IMPROVED PHYSIOLOGICAL MARKERS OF OMEGA-3 STATUS AND COMPLIANCE WITH OMEGA-3 SUPPLEMENTATION IN DIVISION I TRACK & FIELD AND CROSS-COUNTRY ATHLETES

By

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Submitted in partial fulfillment of the
requirements for Departmental Honors in
the Department of Nutritional Sciences
Texas Christian University
Fort Worth, Texas

May 2, 2022

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ABSTRACT

Background: On average, Americans have some of the lowest omega-3 (N3) polyunsaturated fatty acid (PUFA) profiles in the world. Athletes have an increased risk for N3 PUFA deficiency because of their increased energy requirements. As a result, N3 supplementation has shown promise as an ergogenic aid in athletes to help increase performance and enhance recovery.

Objective: The purpose of this study was three-fold. We wanted to determine: 1) if supplementation with Enhanced Recovery™ (ER), would increase N3 index (N3I), AA:EPA ratio, and N6:3ratio compared to a control (CON) ; 2) whether N3 status is related to sport and distance; 3) if supplementation with ER improves palatability, likeability, and compliance compared to CON.

Methods: In this randomized controlled crossover study, two N3 supplements (20g protein/serving each; ER=1.6gN3/serving vs CON=1.66gN3/serving) in twenty-five (male=15; female=10) NCAA Division I track and cross-country athletes (NCAA DI TF-XC) were tested. The entire study lasted 121 days. Participants completed a baseline visit, visits every two weeks (V#1-4) for the 45-day supplementation period and a 33-36-day washout period. After the washout period, participants switched supplementation groups where the same protocol ensued. At baseline, participants completed a food frequency questionnaire and recorded the frequency of consumption of N3-rich foods. At each visit, fasting blood samples were taken, and participants submitted supplement compliance forms. At the end of the study, visual analog scales and an exit survey were collected regarding each treatment.

Results: Results show that N3 profile improves within six weeks of supplementation. The increase in N3 index, and the improvement of N6:3 and AA:EPA ratios were statistically significant. There were no significant changes between supplementation groups, but there were significant changes across time. Compliance was not affected by ER or CON, but males had increased compliance versus females [(males=90.0±17.0%; females=76.5±21.0%) p=0.040]. Likeability, ease of consumption, and preference favored the ER supplementation.

Conclusion: Supplementation, along with dietary consumption of N3-rich foods, increase N3 status in athletes. Consistent compliance with any supplement may depend on palatability, likeability, and ease of consumption. These factors can be determined by pre-season supplement taste tests and freedom of athletes to choose their own form of supplementation.

TABLE OF CONTENTS

CHAPTER I

INTRODUCTION

Omega-3 (N3) polyunsaturated fatty acids (PUFAs) are necessary for proper physiological functioning of the human body. They are an integral part of every human cell plasma membrane, and research has associated N3 PUFAs with fetal development, cardiovascular function, and neurological development and disease.1 Omega-3 PUFAs are known as essential fatty acids (FAs) as they are not readily created *de novo* in the human body, meaning that they must be consumed from dietary sources in substantial amounts. However, global analyses reveal that the N3 PUFA profile among Americans is suboptimal across age groups.[2] It is estimated that the average American consumes 150-400 mg below the current recommendations for eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), two essential N3 PUFAs, per day.[3] This is a direct effect of the evolving western diet, which is now characterized by excessive consumption of saturated fatty acids (SFAs) and omega-6 (N6) PUFA and insufficient consumption of N3s. This imbalance between N6:3 intake and SFA:N3 intake has proven to have detrimental effects on human health, with increases in chronic inflammatory diseases such as cardiovascular disease, rheumatoid arthritis, Alzheimer's disease, obesity, and diabetes.[4] Several experimental studies have shown that an increase in dietary intake of N3 FAs and improvement of N6:3 ratio and N3 index (N3I) could modulate the immune and inflammatory response.[1,3,4]

Table 1. Types of Fatty Acids Along with their Respective Roles, Major Dietary Sources and RDA

	Saturation and Structure *(if applicable)*	**Roles**	**Major Dietary Sources**	**RDA**
N3 PUFA	1st double bond on carbon 3	Anti-inflammatory	Chia seeds, Flaxseeds, Fatty fish	
ALA	(18:3) 18 carbon fatty acid with 3 double bonds		Plant oils: flaxseed, soybean oil, canola oil	1.1 g/day for women 1.6 g/day for men

DHA	22:6, n-3	Regulator of immune system and inflammation. High in retina, brain, and sperm cells.	Fatty fish	250-300mg combined mg of DHA and EPA
EPA	20:5, n:3	Regulator of immune system and inflammation	Fatty fish	250-300mg combined mg of DHA and EPA
N6 PUFA	1st double bond on carbon 6	Healthy inflammation	Soybeans, corn, meat, poultry, eggs	12g for females 17g for males
MUFA	1 double bond	Lower LDL Raise HDL	Avocados, canola, peanut, and olive oil, nuts	10-20% of daily calories
SFA	No double bonds	Raise LDL	Butter and Lard	Less than 10% of daily calories

ALA, alpha linolenic acid; *DHA*, docosahexaenoic acid; *EPA*, eicosapentaenoic acid; *MUFA*, monounsaturated fatty acid*; N3 PUFA*, omega 3 polyunsaturated fatty acid; *N6 PUFA*, omega-6 polyunsaturated fatty acid;*SFA*, saturated fatty acid

Omega-3 PUFAs are crucial for athletes, who face an increased risk of deficiency due to their increased metabolic requirements, oxidative stress, need for muscle repair, and neurological strain.[5] Furthermore, due to the importance of N3 PUFAs in neurological development, adequate consumption is vital for the proper neurological function of young athletes until their mid-20s. However, student athletes on budgets with limited time, knowledge, and dietary preferences are unlikely to consume enough N3 FAs. While it is preferred that N3 PUFAs be obtained from dietary sources, this is not always realistic, warranting supplementation. In January 2019, the NCAA amended Bylaw 16.5.2.7, permitting Universities to provide N3 supplementation for student athletes.[6] With the supplement industry being grossly unregulated, it is imperative that any supplement that is provided to student athletes is safe, effective, palatable, and well researched. Over the past 20 years, the dietary supplement industry has been

gaining attention from both the scientific research and consumer communities; thus, sparking an interest in supplementation in a performance setting.[7]

Literature suggests that optimal FA profiles are not only beneficial for the overall health and physiological functioning for the athletes but can function as an ergogenic aid as well.[3] Sufficient N3I may enhance performance and recovery while promoting injury prevention in athletes.[8] However, a gap in literature exists as this has only been examined in populations of highly trained endurance athletes. Few studies examine college athletes, and no studies examine N3I in NCAA DI TF-XC. Furthermore, there is no consensus regarding the appropriate dosage and length of time of supplementation that is required to improve the FA profile.

