Dietary & Biological Assessment of Omega-3 Status of Collegiate Athletes:

A Cross-Sectional Analysis

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Abstract

Recent changes in the National Collegiate Athletic Association's (NCAA) Division I legislation pertaining to nutritional supplements has resulted in increased interest in the role of omega-3 fatty acids (ω-3 FA's) for athletes. Only minimal information about collegiate athletes' ω-3 FA status is available. The purpose of this project was to assess the ω-3 FA status of male and female NCAA Division I athletes. The assessment was carried out across nine Power 5 athletic programs distributed across the United States prior to the legislation change. The first portion consisted of a food frequency questionnaire (FFQ) assessing dietary intake and supplement use. In the second portion, a subset of participants provided a finger stick blood sample to measure corresponding Omega-3 Index (O3i). In a total sample of 1528 participants (51% male, 19.9 ± 1.4 years of age) from 29 sports, only 6% (n=93) of participants achieved the Academy of Nutrition & Dietetics' recommendation to consume 500 mg DHA+EPA/ day. Use of ω-3 FA supplements was reported by 15% (n=229) of participants. Of those who participated in blood measures (n=298), O3i was 4.33 ± .81%, with zero participants meeting the O3i benchmark of 8% associated with the lowest risk of cardiovascular disease. Every additional weekly serving of fish or seafood was associated with an absolute O3i increase of 0.27%. Dietary DHA + EPA was positively correlated with O3i (p= .441 and .437, p <.001). Sub-optimal ω-3 FA status was observed among this large, geographically diverse group of male and female collegiate athletes.

These results may serve as a baseline for collegiate athletes' ω-3 FA status prior to the NCAA legislation change, and inform future athlete-specific nutrition interventions.

General Audience Abstract

The National Collegiate Athletic Association (NCAA) recently made changes to its supplement policies, allowing Division I schools to provide fish oil/ omega-3 supplements to athletes. Given the relatively small body of research available on the topic, the purpose of this project was to assess the current intake of omega-3 fats in NCAA Division I athletes in their diets and their corresponding Omega-3 Index (O3i) blood levels, a blood measure suggested to be associated with the lowest risk of cardiovascular disease. In a total sample of 1528 participants (51% male, 19.9 ± 1.4 years of age) from 29 sports, only 6% (n=93) of participants achieved the Academy of Nutrition & Dietetics' recommendation to consume 500 mg DHA+EPA/ day. Use of fish oil/ omega-3 supplements was reported by 15% (n=229) of participants. In a subset of 298 participants, zero participants met the suggested Omega-3 Index of >8%.This project produced similar findings to smaller previous studies, suggesting that collegiate athletes are not meeting the general recommendations for omega-3 fats.

Acknowledgements

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Introduction

Omega-3 polyunsaturated fatty acids (ω-3 FA), namely long-chain docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), serve as a significant structural component within phospholipid that form cell membranes. These ω-3 FA's have also been shown to play important physiological roles in the cardiovascular, central nervous and skeletal systems, and the body's inflammatory response. Applications for ω-3 FA's in the sports setting have been a recent topic of interest in scientific literature, specifically in regards to neuroprotection and the management of exercise-associated inflammation.

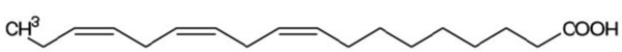
In January 2019, the National Collegiate Athletic Association (NCAA) amended legislation regarding nutritional supplementation, which re-classified ω-3 FA's from "non-permissible" to "permissible", and allows athletic departments to provide these supplements to student-athletes as the institution deems appropriate.[1] As a result of this rule change, both athletes and sports medicine practitioners have taken an increased interest in the application of ω-3 FA-related nutrition interventions. Unfortunately, reports of athletes' intake of ω-3 FA's are minimal to date, providing limited guidance for the implementation of nutrition interventions, protocols and policies in athletic settings. Thus, the purpose of this research was to assess the ω-3 FA status of male and female NCAA Division I collegiate student-athletes who participate in a variety of sports, a population with the potential of being directly impacted by this emerging area of research. Both dietary and blood status findings observed in this research provide insight into collegiate athletes' ω-3 status and may be used to inform future nutrition and supplement-related interventions for the collegiate athlete population.

Literature Review

Structure & The Food Supply: Omega-3 vs Omega-6

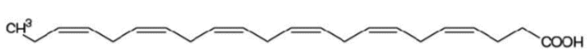
Both ω-3 FA's and ω-6 FA's are classes of polyunsaturated fatty acids (PUFA's), referring to the numerous double bonds involved in their chemical structure. The nomenclature of these PUFA's refers to the position of the final double bond in the structure, occurring at either the third or sixth carbon atom from the methyl tail end of the molecule (Figure 1). The three primary ω-3 FA's include short chain alpha-linolenic acid (ALA) and long chain docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). In comparison, linolenic acid (LA) is the most common of the ω-6 FA's, which can be converted into longer chain fatty acids like arachidonic acid (AA).

Omega-3 (ω-3) Fats



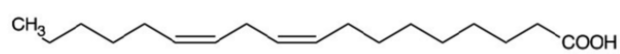
Alpha-linolenic acid (ALA)

Eicosapentaneoic acid (EPA)

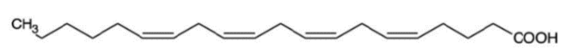


Docosahexaenoic acid (DHA)

Omega-6 (ω-6) Fats



Linoleic acid (LA)



Arachidonic acid (AA)

Figure 1) Common Omega-3 and Omega-6 Fatty Acid Structures

Both of these classes of PUFA's are considered essential fatty acids highlighting their significance in human nutrition as they are unable to be synthesized by the body and must be obtained through the diet. ALA is most prevalent in nuts and seeds such as walnuts, flaxseed, chia seed and plant-based oils including flaxseed, soybean and canola oil. Meanwhile, longer chain DHA and EPA are primarily found in a limited number of food sources including fatty fish and seafood, as a result of their algae-rich diet. While the human body is capable of synthesizing EPA and DHA from ALA, the inefficiency of this conversion further emphasizes the importance of obtaining these two long-chain fatty acids through dietary sources.[2] Alternatively, prevalence of ω-6 FA's in the food supply has increased significantly throughout the 20th century with common sources including vegetable oils, poultry, eggs and cereal-based products.[3]

Physiological Function

While both of these classes of PUFA's are considered essential fats they have distinct variation in their physiological roles. As previously mentioned, ω-3 FA's act as an important structural component of cell membranes. As a whole, the largest body of research related to ω-3 FA's pertains to their cardiovascular role. Increased omega-3 intake and subsequent improved blood serum levels influence parameters of cardiovascular health such as blood cholesterol, triglycerides, blood pressure, and platelet aggregation.[4–6] While there is still some debate, there is recent evidence of associated decreases in relative risk for coronary heart disease with in those with current standards for "optimal" blood status compared to those considered "sub-optimal". [7–9]

While all three of these ω-3 FA's are considered essential nutrients, research has primarily focused on DHA and EPA because of their role in structure and function of the heart, brain and eyes. As an additional obstacle, a lack of association between dietary and blood measures of ALA has been noted in previous research. [10,11] These findings are thought to be linked to competitive inhibition observed with high ω-6 linoleic acid consumption in addition to the increases in ALA oxidation observed with higher ALA intakes, suggesting it serves primarily as an energy source in terms of human metabolism. [12]

DHA in particular is highly concentrated in the brain and plays an essential role in its growth and development. In prior animal studies, significant decreases in DHA levels in the brain have been seen following traumatic brain injury.[13] Omega-3 supplementation applied both prior to and following TBI have been shown to decrease symptom intensity and duration.[13–15]

