.3. **Interventional Study Model:** Parallel
7.4. **Model Description:** Participants will be randomized to treatment (3 grams DHA and EPA (2:1 weight ratio)) or placebo (3 grams high-oleic safflower oil) in a 1:1 allocation ratio based on their position on the football team and roster depth (starter vs. non-starter). Participants will be recruited as a single cohort. After screening, participants will be treated with DHA and EPA or matching placebo for 25 weeks (a full course of an American football season, including summer camp training sessions). A follow-up visit is scheduled 7 weeks after the final dosing at week 32 for recovery and adverse event evaluation.
7.5. **Number of Arms:** Two
7.6. **Masking Roles:** Participant, investigator, care provider, outcomes assessor
7.7. **Masking Description:** Study personnel will be kept blind to treatment assignments. The treatment assignment for each participant will be available to the investigator in a sealed envelope that may be opened only in the case of a serious adverse event which the investigator feels cannot be adequately treated without knowing the identity of the study medication. Code breakers will be collected and reviewed at the end of the study. If the blinding has been broken, then the investigator will provide documentation regarding the fact and indicate the other staff that received this information.
7.8. **Allocation:** Randomized
7.9. **Enrollment Type:** Actual
7.10. **Number of Subjects:** 38

8. **Arms, Groups, and Interventions**

8.1. **Arm 1 Type:** Active comparator
8.2. **Arm 1 Title:** Treatment
8.3. **Arm 2 Type:** Placebo Comparator
8.4. **Arm 2 Title:** Placebo
8.5. **Intervention 1:**
8.5.1. **Intervention 1 Type:** Dietary Supplement
8.5.2. **Intervention 1 Name:** Fish oil supplement containing docosahexaenoic acid (DHA; 22:6n-3) and eicosapentaenoic acid (EPA; 20:5n-3)
8.5.3. **Intervention 1 Description:** Participants consumed a dietary supplement consisting of 3 grams refined fish oils as DHA and EPA ethyl esters in 2:1 ratio 5 days a week for 25 weeks. The product is stabilized with identity preserved mixed natural tocopherols and maintains stability for 24 months when stored in original, unopened, light-resistant containers at a temperature below 30° Celsius. Refined fish oil was encapsulated so six capsules provide 3 gram per dose. Certificate of analysis confirming manufacture date, active ingredient, fill weight, and microbiology safety, and disintegration time was provided. A dose of 3 grams DHA and EPA, 5 days per week, was based on a previous clinical study in a similar population using the same compounds[5]. This dose is considered generally safe, and can provide sufficient bioavailability to significantly reduce plasma NfL level (a biomarker of brain injury and neurodegeneration)[5]. The dose remained constant for each participant during the study period. If a participant experiences an adverse event (AE) considered to be possibly related to the study dietary supplement, the investigator has the option to split up the dose throughout the day, recommend the participant take the supplement with food or to discontinue the participant.
8.6. **Intervention 2:**
8.6.1. **Intervention 2 Type:** Placebo
8.6.2. **Intervention 2 Name:**
8.6.3. **Intervention 2 Description:** Participants consumed a placebo consisting of encapsulated high-oleic safflower oil. Each placebo capsule contains 866.9 mg/capsule active ingredient. The placebo intervention included consumption of 6 capsules 5 days a week for 25 weeks. A certificate of analysis confirming manufacture date (06/03/2019), active ingredient (safflower oil), fill weight (866.9 milligrams), and microbiology safety (all negative), and disintegration time (3 minutes) was provided.
8.7. **Arm/Intervention Cross-Reference:** Arm 1 = Intervention 1; Arm 2 = Intervention 2

9. Outcome Measures
9.1. **Outcome 1: Primary Outcome Measure**
9.1.1. **Title 1:** The effect of DHA and EPA Omega-3 PUFA fish oil supplementation on blood biomarkers of brain injury compared to placebo (high-oleic safflower oil) over a course of

the American football season among collegiate football athletes exposed to repeated subconcussive head impacts.

9.1.2. **Time Frame 1:** 32 weeks from baseline to one month postseason/treatment

9.1.3. **Description 1:** (not required) Blood-based biomarkers for sub-concussion injury (neurofilament light (NFL), Tau protein (Tau), ubiquinone C-terminal hydrolase L1 (UCH-L1)) and can lower inflammatory cytokines (C-reactive protein (CRP), tumor necrosis factor 1 (TNF-α), interleukin 6 (IL6)) will be measured at baseline, mid-treatment, post treatment, and one month (recovery) after the end of treatment and the football season.

9.2. **Outcome 2: Primary Outcome Measure**

9.2.1. **Title 2:** The effect of DHA and EPA Omega-3 PUFA fish oil supplementation on blood cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglyceride levels in collegiate football athletes.

9.2.2. **Time Frame 2:** 25 weeks from baseline to end of treatment

9.2.3. **Description 2:** (not required) A lipid panel will be assessed at baseline and end of treatment.

9.3. **Outcome 3: Secondary Outcome Measure**

9.3.1. **Title 3:** The effect of DHA and EPA Omega-3 PUFA fish oil supplementation on brain imaging markers of sub-concussion injury collegiate football athletes over the course of the American football season.

9.3.2. **Time Frame 3:** 25 weeks from baseline to end of treatment

9.3.3. **Description 3:** (not required) MRI scans were obtained at baseline and end of treatment.

9.4. **Outcome 4: Secondary Outcome Measure**

9.4.1. **Title 4:** The effect of genotype differences on DHA and EPA treatment outcomes on blood based biomarker or brain MRI imaging biomarker as described in specific aim 1.

9.4.2. **Time Frame 4:** 32 weeks from baseline to one month post treatment and post end of football season.

9.4.3. **Description 4:** (not required)

9.5. **Outcome 5: Other Pre-Specified Outcome Measures**

9.5.1. **Title 5:** Conduct non-targeted metabolomic analysis to potentially identify additional potential biomarkers for sub-concussion injuries.

9.5.2. **Time Frame 4:** 32 weeks from baseline to one month post treatment and post end of football season.

9.5.3. **Description 5:** (not required)

10. **Eligibility**

10.1. **Sex:** Male

10.1.1. **Gender-Based:** (not required) No

10.2. **Age Limits:**

10.2.1. **Minimum Age:** 18

10.2.2. **Unit of Time:** Years
10.2.3. **Maximum Age:** NA
10.2.4. **Unit of Time:** NA

10.3. **Accepts healthy Volunteers:** Yes

10.4. **Eligibility Criteria:**

10.4.1. Inclusion Criteria

- University of Arizona
- National Collegiate Athletic Association (NCAA)
- Collegiate football players
- Cleared to participate in university athletics as determined by the team physician.
- >18 years.

10.4.2. Exclusion Criteria

- Chronic daily anti-inflammatory medication (>20 d).
- Medications for blood lipids.
- Active fish oil or omega-3 fatty acid supplementation.
- Consumption of more than two servings of fish per week.
- Injured and unable to participate in regular schedule conditioning or competitions.
- Prior diagnosis acute concussion.
- Allergic to fish or shellfish.