The purpose of this study was three-fold. We wanted to determine if supplementation with ER, would increase N3I levels in the body and determine whether N3I levels were related to sport, and distance covered during a competitive season. We also wanted to determine if supplementation with ER would have improved palatability and likeability compared to control. Therefore, we hypothesized that supplementation with ER would increase circulation of favorable dietary FAs in the body (as measured by N3I, N6:3, and arachidonic acid (AA) to EPA ratio (AA:EPA) and improve palatability and likeability in adult NCAA DI TF-XC athletes. We also hypothesized that N3I would be negatively related to distance throughout a competitive season, whereas sports that cover less distance will have significantly higher N3I levels compared to moderate and longer distance-based sports.

CHAPTER II

LITERATURE REVIEW

Eicosapentaenoic Acid and Docosahexaenoic Acid

Alpha-linolenic acid (ALA) is an essential FA that is converted to EPA and DHA in the liver. However, this process is very limited and does not efficiently produce EPA and DHA from dietary sources. Therefore, consuming EPA and DHA directly from the diet or via supplementation are the only viable ways to increase these FAs in the diet.[14] There are no official recommended daily allowance guidelines for EPA and DHA. However, health professionals have agreed that a protective EPA:DHA ratio is at least 4:1[15]. Guidelines established in literature recommend that individuals consume between 250 and 500 mg combined EPA and DHA per day.[16] Unfortunately, the average consumption of EPA and DHA from dietary sources is only around 90 mg/day from dietary sources in adults in the United States (US) .[17]

There are numerous health benefits to consuming EPA and DHA. Eicosapentaenoic acid and DHA are associated with a multitude of physiological outcomes such as fetal development, cardiovascular function, Alzheimer's disease, and healthy aging.[14,17] EPA and DHA are also the precursors of several metabolites that are potent lipid mediators, considered by many investigators to be beneficial in the prevention or treatment of several diseases.[13]Docosahexaenoic acid levels are especially high in the brain, retina, and sperm.[16] Studies have shown that EPA and DHA may affect many aspects of immune and cardiovascular function including inflammation, peripheral artery disease, major coronary events, and anticoagulation.[8]As a result, having sufficient levels of EPA and DHA have been linked to promising results in disease prevention, weight management, and cognitive function.[17]

In a study using mice models, EPA and DHA supplementation significantly decreased hepatic lipid deposition, body weight, serum lipid profile, and inflammatory directions compared to mice fed a high fat diet. Specifically, a DHA:EPA ratio of 1:2 mitigated inflammatory risk factors more efficiently than ratios of 2:1 and 1:1.[18] This indicates promise for the potential use of EPA+DHA supplements to improve health by reducing inflammation and that a DHA:EPA ratio of 4:1 is likely the most protective.

Consumption of EPA and DHA diet and supplement sources may offer numerous benefits for athletes as well. Multiple studies have reported the effects of EPA and DHA on delayed onset muscle soreness, range of motion, and swelling after excessive muscular exercise[15,16,19]. While the results of these

studies have been controversial, it has been reported that consumption of EPA and DHA takes at least 30 days of ingestion to see results.[18] Thus, shorter duration studies (three weeks or less) with high or low dosage supplementation will likely not produce results indicating any difference in performance, enhanced recovery, or reduction of inflammatory markers. Instead, research shows that a lower dosage for a longer period of time may decrease delayed onset muscle soreness and enables athletes to maintain their range of motion after weightlifting.[20] There is less conclusive evidence regarding swelling reduction. However, studies have indicated that EPA and DHA ingestion can inhibit proinflammatory cytokines after resistance training and running.[20]

In one double blind random crossover study with a similar six-week EPA+DHA intervention period with an eight-week washout period, supplementation significantly increased supplementation significantly increased blood FA levels and decreased the resting levels of inflammatory biomarkers. In the male population, these results did not contribute to a decrease in oxidative stress after aerobic, moderate duration exercise.[15] Much data reiterates these conclusions that supplementation increases blood levels of FAs and decreases proinflammatory cytokines.[7,19,20]

Supplementation of Omega-3

According to a 2011-2012 National Health and Nutrition Examination Survey (NHANES), most people consume appropriate amounts of N3 in the form of ALA. The National Academy of Medicine has established a Recommended Daily Allowance (RDA) of the average daily level of intake required to meet nutrient requirements. The RDA for total N3 as ALA is 1.1 g/day for adult females and 1.6 g/day for adult males. The NHANES survey found that females averaged 1.59 g/day and males averaged 2.06 g/day. However, there is not much consensus for the intake of EPA and DHA. Many health organizations recommend at least 250-500 mg/day of combined EPA and DHA, and the American Heart Association recommends one to two seafood meals per week to meet these requirements.[15] However, in more recent literature, it has been found that this recommendation is insufficient to achieve an N3I $\geq$ 8% without supplementation.[2] It is estimated that the average American consumes 150 mg below the current recommendations per day, even though the recommendations are insufficient to adequately improve the fatty acid profile. As a result, N3 PUFAs seem to be among the most necessary supplements for a large range of populations. Research suggests that these populations include premature infants, elderly with

conditions like sarcopenia, patients with peripheral artery disease or metabolic and inflammatory diseases, and athletes.[2]