In contrast, EPA is considered the most important ω-3 FA in terms of inflammation management. While eicosanoids derived from ω-6 FA's are the primary mediators of cellular inflammation, EPA has been shown to competitively inhibit AA metabolism.[16–18] The primary mechanism for this inhibition relates to the observation that increased EPA intake in the diet demands additional desaturase and elongase enzymes considered necessary substrate for the formation of these ω-6 derived pro-inflammatory eicosanoids. [16]

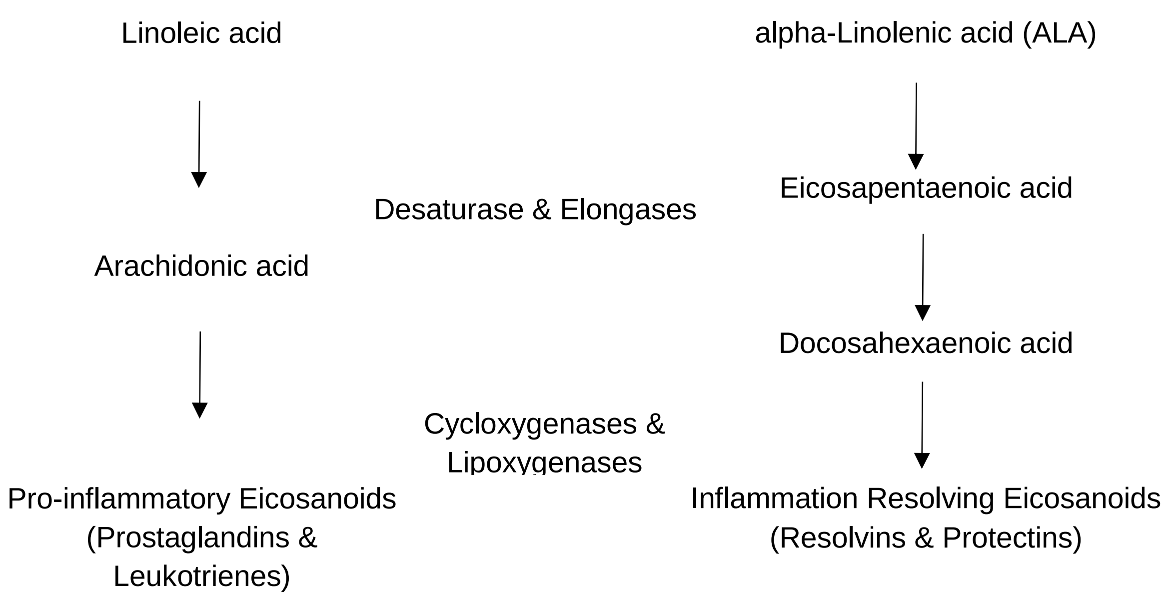


Figure 2) Overview of Omega-6 and Omega-3 Metabolism Pathways

Health and Performance Applications

Despite accumulating evidence suggesting ω-3 FA status in many athletes is relatively low, ω-3 FA's have become a popular topic in a variety of areas including recovery from exercise,[12,19–23] neuroprotection,[24,24–29] skeletal muscle health[30–34] and both aerobic and anaerobic exercise performance.[35,36]

Of particular interest in research regarding contact sport, the potential neuroprotective role of DHA as related to concussion has been a recent topic of investigation.[24–28,37] Prophylactic DHA supplementation has been suggested to provide a neuroprotective effect to athletes, as observed by attenuated increases in serum neuro-filament light (NFL), a biomarker of axonal injury associated with accumulating sub-concussive injuries during the American football season.[24] These benefits may still be of interest even long after the initial incidence of the injury, as evidenced by ω-3 supplementation in retired athletes showing improvements in cerebral blood flow and cognitive function.[28]

Increased oxidative stress and inflammatory response in competitive athletes as a result of their intensive training regimen has made EPA's role in inflammation management a popular area for sports-related research as well. Supplementation of ω-3 FA's has been observed to reduce pro-inflammatory markers and attenuate the rise of acute phase proteins involved in the post-exercise inflammatory response.[19,20,38,39] EPA's inflammation resolving characteristics have also been applied to the concept of muscle recovery as several studies have observed improvements in delayed-onset muscle soreness using an ω-3 supplement.[21,22,40]

As it relates to cardiovascular health, research has suggested ω-3 FA's have potential as an ergogenic acid as improvements in anaerobic endurance capacity[35] and increased oxygen efficiency during exercise.[36]

Current Dietary Trends

While there is currently no consensus for dietary recommendations in regards to ω-3 FA's, (Table 1) low consumption appears to be prevalent in North America, primarily attributed to a limited number of ω-3 FA dietary sources and long-term changes in the food supply resulting in the increased prevalence of ω-6 FA.[3,41] Research indicates relative levels of EPA and DHA in red blood cells have decreased 40-50% in the modern Western population over the course of the 20th century.[41] Even with improvements in intake related to recent media attention and the popularization of fish oil supplements, most Americans are still not meeting current recommendations as illustrated by an examination of NHANES 2003-2008 suggesting an average of 4.27 oz/ week of total fish intake and only 1.05 oz/week of fish rich in ω-3 FA's.[42] In comparison, the primary standard for this assessment was the Academy of Nutrition & Dietetics' recommendation for at least 2-3.5 oz (7 oz total) servings of fatty fish/ week.[43]

Table 1) Dietary Recommendation for ω-3 FA's

	Year of Publication	Population	Dietary Recommendation
Academy of Nutrition & Dietetics	2014	General Public (adults)	500 mg EPA+DHA/ day[43]
American Heart Association	2002	General Public	At least 2 fish servings weekly (3.5 oz per serving)[44]
World Health Organization	2003	General public (adults)	1-2% of energy/ day[45]
National Academy of Medicine	2005	Adult men	1.6 g/ day of ALA, of which ~10% EPA+DHA[46]
National Academy of Medicine	2005	Adult women	1.1 g/ day of ALA, of which ~10% EPA+DHA[46]

ω-6:ω-3 ratios in the Western diet have also increased 77% in the last century.[3] This is primarily attributed to the rise of processed, "convenience" foods significantly increasing the use of ω-6 fat sources such as soybean oil, noted for their favorable stability as a replacement for much quicker perishing ω-3 sources.[18] Unfortunately as previously discussed, this balance between ω-6 FA's and ω-3 FA's in the diet appears to have potential health implications. For example, the ω-6:ω-3 ratio has actually being proposed as a potential measure of health status as it relates to cardiovascular health and systemic inflammation.[18,47] While both classes are essential nutrients, an unfavorable shift in the ω-6:ω-3 ratio has been associated with the development of chronic conditions like atherosclerosis and diabetes stemming from poor management of the pro-inflammatory eicosanoid end-products resulting from a combination of excessive intake of ω-6 FA's and/ or inadequate intake of long chain ω-3 FA's.[18,47,48]

Athlete-Specific Considerations

Particularly at the collegiate level, athletes are often at risk of developing nutritional deficiencies as a result of factors such as limited food access, busy schedules and a newfound sense of independence all in combination with the physical and mental demands of preparation for and competition in their sport..[49,50] At the Division III level for example, less than 50% of football players participating in a survey reported consuming fruits and vegetables daily.[51] To the authors' knowledge there is currently only one assessment available of dietary ω-3 FA intake in athletes, which observed intakes of EPA and DHA below 100 mg daily in a group of 58 collegiate athletes.[10] This is significantly less than the recommendation from the Academy of Nutrition & Dietetics (AND) of two fish servings weekly, providing a daily average of 500 mg EPA + DHA.

Assessment of Blood Status

In addition to assessing dietary intake, ω-3 status may be evaluated using the Omega-3 Index (O3i), which reflects the EPA and DHA content of erythrocyte membranes.[7] In comparison to the traditional ω-3 assessment method requiring a conventional blood draw, the dried blood spot system requires a minimal blood volume adding convenience in terms of data collection and transportation logistics.[52] Additionally, O3i via the dried blood spot system has a low biological variability,[53] is less affected by acute feedings to better reflect long-term intake of ω-3 FA's compared to plasma and plasma phospholipid measurements,[54] and has been shown to correspond with concentrations of ω-3 FA's in the heart, brain, and a variety of other tissues.[55,56]

Cardiovascular health has been the primary focus of ω-3 blood status investigations to this point. One particular point of interest has been the relationship between EPA and DHA levels, and more recently, O3i and risk for adverse cardiovascular events, which has been examined in a variety of studies comparing the associated risk between standard deviations in populations.[7,57–62] The most common benchmarks in current literature suggest an O3i <4% as a marker for high risk of the development of cardiovascular disease and 8% in the lowest risk classification. [7–9] These benchmarks were initially established with the help of a 19-cohort meta-analysis including over 25,000 individuals to estimate hazard ratios across the data set, with the authors estimating a 30% reduction in risk for fatal coronary heart disease by moving from the high risk to the lowest risk classification.[9] In comparison, research examining blood status suggests the average American adult has an O3i between 3-5%.[41,63,64]

O3i averages ranging between 4-5% have also become a common observation across assessments in both elite and collegiate settings, however sample sizes remain relatively small.[10,65,66] Recently, an average O3i of 4.4% was observed among collegiate football athletes at four U.S. universities.[67] A large scale assessment examining differences in variables such as gender, sport and geographic location has not been performed to the investigators' knowledge. Additionally, while cardiovascular health is an important consideration for athletes, further research is needed to determine athlete-based O3i recommendations specific to the performance and recovery outcomes being currently investigated.

While there are still a variety of questions that need to be answered in terms of ω-3 FA's, the wide-reaching functions of DHA and EPA specifically in regards to the heart, brain, eyes and the management of inflammation, suggest they may be particularly important for athletes. Unfortunately, current research suggests American intake is largely inadequate and research documenting athlete-specific intake of ω-3 FA's is scarce. Further research is needed to establish a clearer picture of the current state of ω-3 intake in athletes and continue to investigate the magnitude of the nutrient's health and performance implications.

Project Methodology

Study Design

A multi-site, cross sectional design was implemented to assess the ω-3 dietary intake, ω-3 supplement use, and O3i of collegiate student-athletes. These assessments were carried out during the 2018-2019 academic year, prior to any participating institution adopting a supplementation protocol as a result of the NCAA legislation change designating ω-3 supplement acids as permissible.

Participants

Student-athletes from nine NCAA Division I institutions were invited to participate in the project. In order to achieve geographical diversity, institutions were located throughout the U.S., with one institution representing each region shown in Figure 3. All nine institutions were classified as Power 5 programs, that is, part of the Football Bowl Subdivision of NCAA Division I and a member of one of the following conferences: Atlantic Coast Conference, Big Ten Conference, Big 12 Conference, Pac-12 Conference, and Southeastern Conference. All male and female student-athletes who were over the age of 18 years and on a current roster for any NCAA Division I sport were eligible to participate.

Omega-3 Dietary Assessment

A 26-item food frequency questionnaire (FFQ) validated to assess ω-3 dietary intake[10,11] was administered to participants (Appendix A). We modified the questionnaire to also include demographic characteristics of participants (sex, age, academic year, and sport) and ω-3

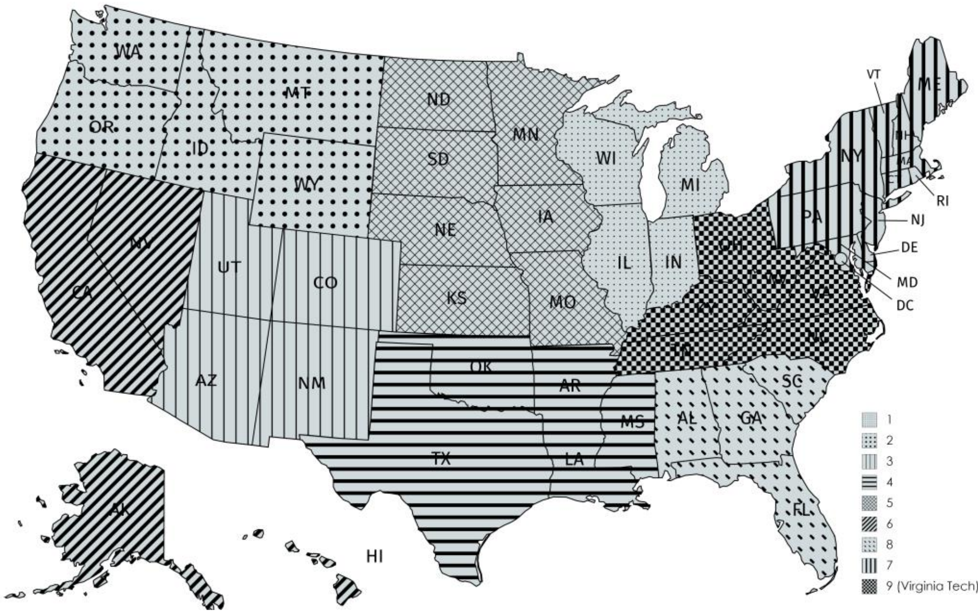


Figure 3) Regions Represented by Participating Institutions

supplement use. Within the questionnaire, participants reported the frequency of consumption and average portion size for an extensive list of ω-3 food sources including fish, shellfish, walnuts, canola oil, flaxseed, flaxseed oil, and cod liver oil. For participants who indicated that they consumed ω-3 supplements, information about brand, form, dosage, and frequency taken was requested.

To analyze responses, ω-3 content of dietary sources was referenced using a database provided by the survey developers,[11] originally derived from the U.S. Department of Agriculture Addendum A: EPA and DHA Content of Fish Species[68] and from *Bowes and Church's Food Values of Portions Commonly Used.*[69] Intake of ALA, EPA and DHA was calculated based on these reference values in relation to participants' sex and reported frequency and portion size as described in detail by Sublette et al.[11]

Blood Fatty Acid Analysis

Following completion of the dietary assessment portion of the study, participants were offered the opportunity to volunteer for a second portion of the study: analysis of blood fatty acids. For the collection, a single drop of whole blood was withdrawn onto a blood spot card pre-treated with an antioxidant cocktail (Figure 4).

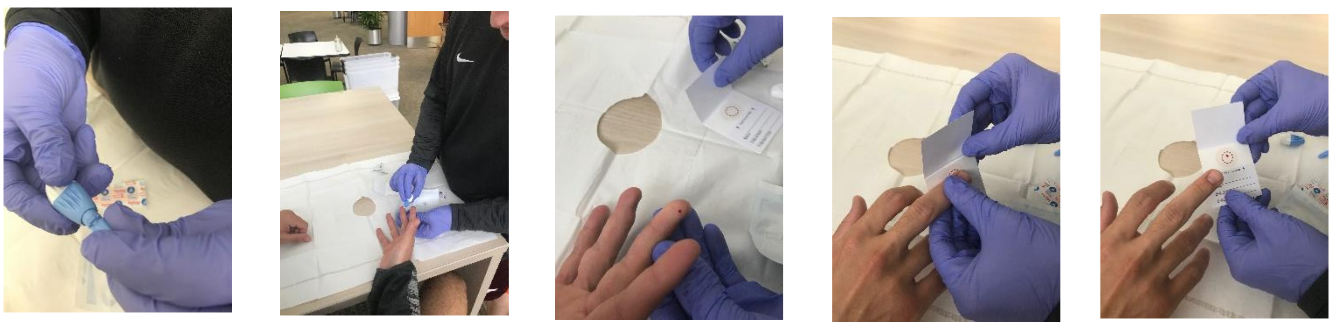


Figure 4) Step-by-step illustration of O3i fingerstick collection method

Samples were shipped to a central laboratory (OmegaQuant, Sioux Falls, SD) for a full fatty acid analysis (Appendix B) in addition to the calculation of the Omega-3 Index using gas chromatography. This methodology is described in detail by Harris and Polreis.[70] The fatty acid analysis included O3i, EPA, DHA, ALA, Arachidonic Acid (AA), and omega-6 fatty acids (ω-6 FA). Calculations of ω-6:ω-3 and AA:EPA ratios were also conducted.