Long chain polyunsaturated fat levels are especially important to monitor in athletes because they experience increased inflammation and oxidative stress. Omega-3s are thought to be protective in our target population, due to their ability to regulate inflammation and stress incurred from exercise. Additionally, since the RDA was created for the general healthy population, it is reasonable to assume that athletes require even higher amounts due to increased oxidative stress and muscle repair that they experience. It is also it is reasonable to assume that because their demands are higher, they do not consume adequate amounts of N3 PUFAs either, considering their increased energy needs and metabolic flux.[15] In fact, in studies analyzing dietary habits of athletes, that a substantial number do not meet their macro and micronutrient needs, including EPA and DHA. Studies show most athletes having an N3 intake below 100mg per day. [15] However, research suggests that this deficiency can be reversed easily with supplementation.[12,15]

Omega-3 Index

Omega-3s have been linked to a multitude of critical biological functions in the human body. Low intake of dietary EPA and DHA is thought to be associated with increased inflammatory processes as well as poor fetal development, general cardiovascular health, and risk of the development of Alzheimer's disease. However, sufficient levels of EPA and DHA can be protective, affecting many aspects of human physiology and metabolism, and can impact outcomes related to enhanced performance, recovery, and prevention of illness or injury in athletes.[9] Although major health benefits come from EPA and DHA, studies show that many populations do not obtain sufficient amounts in their diet.[2,15,16,17] Because sufficient levels of EPA and DHA are so important to maintain, they are measured by the N3I, in addition to the EPA:DHA ratio. Studies have shown the N3I is an important risk factor and indicator for death due to disease.[10] An N3I of 8% or above is considered optimal, while an index below 3% indicates a higher risk for disease development. In a study that examined the N3I levels across the globe, adults in the United States were in the lowest category at levels < 4%, on average.[8] One reason why N3I may be low in the US is because EPA and DHA are found in limited food sources and are not often consumed in the western diet. Some sources of EPA and DHA include cold water fish and other marine sources such as krill, algae, and crustaceans.[9]

Research shows that an N3I can be raised to a protective level with supplementation.[11,12,13,15] One study demonstrated that 12 weeks of supplementation of 3g/d of combined EPA and DHA resulted in achievement of N3I of ≥8% in most subjects with low basal EPA and DHA status.[12] It also showed that the participants had increased metabolic rate and fat oxidation during rest and exercise, decreased resting and exercise heart rate, and increased physical function and decreased fasted blood triglycerides. While these are exciting results, this study was conducted on a specific population of a female population with an average age of 66±1years, and BMI measurements indicated that many were overweight.[12] Much more research must be done to reach a consensus on the amount of N3 PUFA to supplement, length of time required for supplementation, and how long it takes for levels to return to baseline. Unfortunately, there is very little data on college aged athletes, particularly track and cross-country runners.

Interestingly, another study showed that just eight weeks of supplementation of 0.94 DHA/d in 105 (F=87; M=17) otherwise healthy vegetarian participants with low basal EPA and DHA status raised the N3I of the majority to ≥8%.[12] Another study showed that combined EPA and DHA supplementation of 2.6g EPA/d and 1.8gDHA/d supplementation improved the N3I in just one month in populations with peripheral arterial disease.[14]

Omega-3s in Athletics

Omega-3s have received much attention in the sports nutrition arena in the context of benefitting performance and recovery in sport. Omega-3s have potential anti-inflammatory and antioxidant activity that may aid in immune function, decrease recovery time, and improve performance in sport. Nutritional strategies such as supplementation with nutrients like N3 PUFAs can result in optimal training gains, enhanced recovery, reduced risk of illness, and a high-level competition performance.[2] Because of this, there is increasing support for the potential use of N3 PUFAs as an ergogenic aid for athletes. We are especially interested in the potential of N3s to enhance the recovery process in athletes. The healing process is governed by FAs of the N3 and N6 series. In order to facilitate healing (or recovery in athletes), FAs have to be present in significant amounts in the affected tissues before the trauma occurs. Because of this, it is extremely important that athletes are able to obtain sufficient levels of N3 PUFAs.[8]

In a study examining college football athletes, about 34% of athletes (n=138) had an N3I considered high risk (<4%), and 66% (n=266) had a risk considered intermediate (4%-8%).[5] None of the athletes had an N3I in the low-risk zone, concerning finding.[8] This is not just an isolated population that has inadequate N3 PUFA status, but a pattern across many NCAA Division I Sports. This concludes that the NCAA football athletes had a low level of EPA and DHA, both vital for biological function. Compared to the general population, these football athletes are more prone to neurological injury and repetitive head impacts, suggesting that because of their low N3 levels, the athletes are more at risk for early cardiovascular disease and neurological damage.[5] Multiple studies demonstrate that supplementation can improve these statistics.

A study by Thielecke et al compares the effect of N3 PUFAs on both athletes and amateurs. In amateurs in which EPA+DHA were administered in doses of 0.6 g and 0.3 over 8 weeks, led to a significant increase in VO_2 max and an improved O_2 uptake.[8] In this same study, 26 competitive soccer players, were supplemented with 4.9 g EPA and 1.4 g DHA per day over four weeks and found that increases in leg strength, sprint speed, explosive power and anaerobic endurance were left unaffected.[8] Studies of less than eight weeks duration in athletes showed no clear picture of the benefits of N3 PUFAs, and only two out of five studies reported beneficial outcomes. However, when supplementation was longer than eight weeks, six out of six studies reported positive changes to N3I levels due to the EPA+DHA supplementations.[8] These findings indicate that athletes need a higher dosage of N3 PUFAs than amateurs in order to see effects on their N3I, and both groups show more consistent results when the supplementation time is longer than eight weeks.

Omega-3 Palatability

Palatability and likability are other aspects of supplementation that affects consumer compliance. It is cited that 50% of people do not take medication as prescribed.[22] In a study examining cancer patients, Schmidt et al found that fish oil capsules and N3 supplement beverages had the same effect of improving N3 blood levels[24]. However, an unrealistic number of pills were required to achieve sufficient N3 blood levels.[24] As a result, supplementation with a beverage may be a viable alternative to supplementation with pills.