Statistical Analysis

Data were analyzed using IBM Statistical Package for the Social Sciences (SPSS) 26. Descriptive statistics are expressed as means and standard deviations for continuous data, and frequencies and percentages for categorical data. Differences in diet and blood measures between demographic groups were calculated using chi-square or analysis of variance

(ANOVA). Relationships between variables were analyzed using Pearson's correlations. Multiple regression analysis was used to assess the effects of diet on O3i, after adjusting for demographic covariates. Significance was set at a level of $p<0.05$.

Ethical Considerations

This study was approved by the Institutional Review Board of Virginia Tech (IRB# 18-606) and respective institutional research review committees. Consent for the dietary assessment portion of the study was inferred based on voluntary completion. Written and informed consent was provided by participants before starting the blood fatty acid portion of the study (Appendix C).

Project Results

In all, 1528 participants (51% males) completed the dietary assessment portion of the study, and 298 (55% males) completed the blood analysis portion. Participants represented 29 different athletic teams from nine institutions. Descriptive characteristics are shown in Table 2.

Table 2) Descriptive Characteristics of Participants

	Dietary Assessment	Blood Fatty Acid Analysis
n	1528	298
Sex (male/female)	780/748	163/115
Age (years)	19.9 ± 1.4	20.0 ± 1.3
Academic year =n (%)	*Freshman:* 442 (28.9%) *Sophomore:* 373 (24.4%) *Junior:* 377 (24.7%) *Senior:* 270 (17.7%) *5th year or Graduate:* 63 (4.1%)	*Freshman:* 88 (29.5%) *Sophomore:* 73 (24.4%) *Junior:* 70 (23.5%) *Senior:* 58 (19.4%) *5th year or Graduate:* 9 (3.0%)
Sport =n (%)	*Football:* 303 (19.8%) [a] *Non-football Male Sport:* 477 (31.2%) [b] *Female Sport:* 748 (49.0%)	*Football:* 81 (27.2%) [a] *Non-football Male Sport:* 82 (27.5%) [b] *Female Sport:* 115 (38.5%)

A total of 1345 (88%) participants reported eating fish and/or shellfish at least once during the previous 6 month timeframe (Table 3). Fish and/or shellfish was consumed two or more times per week by 601 (39%) participants (Table 3). Salmon and shrimp were the most common sources of DHA and EPA consumed (Table 3). ALA food source consumption included canola oil (1302/ 85%), walnuts (824/ 53.9%), chia (666/ 43.6%), flax or flax oil (534/ 34.9%), and cod liver oil (51/ 3.3%).

Table 3) Fish and Seafood Consumption

Frequency of Fish/Seafood Consumption	n (%)
never	183 (12.0%)
Less than once/month	142 (8.9%)
2-3 /month	157 (10.3%)
1/week	445 (29.1%)
2/week	247 (16.2%)
3-4/week	235 (15.4%)
5-6/week	100 (6.2%)
Daily	15 (1.0%)
2 or more/day	4 (0.3%)

Fish/Seafood Source	Reported Consumption n (%)
Salmon	1016 (66.5%)
Shrimp	849 (55.6%)
Crab	507 (33.2%)
Tuna	464 (30.4%)
Tilapia	423 (27.7%)
Catfish	251 (16.4%)
Lobster	232 (15.2%)
Cod	204 (13.4%)
Mahi Mahi	168 (11.0%)
Scallops	141 (9.2%)
Whitefish	93 (6.0%)
Oysters	90 (5.9%)
Halibut	88 (5.7%)
Clams, Mussels, Bass, Flounder, Snapper, Trout, Haddock, Swordfish, Sardines, Mackerel, Shark, Whiting, Bluefish, Kingfish, Sole, Tilefish, Turbot	<5%
Herring, Skate	0%

Use of ω-3 FA supplements was reported by 229 (15%) participants. Of supplement-users, 153 (67%) purchased the supplement on their own, while 76 (33%) received supplements via their respective athletic program. Almost all participants provided no response to brand, type, and dose of ω-3 supplements consumed.

Dietary consumption of total ω-3 FA, EPA, DHA, EPA + DHA, and ALA are shown in Table 4. Males consumed significantly more EPA and DHA than females (53.4 vs. 40.4 mg and 106.4 vs. 83.9 mg, respectively; $p<0.05$), while females consumed significantly more ALA than males (626.6 vs. 530.4 mg, respectively; $p<0.05$). Compared with participants representing non-football sports, football participants consumed significantly more EPA and DHA (57.3 vs. 43.6

mg and 111.8 vs. 89.7 mg, respectively; $p<0.05$) and similar ALA (622.7 vs. 587.8 mg, respectively). Virginia Tech participants reported consuming a daily EPA + DHA average of 107.5 mg, the lowest of all 9 participating institutions (Table 6). Total ω-3 FA did not differ based on sex or sport. No differences in dietary fatty acid intake based on age or academic year were observed. 93 of participants (6%) met AND's 500 mg EPA+DHA/ day recommendation. There were no differences in blood Total ω-3 FA, EPA, DHA, EPA + DHA, or ALA based on sex, sport, age, or academic year. O3i ranged from 2.25 to 7.23 (Figure 5), with 114 (38%) in the high risk category, 184 (62%) n the moderate risk category, and 0 (0%) in the low risk category. Virginia Tech had an average O3i of 4.29 with 25 (57%) in the high risk category and 19 (43%) in the moderate risk category and no participants (0%) in the low risk category. Higher ω-6:ω-3 and AA:EPA were observed in football participants compared with participants in other sports (12.1 vs. 8.7 and 32.4 vs. 25.5, respectively; $p<0.05$ and $p<0.001$, respectively). Virginia Tech participants had an average ω-6:ω-3 ratio of 6.5, the best of all 9 participating institutions.

Table 4) Dietary Consumption of ω-3 FA's

	Daily Intake (mg)
EPA	46.8 +/- 86.9
DHA	94.8 +/- 164.9
ALA	571.8 +/- 1151.5
EPA + DHA	141.7 +/- 250.6
Total ω-3 FA	713.4 +/- 1214.2

Table 5) Blood Fatty Acid Analysis

	Blood Measures
EPA (% FA)	0.45 ± .19
DHA (% FA)	2.19 ± .59
ALA (% FA)	0.49 ± .19
ω-3 Index	4.33 ± .81
ω-6/ ω-3	9.2 ± 1.9
AA/EPA	27.7 ± 12.1

Table 6) ω-3 FA measures by region

Region	**Avg EPA + DHA (mg/day)**	**Omega-3 Index (O3i)**	**Avg O6:O3 ratio**
1	195.8	4.22%	10.8
2	180.3	4.25%	9.4
3	180.3	4.81%	7.7
4	175.5	4.14%	8.8
5	135.4	4.22%	10.8
6	129.0	4.41%	8.4
7	138.4	-	-
8	125.1	4.12%	12.7
Virginia Tech (9)	107.5*	4.29%*	6.5

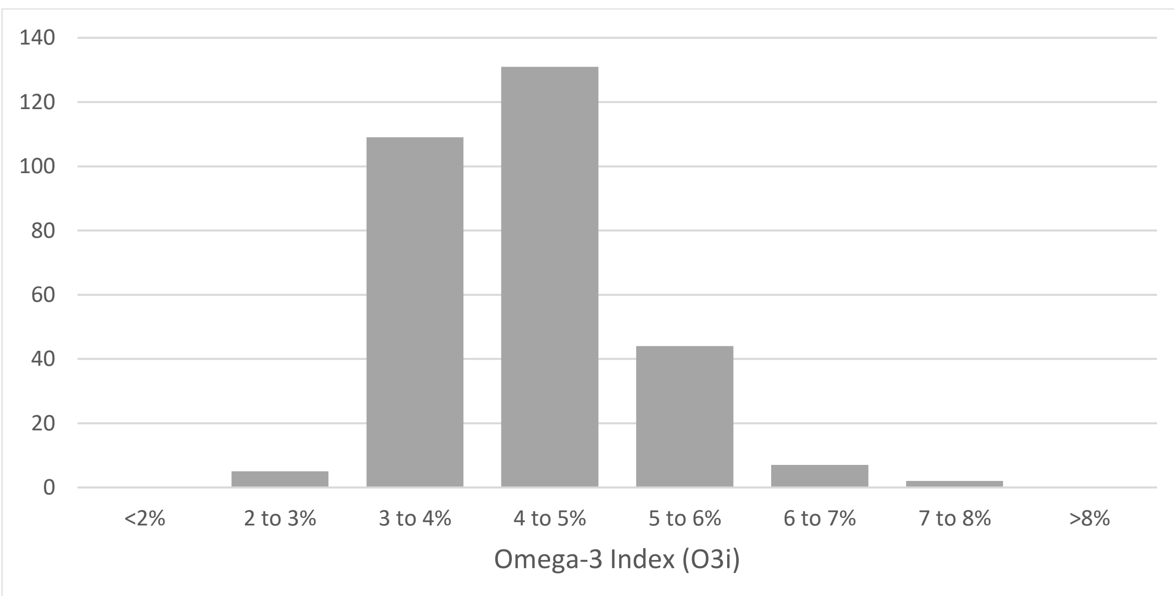


Figure 5) Distribution of O3i results

Dietary intake of both DHA and EPA were positively correlated with blood EPA, DHA, O3i and negatively correlated with ω-6:ω-3 (Table 7). The only significant association related to dietary ALA intake was a negative correlation with blood ω-6:ω-3. A significant negative correlation was also observed between both ω-3 Index and ω-6:ω-3 and ω-3 Index and AA/EPA (Figure 6 & 7). After controlling for age and sport (football vs. non-football), frequency of seafood consumption was a significant predictor of O3i (R^2=.3790, $p<0.01$). Each additional serving of seafood was associated with a O3i increase of .27% (Figure 8). Participants who reported taking ω-3 supplements had significantly higher ω-3 Index compared with those not taking supplements (3.7 vs. 4.7%, respectively; $p<0.05$).

Table 7) Diet and Blood Fatty Acid Correlations

	Diet EPA	Diet DHA	Diet EPA + DHA	Diet ALA	Diet Total ω-3	Blood EPA	Blood DHA	Blood ALA	Blood ω-3 Index	Blood ω-6:ω-3	Blood AA:EPA
Diet EPA	1	0.977 **	0.990 **	0.134	0.332 *	0.342 *	0.397 *	0.072	0.437 *	-0.368 *	-0.259
Diet DHA	.977 **	1	0.997 **	0.154	0.352 *	0.334 *	0.404 *	0.080	0.441 *	-0.366 *	-0.263
Diet EPA + DHA	0.990 **	0.997 **	1	0.148	0.299	0.403 *	0.338 *	0.078	0.442 *	-0.358 *	-0.263
Diet ALA	0.134	0.154	0.148	1	0.979 **	0.296	0.214	0.090	0.271	-0.358 *	-0.186
Diet Total ω-3	0.332 *	0.552 *	0.347 *	0.979 **	1	0.339 *	0.272	0.098	0.332 *	-0.776 **	-0.222
Blood EPA	0.342 *	0.334 *	0.338 *	0.296	0.339 *	1	0.402 *	0.072	0.648 **	-0.582 **	-0.762 **
Blood DHA	0.397 *	0.404 *	0.214	0.403 *	.273	.402 *	1	-.122	0.958 **	-0.770 **	-.192
Blood ALA	0.072	0.080	0.078	0.090	0.098	0.072	-0.122	1	-0.079	-0.198	-0.249
Blood ω-3 Index	0.437 *	0.441 *	0.442 *	0.271	0.332 *	0.648 **	0.958 **	-0.079	1	-0.823 **	-0.398 *
Blood ω-6:ω-3	-0.368 *	-0.366 *	-0.245	-0.358 *	-0.776 **	-0.582 **	-0.770 **	-0.198	-0.823 **	1	0.488 *
Blood AA:EPA	-0.259	-0.263	-0.263	-0.186	-0.222	-0.762 **	-0.192	-0.249	-0.398 *	0.488 *	1