According to a study on 582 Canadian athletes, extensive dietary supplement use was found, with 88.4% of participants taking ≥one dietary supplement during the previous six months. Overall, sport

drinks (22.4%), sport bars (14.0%), multivitamins and minerals (13.5%), protein supplements (9.0%) were most frequently reported.[22] If two of the top three dietary supplements (sports drinks and protein supplements) could be combined with N3 PUFAs, compliance would then be increased in N3 supplementation and palatability. Taken together, the literature suggests that N3 supplementation may mediate the inflammatory response and improve recovery in athletes.[18,25,26,28,30] Yet studies focused on EPA+DHA supplementation in DI athletes of various sports are few and far between. Moreover, the study designs are inconsistent, obscuring clear translation and correlation between data sets. Therefore, researchers have not been able to make strong conclusions regarding the optimal dose, supplementation period, and conceivable benefits of EPA and DHA on athletic performance factors.[15] Omega-3 levels in sport have been studied, but very few have proven the direct effects of N3 on NCAA DI TF-XC athletes. Furthermore, since athletes of different sports have varying energy and nutrient requirements, it is important to determine an appropriate and/or effective N3 dose, as well as the appropriate length of supplementation time for athletes of different sports.

CHAPTER III

METHODS

Study Design

This study, a longitudinal randomized placebo-controlled parallel crossover trial, was designed to test N3I response to ER versus CON. In a series of eight visits that took place in the TCU Sports Medicine Walsh Athletic Training Facility, eight fasting blood samples were taken via finger prick method over 121 days.

Participants were block-randomized into intervention and control groups. The intervention group was given an ER beverage (liquid N3 with protein) and the CON was given N3 PUFA soft gel capsules and a protein beverage with equivalent N3 dosage (Tables 2 and 3). Participants were assigned to their test group for 45 days, after which a 33-36-day washout period was enforced. During the final 45 days, participants were assigned to the other test group.

Table 2. Enhanced Recovery Nutritional Facts

Enhanced Recovery™

(1 Enhanced Recovery™ tetra pak per day)

Product	Calories	Total Omega 3s (mg)	DHA (mg)	EPA (mg)	ALA (mg)	Other Omega 3s (mg)	Protein (g)
Enhanced Recovery™	250	1,600	820	550	230	0	20
Total:	*250*	*1,600*	*820*	*550*	*230*	*0*	*20*

Table 3. Control Regimen Nutritional Facts

Control

(2 capsules Nordic Naturals DHA Xtra + 1 bottle (20 oz) BiPro protein water)

Product	Calories	Total Omega 3s (mg)	DHA (mg)	EPA (mg)	ALA (mg)	Other Omega 3s (mg)	Protein (g)
Nordic Naturals – DHA Xtra	20	1,660	960	410	0	290	0
BiPro Protein Water	90	0	0	0	0	0	20
Total:	*110*	*1,660*	*960*	*410*	*0*	*290*	*20*

Participants

Thirty-one male and female TCU track and field and cross-country athletes (n=20 male, n=11 female) participated in this study. Students were recruited via email and announcement. All involvement was volunteer based. Participants arrived at each visit having fasted from all food, supplements, medication, and refrained from vigorous exercise for at least 12 hours. Informed consent was given and disclosed with participants. Inclusion criteria included the participants must be 18-25 years of age, a student at TCU, a track & field or cross-country athlete at TCU, and willing to maintain their current training and nutrition regimen through the course of the study. Exclusion criteria included the following recent altitude training or use of a hypobaric chamber (within the last 6 months), the use of other supplements or ergogenic aids (excluding contraindicated supplements), current or recent pregnancy, breastfeeding, or plans to become pregnant before the study ends, blood transfusions or blood donation in the past 16 weeks, chronic diseases such as cardiovascular disease, hepatic disease, renal disease, diabetes, or having a pacemaker.

Study Protocol

Table 4. Timeline of Visits and Measurements

	Visit 1 Day 0-2	Visits 2-3 Days 15-31	Visit 4 Days 43-45	Washout Days 43-79	Visit 5 Day 79	Visits 6-7 Days 94-108	Visit 8 Days 120-122
Time	30 min	15 min	15 min	15 min	30 min	15 min	15 min
COVID Screening	X	X	X	X	X	X	X
OmegaQuant ™ blood sample	X	X	X	X	X	X	X
Skulpt® body composition	X				X		
Height	X						
Weight	X	X	X	X	X	X	X
Compliance Log Given	X	X	X		X	X	X
Compliance Log Collected		X	X	X		X	

Supplements Given	X	X	X		X	X	
Intake Form	X	X	X	X	X	X	X
Food Frequency Questionnaire	X						
Palatability and Likability VAS			X				X
Exit Survey							X

min, minutes; *VAS*, Visual Analog Scale; *Visit 1 (V#1, Day X); X*, protocol included in visit

Pre-Study

Preceding the first visit, all potential participants received a screening and demographic information form. This form focused on the inclusion and exclusion criteria. If athletes met the screening criteria, they were admitted into the study and booked visit 1 (V#1) on days 0-2. To increase compliance, participants received bi-weekly reminders through email and text notifying them about upcoming study visits and pre-data collection procedures.

Visit 1 (V#1, Day 0-2)

Upon arrival, all participants were screened for COVID-19, which included a temperature check, and questions about having COVID symptoms. Next, participants completed a validated Food Frequency Questionnaire (FFQ). The FFQ included foods that contained N3 PUFAs (ex. fish, walnuts, and flax) to check for confounding variables that may alter N3I results. Participants also completed an intake form which included a COVID screening, and questions about supplemental intake and menstrual cycle for females.

Baseline measurements of height, weight, and body composition (Skulpt™ Aim, Skulpt™ Inc., San Francisco, California) were collected. Women were asked the start date of their last menstrual cycle. After anthropometrics, a capillary blood sample (six-nine drops) was taken via finger stick for OmegaQuant™ (OmegaQuant LLC, Sioux Falls, South Dakota) analysis. Finally, each participant was provided with a 14 to 16-day supply of either ER or Nordic Naturals DHA supplements and BiPro beverages. Participants were given a compliance log to track their supplement intake, consumption of N3

foods, and distance ran during practice/competition per day. Honesty in filling out the compliance log was emphasized. Additionally, each participant was given an at-home capillary blood collection kit (N3I Complete Test, OmegaQuant LLC, Sioux Falls, South Dakota) and was instructed on how to use it if they became ill or had to quarantine due to a COVID-19 shutdown.

Visits 2-3 (V#2-3, Days 15-31)

The second (V#2) and third (V#3) visits occurred on days 15-17 and 29-31, respectively. Participants were reminded to bring their completed compliance logs and any unused supplements. Research personnel collected the compliance logs upon arrival, where the same testing procedures (except body composition and FFQ) from V#1 were followed (COVID-19 screening, anthropometrics, capillary blood sample) At the end of each visit, the athletes were given a new compliance log and another 14- to 16-day supply of supplements.

Visit 4 (V#4, Day 43-45)

The aforementioned study procedures were followed at V#4. Additionally, participants were given a visual analog scale (VAS) to assess palatability and likeability of the supplement in which they were assigned.

Washout Period

Following V#4, the researchers instituted a 33-36-day washout period, which occurred on days 43-79. During this time, no supplement was administered to the participants, but consumption of any foods containing N3 PUFAs and mileage covered were recorded by each participant using the washout log. This washout log does not include the amount of supplement taken, as no supplements were given during this time.

Visits 5-7 (V#5-7, Days 78-108)

After the washout period, participants were reassigned to the opposite research group where the exact same procedures for visits 1-3 were followed for visits 5-7. On V#5, the FFQ was given again, to assess participant's dietary intake after the washout period.

Visit 8 (V#8, Day 120-122)

On the eighth visit, the same protocol was followed as visits 6-7. During this visit, participants also completed a palatability and likeability VAS, the same form as V#4. Additionally, participants completed an exit survey. The exit survey compared the two regimens and asked which the participant liked better and which they thought was more effective.

Post-Study

Thirty days after completion of the study, participants were given the opportunity to pick up a hard copy of their personal data which included their OmegaQuant^{TM} results, anthropometrics, and compliance log data that was collected. If the participant did not want this, their personal results were shredded.

Statistical Analyses

The current study design utilizes one independent variable with two levels: The ER treatment and the CON treatment. There are five dependent variables: compliance, palatability ratings, N3I, N6:3 ratio, and AA:EPA ratio. Descriptive measures include height, weight, body composition, age, sex, and other data collected from the questionnaires. In order to assess the potential impact of the treatments on the dependent variables, the following statistical analyses was used: compliance and ratings of palatability was analyzed using paired samples t-test between conditions; N3I, N6:3 ratio, and AA:EPA ratio was analyzed using an ANOVA with repeated measures; Pearson product-moment correlations were used to determine a relationship between distance covered prior to each visit and N3I, N6:3 ratio, and AA:EPA ratio. Descriptive statistics were also collected and used to describe our population and their practices, but no inferential statistics were ran. Covariates were used as appropriate based on theoretical reasoning for their inclusion.

Following an intent-to-treat approach, the mixed model analysis relied on data from all participants who had N3I levels at baseline, regardless of whether or not they completed the study. All data was analyzed using SPSS Statistics software (IBM, Version 26.0). The results were utilized to discern any significant differences or relationships among variables. Incremental area under the curve (iAUC) was calculated, N6:3 ratio, and AA:EPA ratio, and compared using an ANOVA. Statistical significance was set at $p<0.05$. Data are presented as mean ± SE unless specified otherwise.

CHAPTER IV

RESULTS

Participant Characteristics

A total of 25 Division I track and cross-country student athletes completed all study visits. The mean age was 20.1± 1.9. The majority of participants were sprinters and jumpers (64%). Participant characteristics at screening are shown in **Table 5**. There was no difference in BF% between baseline and post treatment measurements.

Table 5. Participant Characteristics at Baseline

	Total (n=25)	**Males (n=15)**	**Females (n=10)**
Age (y)	20.1±1.9	20.3±2.1	19.7±1.7
Height (cm)	176.4±10.6	181.9±8.6	168.2±7.6
Weight (kg)	71.4±16.0	77.8±16.3	61.9±9.8
BMI (kg/m^2)	22.7±2.9	23.4±3.3	21.7±2.1
Body Fat (%)	17.4±7.7	14.6±7.4	21.7±6.2
Fat mass (kg)	12.7±8.0	12.1±9.6	13.6±5.3
Fat-free mass (kg)	58.8±12.5	65.7±10.0	48.3±7.7
Sport			
Sprinter	36%	40%	30%
Jumper	28%	20%	40%
Thrower	12%	13%	10%
Cross-Country	24%	27%	20%
Race			
White	40%	40%	40%
African American	52%	47%	60%

Other	8%	13%	0%
Education Classification			
Freshman	32%	34%	30%
Sophomore	28%	20%	40%
Junior	8%	13%	0%
Senior	20%	20%	20%
5th Year (Graduate or Senior)	12%	13%	10%

Results are presented as Mean±SD.
BMI, body mass index; *cm*, centimeter; *kg*, kilogram; *m*, meter; *y*, year.

Compliance, Palatability, Likeability

Overall compliance was greater than 80%. Supplement compliance was slightly less for the ER™ intervention (84±24%) than the control intervention (87±15%). Compliance was higher for males (90±17.0%) than females (76.5±21.0%). Compliance was dependent on likeability and ease of consumption ratings. In addition, supplement compliance was higher during the first treatment (92%) compared to the second treatment (77%). Compliance was lower for each supplement if participants believed that the other was more successful.

Athletes rated both supplements in terms of taste, drinkability, willingness to consume, and ease of consumption on a visual analog scale. There were no differences between ER and CON for these ratings (ns). All conditions were rated higher in athletes who preferred the ER compared to those who preferred the CON, and a greater number of athletes preferred the ER (60.9%) over the CON (39.1%). Participants who preferred the ER and believed that it was a more successful supplement were more compliant with the ER than the CON (p=0.082). The VAS ratings and compliance for the CON were not dependent on supplement preference. However, participants who believed that the CON was an equally or more effective supplement than the ER treatment had a lower compliance with the ER supplement (p=0.066).

Fewer participants believed that the ER was more effective than the CON (21.7% vs. 39/1%). However, 39.1% of the athletes believed that they were equally effective. Interestingly, 65.2% of athletes

reported the ER was easier to consume versus the CON (34.8%) and found it more realistic to incorporate into their daily routine. These athletes were more willing to continue taking the ER after the study compared to those who preferred the CON (75.5±21.2% vs. 45.1±27.4%). Willingness to continue taking the CON was unchanged across preference classifications. But those who believed ER was equally effective as the CON demonstrated a greater willingness to continue taking the ER than the CON (p=0.007).