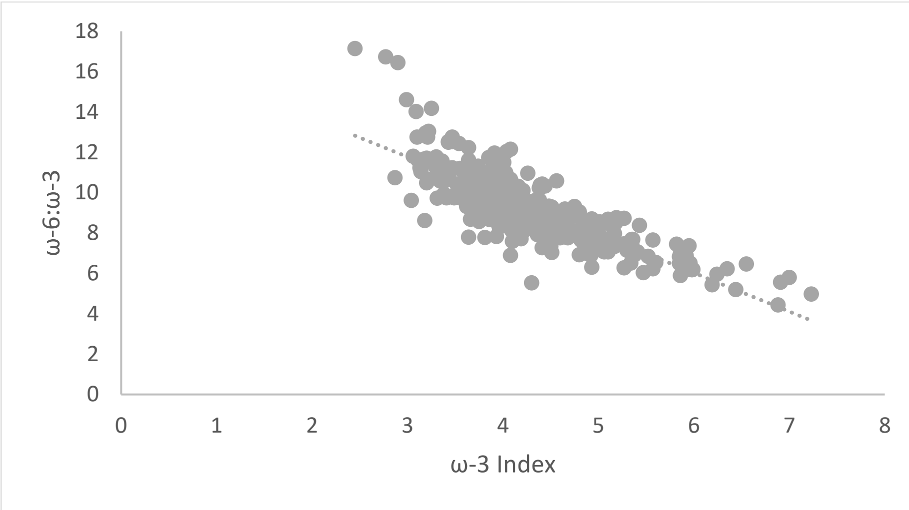


Figure 6) Correlations between O3i and ω-6:ω-3 ratio

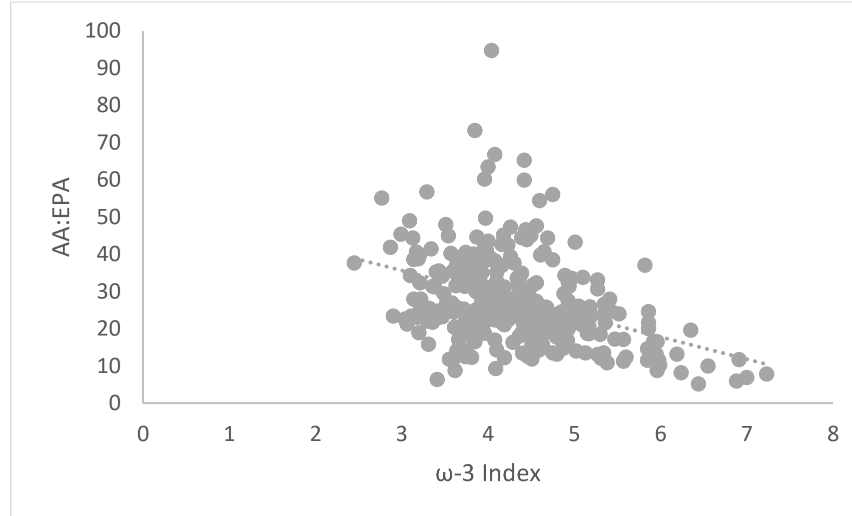


Figure 7) Correlations between O3i and AA:EPA ratio

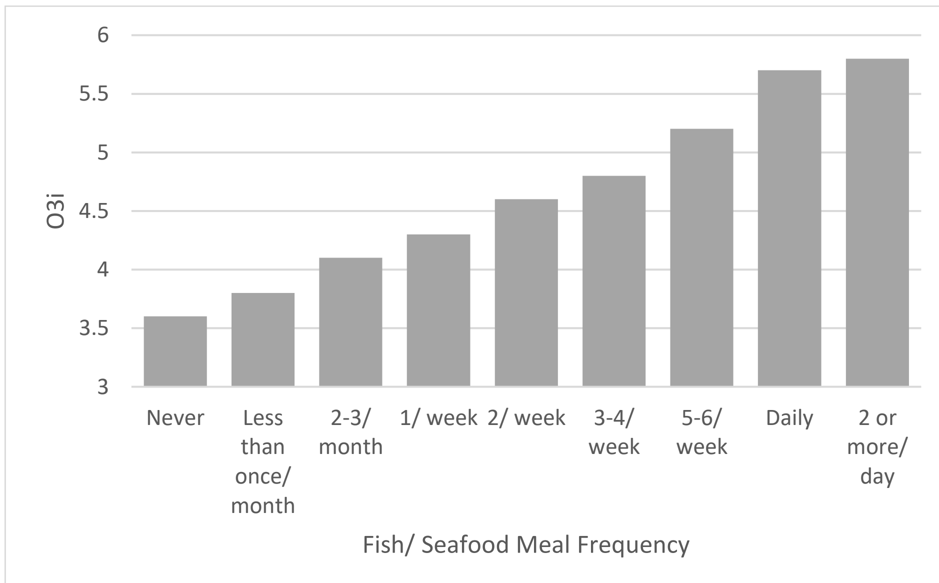


Figure 8) Relationship between fish and/or seafood meal frequency and O3i

Discussion

The primary goal of this assessment was to describe the ω-3 FA status of NCAA Division I athletes. The findings indicate that, as a group, U.S. collegiate athletes are not meeting current dietary recommendations for ω-3 FA and have sub-optimal O3i. To the authors' knowledge, this is the first large scale assessment of ω-3 FA status in both male and female collegiate athletes from a variety of sports. While further research is needed to more clearly identify athlete-specific needs and the extent of performance and health implications, these findings suggest a pattern of ω-3 FA deficiency among U.S. collegiate athletes.

These results also parallel those of other investigations that have observed a positive correlation between dietary ω-3 FA intake and O3i.[71–73] Strong positive correlations were observed between dietary DHA and EPA and corresponding blood values (DHA, EPA and O3i). Additionally the lack of association between dietary ALA and various blood measures, is also consistent with previous findings [31,43] The only exception, a negative association between ALA and blood ω-6: ω -3, can likely be explained by competitive inhibition linked to the desaturase enzymes involved in the metabolic pathways of both PUFA classes. The lack of association between dietary ALA and DHA, EPA and O3i can likely be explained by the relatively low conversion rate of ALA to its longer chain sister ω-3 FA.[2] For practitioners, this would suggest the most efficient method for improving ω-3 FA status would be to prioritize dietary intake of DHA and EPA using rich food sources like fish, seafood, fish oil and algae-based products which contain higher concentrations of these long chain ω-3 FA.

Participants in this project did not meet dietary ω-3 FA recommendations described in Table 1. Specifically, only 6% consumed 500 mg EPA + DHA/day recommended by AND,[43] 39% consumed two or more fish servings weekly as recommended by the American Heart

Association, [44] and 4% met the Institute of Medicine's recommendation of 1.6 g ALA (men) or 1.1 g ALA (women) with 10% coming from EPA + DHA.[46] Of note, 45% of participants reported consuming no fish, the richest source of EPA and DHA, during the previous six months. Participants consumed insufficient ω-3 FA's at a similar rate to the U.S. general public[41,42] and similar to that observed in a small group of athletes studied by Wilson and Madrigal.[10]

Importantly, none of the Table 1 dietary recommendations are specific to athletes, and it is plausible that athletes may have higher ω-3 FA needs than the general population. In a recent study examining non-elite runners, for example, a lower O3i average was noted in those who reported higher training mileages.[74] This should be an important variable to consider when determining athlete recommendations given the metabolic adaptations associated with intensive training regimens[74,75] in combination with unique risk factors related to cardiovascular disease[6,76,77] and neuroprotection.[24,37,78] Nevertheless, consumption of two or more fish servings weekly was insufficient to achieve optimal O3i. A limited number of participants (15%) reported taking ω-3 supplements in the current investigation, and few provided specific information about brand, form, dosage, and frequency used. However, participants who took a supplement did have higher O3i than those who did not. Further research on dietary and supplementation interventions for athletes is needed to better understand the athlete-specific needs.

These findings are comparable to previous research in athletes in both the collegiate and professional setting with average basal O3i measures in the range of 4-5%.[10,65–67] To our knowledge, no U.S.-based athletes have been documented in the peer reviewed literature as having ω-3 Index greater than 8%,[10,67] the benchmark considered optimal for cardiovascular health.[7–9] Although O3i is positively correlated with the concentration of ω-3 FA's in a variety of tissues, and ω-3 status is associated with a number of health and performance factors for athletes,[17,20–28,30–33,35–37,39,40] target O3i for non-cardiovascular conditions is not well-established. Research to investigate the impact of O3i on health and performance measures in athletes is needed.

While males consumed significantly more long-chain ω-3 FA's compared to females, it is important to note that general body mass and calorie needs are generally higher in males driving the increases in intake. This seems a more likely explanation, as no significant differences were observed in O3i between sexes. Similar findings were also seen between football and non-football participants, with higher intakes in football but similar O3i. Given the elevated risk of cardiovascular disease observed in American football players[76] and questions surrounding the long-term brain health of those experiencing repetitive, high-speed impacts,[37,79] football, in addition to other contact sports, will likely continue to be a priority for ω-3 investigations and nutrition interventions.

While research surrounding AA:EPA and ω-6:ω-3 ratios is still limited, the sample population had significantly higher averages than those documented in previous research related to general American[72] and athlete populations.[74] The AA:EPA and ω-6: ω-3 ratios are both direct measures of the balance between these two classes of PUFA and resulting inflammatory status. Similar to O3i, recent research has also identified associations between an elevated AA:EPA ratio and increases in cardiovascular disease risk.[80,81]. This may be of particular interest to athletes, given the discussions around oxidative stress, inflammation and muscle damage associated with high level training. The fact that football participants had significantly higher ω-6:ω-3 and AA:EPA ratios is further support that these athletes in particular would likely benefit from increased ω-3 intake and may even have higher needs compared to other athletes due to their combination of risk factors.

Virginia Tech-specific findings indicate potential for interventions focused on improving dietary intake of ω-3 FA's, EPA and DHA in particular. Interestingly, Virginia Tech athletes participating in the blood assessment portion of the study also measured the lowest ω-6: ω-3 ratio averages. While a further examination is needed to explain this observation, this could potentially be a result of lower intake of processed foods containing higher levels of ω-6 FA's or higher intake of ALA, contributing to the competitive inhibition of ω-6 FA metabolism. Findings like this highlight the potential for a two-pronged approach when creating nutrition interventions aimed at improving fatty acid status, specifically in regards to inflammation management: both an emphasis centered on increasing intake of ω-3 rich sources likes fish/ seafood and supplementation while also monitoring intake of ω-6 rich sources such as processed foods.

Strengths & Limitations

Collaboration with a diverse group of Power 5 institutions enabled us to study a large sample of athletes from nearly every NCAA sport with presumably varying dietary habits and available resources. Given the timing of the NCAA legislation changes in relation to the timeline of our assessment, this investigation also serves as a baseline for ω-3 FA intake among collegiate athletes allowing for the assessment of future nutrition interventions and supplement protocols aimed at elevating ω-3 FA status in athletes. While the FFQ is a validated tool, there are limitations in terms of precisely quantifying the content of ω-3 FA's in foods consumed by participants due to variations in factors like diets of the fish and seafood consumed, location, and time of year. Also, we did not collect data related to race/ethnicity, height, and body weight in effort to assure anonymity of participants. However, this information may have been insightful in data analysis. The lack of universally accepted dietary recommendations and blood measure standards provided an additional obstacle in terms of comparing our outcomes, which should be a primary motive for future research.