Figure 1: VAS Ratings by Supplement Preference

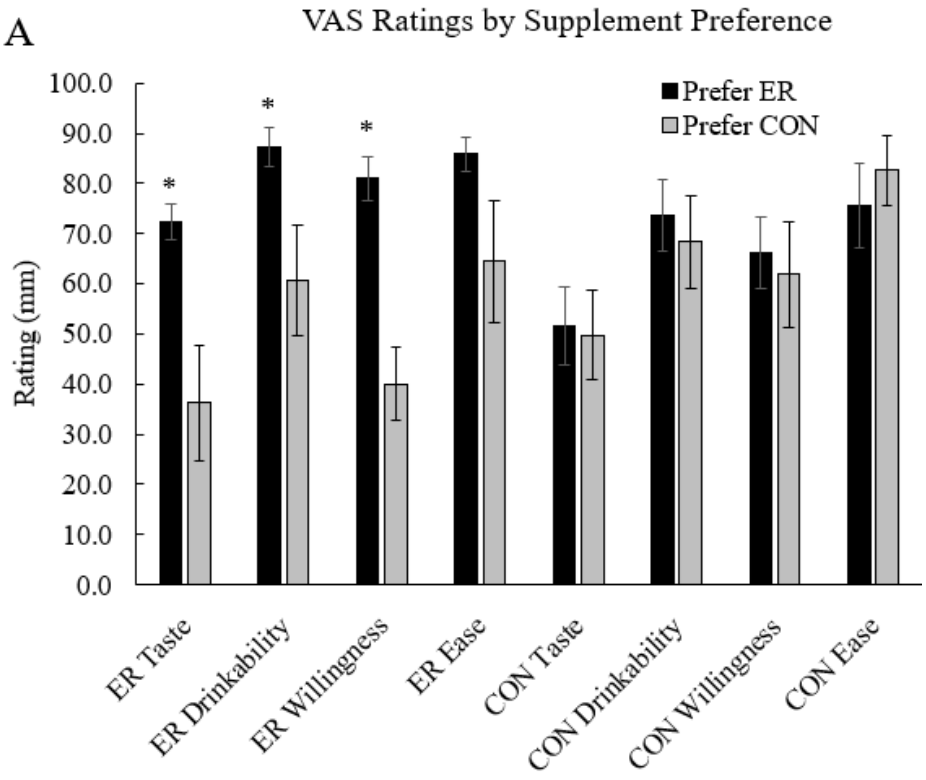
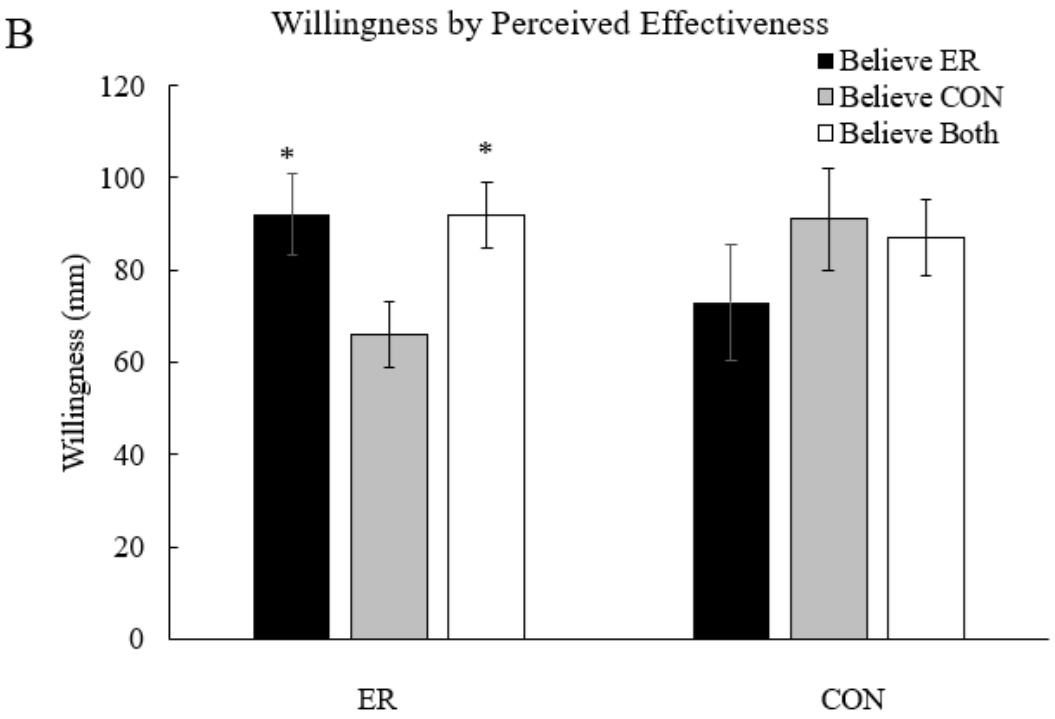


Figure 2: Willingness by Perceived Effectiveness



Omega-3 Index Levels

All of the participants were considered high and intermediate risk. Those that were in the intermediate group at baseline (88%) become low risk by the end of the intervention and those who began in the high-risk (12%) group became intermediate risk.

Omega-3 index levels improved most dramatically from visits one to two, with the highest percent change of 41% for the CON and a 26% change for the ER. This drastic difference in the percent change interventions could be due to the fact that CON had a lower mean at baseline, since by the end of the intervention, the CON and the ER groups had almost identical means.

Figure 3: Mean Omega 3 Index by Treatment (%)

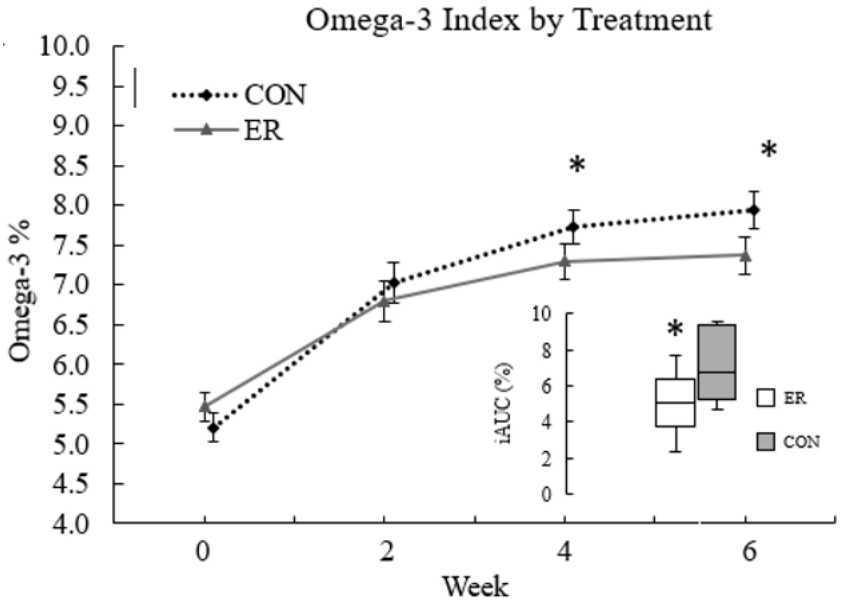
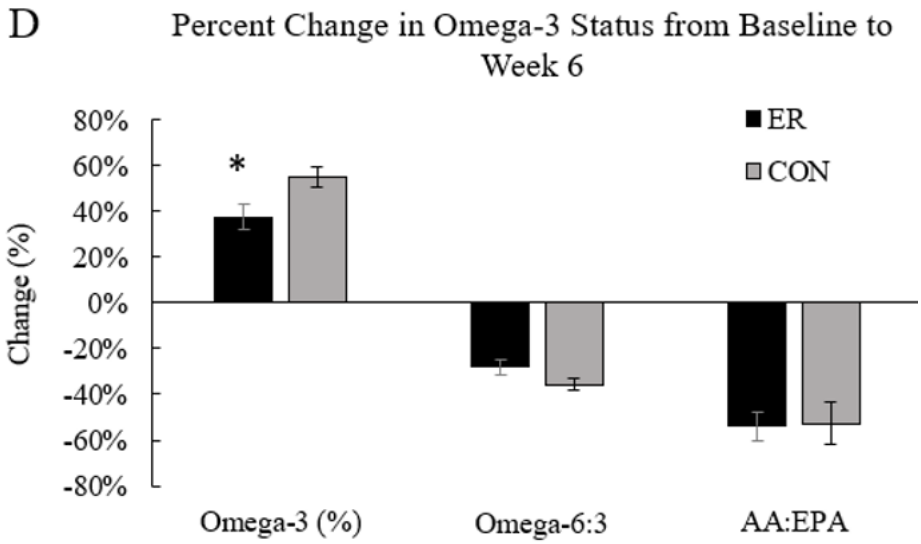


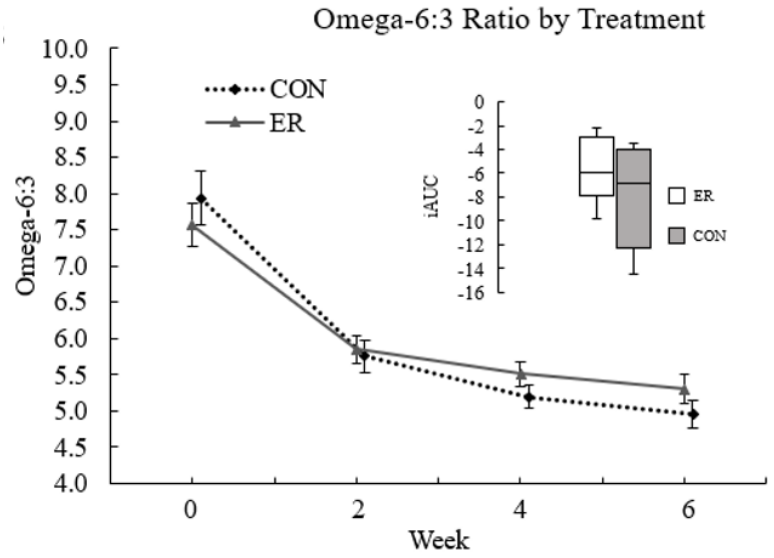
Figure 4: Percent Change in Omega-3 Status from Baseline to Week Six



Omega-6:3 Ratio

The n6:3 ratio changed equally as a function of time across interventions. The change was not dependent on treatment type. While the percent change for the ER was much higher, the mean N6:3 values were very comparable at the end of the study.

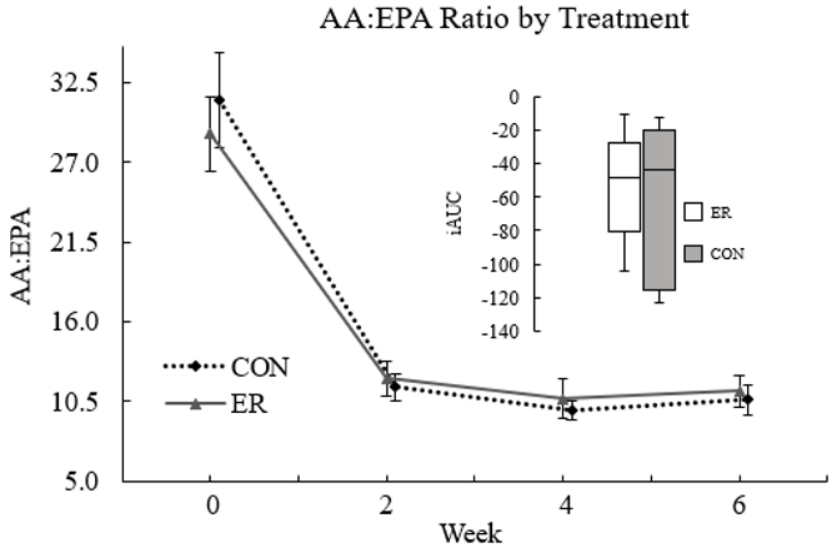
Figure 5: Omega 6:3 Ratio by Treatment



AA:EPA Ratio

The AA:EPA ratio was affected by time and not the intervention. The highest percent change occurred between visit 1 and visit two with a 55% change for the ER and a 51% change for the CON, but the mean ratio did not change after visit two. The ER intervention for AA:EPA ratio overall experienced a larger percent change.