Practical Applications

Similar to the general public, U.S.-based collegiate athletes consume low quantities of ω-3 FA's. This information may be used to better inform nutrition counseling, dietary recommendations, menu planning, and dietary supplement protocols for athletes. Specific to collegiate athletes, the recent changes in NCAA legislation provide a direct opportunity to impact collegiate athletes' ω-3 FA status through supplementation. This project additionally provides a baseline in order to measure the significance of the impact of nutrition interventions created as a result of this legislation change.

Dissemination Plan

Following data collection, preliminary results for this study were presented at the annual Collegiate and Professional Sports Dietitians' Association (CPSDA) Conference. This event provided an opportunity to present our findings to over 500 sports dietitians and health care professionals working across the United States and abroad in a variety of sports settings. The data collected in this assessment is also in the process of being submitted for publication to a sports medicine-focused journal.

Conclusion

Prior to the NCAA legislation change in regards to ω-3 supplementation, we observed relatively low ω-3 FA status in NCAA Division I athletes in the form of both dietary and blood assessment. Provided that ω-3 FA's are being investigated to influence such a wide variety of physiological functions connected to athletic performance, dietary and supplement interventions aimed at increasing intake in athletes may be beneficial. Assessments in both the college and professional setting displaying the hypothesized gap between the current body of recommendations and athlete intake should encourage both practitioners and athletes to consider the potential merit of increasing ω-3 intake as part of their nutrition strategy. Further investigation, however, is necessary to establish athlete-specific recommendations and the magnitude of proposed benefits associated with optimizing ω-3 intake.

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Appendices

Appendix A) Food Frequency Questionnaire

Assessment of Omega-3 Fats in the Diets of Collegiate Athletes
Researchers within the Department of Athletics and the Department of Human Nutrition, Foods, and Exercise at Virginia Tech are conducting a research study aimed at learning more about intake of omega-3 fats by collegiate athletes. This research study involves completion of a questionnaire. Participation is completely optional, you are free to withdraw at any time, and all responses will remain anonymous.

This survey requires less than 10 minutes to complete.
There are no direct benefits for participating in this research and no promise or guarantee of benefits have been made to encourage you to participate. You will not be compensated for participating. If you should have any questions about the study, contact Peter Ritz, RD at pritz6@vt.edu . If you have questions about protection of human research participants regarding this study, contact the Virginia Tech Institutional Review Board at irb@vt.edu or (540) 231-3732.

Taking the survey indicates consent to voluntarily participate in the study.

1. Student ID # ____________________
2. Age: _________________
3. Year in school (freshman, sophomore, etc.): ________________
4. Sex: ___ Male ___Female
5. What NCAA Division 1 sport do you participate in? ________________
6. How many times have you eaten **fish or shellfish** in the past week?
   ___0 times ___1-3 times ___More than 3 times
7. Over the past 6 months have you eaten **fish or shellfish** in any form?

____ Never ____ Less than 1 time/ month ___1 time/ month

____2-3 times/ month ____ 1 time/ week ___2 times/ week

____3-4 times/ week ____ 5-6 times/ week ___1 time/ day

____2 or more times/ day ***If your response is "Never", advance to question 10***

8. Each time you ate **fish or shellfish**, how much did you eat?

____Less than 2 ounces (Less than one filet or less than 4 pieces of sushi)

____2-7 ounces (About 1 filet or 4-14 pieces of sushi)

____More than 7 ounces (More than 1 filet or more than 14 pieces of sushi)

9. Please check off the types of **fish or shellfish** you eat most frequently. (*Check off as many as are appropriate for you*.)

____Bass ____Bluefish ____Catfish ____Clams ____Cod
____Crab ____Flounder ____Haddock ____Halibut ____Kingfish
____Lobster ____Mackerel ____Mahi Mahi ____Mussels ____Oysters
____Salmon ____Sardines ____Scallops ____Shark ____Shrimp
____Skate ____Snapper ____Sole ____Swordfish ____Tilapia ____Tilefish
____Sea Trout ____Fresh Trout ____Tuna ____Turbot
____Whitefish ____Whiting

10. In the past 6 months, about how often did you eat **walnuts**?

_____ Never _____ Less than 1 time/ month ____1 time/ month

_____2-3 times/ month _____ 1 time/ week ____2 times/ week

_____3-4 times/ week _____ 5-6 times/ week ____1 time/ day

_____2 or more times/ day ***If your response is "Never", advance to question 12.***

11. Each time you ate **walnuts**, how much did you eat?

_____Less than ¼ cup _____¼- ½ cup _____More than ½ cup

12. In the past 6 months, about how often did you use or consume **canola cooking oil**?

_____ Never _____ Less than 1 time/ month ____1 time/ month

_____2-3 times/ month _____ 1 time/ week ____2 times/ week

_____3-4 times/ week _____ 5-6 times/ week ____1 time/ day

_____2 or more times/ day ***If your response is "Never", advance to question 14.***

13. Each time you had **canola cooking oil**, how much did you have?

_____Less than 1 teaspoon _____1-2 teaspoons

_____3 teaspoons (1 tablespoon) _____More than 1 tablespoon

14. In the past 6 months, about how often did you eat **flaxseed**?

_____ Never _____ Less than 1 time/ month ____1 time/ month

_____2-3 times/ month _____ 1 time/ week ____2 times/ week

_____3-4 times/ week _____ 5-6 times/ week ____1 time/ day

_____2 or more times/ day ***If your response is "Never", advance to question 16.***

15. Each time you had **flaxseeds**, about how much did you eat?

_____Less than 1 teaspoon _____1-2 teaspoons

_____3 teaspoons (1 tablespoon) _____More than 1 tablespoon

16. In the past 6 months, about how often did you have **flaxseed oil**?

_____ Never _____ Less than 1 time/ month ____1 time/ month

_____2-3 times/ month _____ 1 time/ week ____2 times/ week

_____3-4 times/ week _____ 5-6 times/ week ____1 time/ day

_____2 or more times/ day ***If your response is "Never", advance to question 18.***

17. Each time you used **flaxseed oil**, how much did you have?

_____Less than 1 teaspoon _____1-2 teaspoons

_____3 teaspoons (1 tablespoon) _____More than 1 tablespoon

18. In the past 6 months, about how often did you eat **chia seeds**?

_____ Never _____ Less than 1 time/ month ____1 time/ month

_____2-3 times/ month _____ 1 time/ week ____2 times/ week

_____3-4 times/ week _____ 5-6 times/ week ____1 time/ day

_____2 or more times/ day ***If your response is "Never", advance to question 20.***

19. Each time you ate **chia seeds**, about how much did you eat?

_____Less than 1 teaspoon _____1-2 teaspoons

_____3 teaspoons (1 tablespoon) _____More than 1 tablespoon

20. In the past 6 months, about how often did you use **cod liver oil**?

_____ Never _____ Less than 1 time/ month ____1 time/ month

_____2-3 times/ month _____ 1 time/ week ____2 times/ week

_____3-4 times/ week _____ 5-6 times/ week ____1 time/ day

_____2 or more times/ day ***If your response is "Never", advance to question 22.***

21. Each time you used **cod liver oil**, how much did you have?

____Less than 1 teaspoon ____1-2 teaspoons

____3 teaspoons (1 tablespoon) ____More than 1 tablespoon

22. In the past 6 months, have you used an **omega-3 fatty acids or fish oil supplement** at least once each week?

____No ***(YOU ARE FINISHED WITH THIS QUESTIONNAIRE)***

____Yes

Please write the name of the supplement below:____

23. Was the **omega-3 or fish oil supplement** provided by the athletic department?

____Yes, my physician wrote a prescription

____No, I purchased the supplement on my own

24. Is the **omega-3 or fish oil supplement** in pill/ capsule form?

____No

____Yes- How much did you take?

____1 pill or capsule each week ____1 pill or capsule each day

____2 pills or capsules each week ____2 pills or capsules each day

____3-4 pills or capsules each week ____3-4 pills or capsules each day

____5-6 pills or capsules each week ____5 or more pills or capsules each day

25. Is the **omega-3 or fish oil supplement** in liquid form?

____No

____Yes- How much did you take?

____ Less than 1 tablespoon/ week ____1 tablespoon/ day

____1 tablespoon/ week ____2 tablespoons/ day

____2 tablespoons/ week ____3-4 tablespoons/ day

____3-4 tablespoons/ week ____5 tablespoons/ day

____5-6 tablespoons/ week

26. Please record the dosage of the **omega-3 or fish oil supplement** you take, if you know it:

____ Don't know dosage

Pills/ capsules: __________mg per pill/ capsule

Liquid: __________mg per tablespoon

Appendix B) Sample Fatty Acid Analysis Report

FULL FATTY ACID PROFILE REPORT

NAME: Peter Ritz
DOB: 11/06/1992
ID: PRItz

COLLECTION DATE: 09/24/2018
RESULT DATE: 10/02/2018
PROVIDER:
ACCOUNT: Virginia Tech-NCAA Athlete Screen

Dried Blood Spot Fatty Acid Profile

Fatty Acid Group	Total	Percentile Rank	Reference Range*
Omega-3 Fatty Acids	**6.65%**	**63rd**	**2.92-13.29%**
Omega 3 Index	6.74%	64th	2.90-12.90%
Alpha-Linolenic (18:3n3)	0.54%		
Eicosapentaenoic (EPA, 20:5n3)	1.17%		
Docosapentaenoic-n3 (22:5n3)	1.35%		
Docosahexaenoic (DHA, 22:6n3)	3.59%		
Omega-6 Fatty Acids	**35.57%**	**26th**	**26.35-45.15%**
Linoleic (18:2n6)	23.74%		
Gamma-Linolenic (18:3n6)	0.21%		
Eicosadienoic (20:2n6)	0.21%		
Dihomo-γ-linolenic (20:3n6)	1.77%		
Arachidonic (AA, 20:4n6)	8.29%		
Docosatetraenoic (22:4n6)	1.01%		
Docosapentaenoic-n6 (22:5n6)	0.34%		
cis-Monounsaturated Fatty Acids	**21.78%**	**56th**	**15.65-32.26%**
Palmitoleic (16:1n7)	0.67%		
Oleic (18:1n9)	20.57%		
Eicosenoic (20:1n9)	0.20%		
Nervonic (24:1n9)	0.34%		
Saturated Fatty Acids	**35.18%**	**86th**	**29.52-37.74%**
Myristic (14:0)	0.71%		
Palmitic (16:0)	21.95%		
Stearic (18:0)	11.52%		
Arachidic (20:0)	0.13%		
Behenic (22:0)	0.36%		
Lignoceric (24:0)	0.51%		
Trans Fatty Acids	**0.82%**	**21st**	**0.35-2.69%**
Trans Palmitoleic (16:1n7t)	0.11%		
Trans Oleic (18:1t)	0.24%		
Trans Linoleic (18:2n6t)	0.47%		
Trans Fat Index	0.71%	20th	0.30-2.42%
Ratios			
AA:EPA	7.1:1	28th	1.4 – 52.6
Omega-6:Omega-3	5.4:1	31st	2.3 – 14.5

Appendix C) Blood Portion Consent Form

Consent Document

Department of Human Nutrition, Foods, and Exercise
Virginia Tech

TITLE: **College Athletes' Omega-3 Fatty Acid Profile**

INVESTIGATORS: Michelle Rockwell, Peter Ritz, Jennie Zabinsky, Alexandra Bechard, Janelle Labiaga, Alyson Onyon, Rachel Steinbach, Valisa Hedrick, Samantha Kostelnik, Nikki Jupe

PURPOSE

We are interested in learning more about athletes' blood fatty acid levels and how these compare to dietary fatty acid intake.

SUBJECTS

Up to a total of 450 NCAA Division 1 student-athletes from nine different universities who volunteer to complete the fatty acid intake questionnaire will be offered the opportunity to participate in a finger stick fatty acid blood analysis.

PROCEDURES

If you agree to participate, you will choose which finger (right or left index or ring finger) will be used for a finger stick. Your hand will be gently massaged and then the side of the chosen finger will be cleaned with an alcohol pad. After the area dries, a lancet (tool used to prick fingers) will be applied to the chosen finger to draw a single drop of blood. This blood will be withdrawn onto a cardboard card that has been pre-treated with special preservative. We will cover your finger with a band-aid. The card will be stored in a sealed envelope and mailed to a laboratory where it will be analyzed along with other samples for content of specific fatty acids, such as omega 3 fatty acids. The laboratory analyzing the samples (Omega Quant, South Dakota, U.S.) will discard of your sample two weeks following analysis.

In the event a researcher or other staff person is inadvertently exposed to your blood, your blood will be tested for the presence of HIV, the Hepatitis B Virus, and the Hepatitis C Virus. There will not be any cost to you for this test. The research team will follow proper procedures for testing and reporting as outlined by Oregon State Law, which includes sending the sample to a certified laboratory. Please note that, should your blood require testing, you will be informed of your test results and provided with the opportunity to receive appropriate and timely counseling. In addition, positive test results will be sent to the local health department.

SUMMARY OF SUBJECT RESPONSIBILITIES

- Choose finger for stick.
- Remain still during fingerstick.

RISKS OF PARTICIPATION

- Risks of participation include pain or discomfort when the lancet is inserted and/or bruising at the site where the lancet is inserted. A small scar may remain on the finger for a few weeks. The risk of developing an infection at the site of the lancet insertion is less than 1/1000.

COMPENSATION

- There will be no compensation for your participation.

BENEFITS OF PARTICIPATION
Your participation will provide you with:

- A sheet showing information about your blood levels of different fatty acids.

CONFIDENTIALITY
The data from this study will be kept strictly confidential. No identifiable data will be released to anyone but those working on the project. Subject numbers without anything to identify your name will identify data.

FREEDOM TO WITHDRAW
You are free to withdraw from the study at any time for any reason. Simply inform the researchers of your intention to cease participation.

INJURY DURING PARTICIPATION
Neither the researchers nor the university have money aside to pay for medical treatment that would be necessary if injured as a result of your participation in this study. Any expenses that you incur including emergencies and long-term expenses would be your own responsibility. You should consider this limitation before you consider participating in this study.

APPROVAL OF RESEARCH
This research has been authorized, as required, by the Institutional Review Board for Research Involving Human Subjects at Virginia Tech. Several other universities, including University of Oregon, are working with Virginia Tech to collect data. You will receive a copy of this form to take with you.

SUBJECT PERMISSION
I have read the informed consent and I have had all my questions answered to my satisfaction. I hereby give my voluntary consent to be a participant in this research study. I understand that I may withdraw from the study at any time.

If you have any questions about the study, you may contact:

- Michelle Rockwell, Department of Human Nutrition, Foods, and Exercise. (540) 231-9572
- Nikki Jupe, University of Oregon. (541) 346-4481

If you have any questions about your rights as a participant, you may contact:

- Institutional Review Board, Office of the Vice President for Research and Innovation, Virginia Tech: (540) 231-3732
- Research Compliance Services, 5237 University of Oregon: (541) 346-2510

Name of Subject (please print)____

Signature of Subject _______________________________________ Date _________