Figure 6: AA:EPA Ratio by Treatment



CHAPTER V

DISCUSSION

The aim of this study was to determine if supplementation with ER, would improve the FA profile in NCAA DI TF-XC athletes during a competitive season. Additionally, we wanted to determine if supplementation with ER would have improved palatability and likeability compared to a CON. We found that the N3 status could be improved within six weeks of N3 supplementation, regardless of supplement type, indicating that short duration N3 supplementation could improve the N3I in collegiate athletes. Moreover, dietary consumption of N3 in conjunction with consistent intake and supplement compliance were the most important factors in improving N3 status. The importance of consuming N3 in a supplement form in conjunction with dietary sources is well supported in literature. We found that while both supplements were equally effective, athletes had a greater preference for ER compared to CON (traditional supplementation) and compliance was driven by likeability and perceived effectiveness. Thus, the results of this study demonstrate that consistent N3 supplementation improves N3 status, over a short period of time when compliance is high.

Interestingly, the present study found that all (100%) of our Division I athlete participants were classified as having either high or intermediate N3I risk prior to supplementation. However, 72% of athletes in our sample achieved a low-risk classification by week six of supplementation. McDonnel et al found that even among those who consumed dietary sources of N3s each week, only those who consumed oral supplements had a sufficient N3I.[13] To our knowledge, research regarding dosage and length of time of supplementation is inconclusive. Lewis et al found that 21 days of N3 supplementation improved N3I, N6:3 ratio, and AA:EPA ratio.[23] Our study revealed that improvements in the N3I were observable beginning in week two (or 14 days) into the study. The N3I continued to improve until week four but plateaued thereafter. McDonnel et. al examines a long-term dosage of combined EPA and DHA for one and a half years.[13] It was reported that consumption of fatty fish with a supplement of at least 400 mg was effective in raising N3I to optimal levels. Raatz et al, on the other hand, used a supplementation protocol of 48 hours and observed N3 absorption in blood samples.[14]

There is also no consensus of the amount of time for FA profiles to decline after abstention of supplementation. McDonnel et al and Raatz et al both used six weeks between crossover to ensure adequate washout. In our study, however, after only 33-36-day supplement abstention during washout, all athletes who achieved a low-risk classification returned to intermediate-risk suggesting the importance of continuous supplementation despite rapid improvements.

Supplement dosage is another factor of N3 research that has not been defined well. A study done by Luo, suggests that high-dose N3 supplementation may be beneficial for mitigating neurological symptoms such as depression.[27] In contrast, a 21-day trial of 375 mg EPA, 230 mg DPA trial revealed no significant change in EPA and DHA. Our study used a 1600 mg intervention protocol to determine N3 status after a six-week supplementation period, which revealed that N3 status was improved in all participants.

The study results also displayed decreases in N6:3 and AA:EPA. However, longer duration and more consistent supplementation may be necessary to raise all participants to a low-risk category. Increases in the N3I were observed for both treatments from week two to week four but plateaued thereafter. Our study observed a final N3I of 7.4-7.9% at week six. At the dosing strategy presented in our study, improvements in N3I similar to other studies of longer duration may be observed in four to six weeks. This suggests that a longer duration of N3 supplementation in Division I athletes is not necessarily required to observe improvements in N3I.

There were no significant differences between treatments, however males showed higher compliance overall in both treatment groups. When evaluating compliance with supplement usage by sex, males are often reported to have a higher compliance rate than women, most notably when consuming protein-centered supplements.[24] In addition, males are more likely to use a drug or supplement if it is considered to be performance enhancing, while females are more likely to use a drug or supplement if it is prescribed specifically to them.[25] Previous studies have found that compliance is driven by likability and ease. In addition to this, palatability also determines the potential effectiveness of a supplement.[26] If the individual does not find what they are consuming to be palatable, they will not continue to consume it,

and will therefore hinder compliance. Since it is difficult to compare a beverage to a pill in regard to palatability, we used the term "likeability" and combined the N3 pills with a protein beverage that is regularly provided to the athletes in the CON for an adequate comparison. Although our athletes had more familiarity with the CON, VAS ratings were similar.

The current study showed that more athletes preferred ER, and thought it was easier and more realistic to consistently consume. Moreover, VAS ratings of taste, drinkability, and willingness to consume were greater for ER in those who preferred ER, an effect not observed for the CON. Compliance to ER was also higher in those who preferred ER whereas compliance to the CON was similar regardless of preference. Interestingly, those that preferred ER were equally as compliant to the CON, possibly due to familiarity.[27] This, however, was not observed for the CON and may be due to suspicion of new dietary practices. To boost adherence, athletes should be offered a variety of choices for N3 supplementation, and taste tests/trial periods may help adequately assess individual preference and avoid non-compliance.

Limitations

This study had a few limitations that warrant discussion. Firstly, the study did not control for N3 consumption, but instead relied on compliance logs, which were monitored through supplement counts and research staff. Secondly, there was an order effect for compliance, in which the athletes were more compliant earlier in the study compared to later in the competitive season. The order effect should cause for extra precautions to arise later in future studies, to keep compliance high. Thirdly, our study also did not utilize a true control. The study only sought to examine if this new type of N3 supplement (ER) was comparable to the current "standard of care," regular supplementation. Fourthly, double blinding and single blinding protocols were not implemented due to the inherent difference of the supplement protocols, as one was a completely liquid substance and the other was a liquid and pills. Lastly, a limitation in this study is the long duration. As the competitive season progressed, compliance waned, which could have affected the data. However, the athletes received biweekly automated text messages and email reminders to consume supplements and uphold testing appointments.

CONCLUSIONS

Supplementation from both the ER and CON improved N3 status in a short period of time but should also be supported with consistent compliance and an N3 foods-first approach. Compliance may depend on likability, ease of consumption, personal preference, and perceived effectiveness. As the D1 season progresses, compliance may be lowered by additional barriers. Athletes should be given the options between modalities, to increase compliance through likeability, ease, and preference.

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