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ORIGINAL ARTICLE

A Low Omega-3 Index and High AA/EPA Ratio in American College Football Players are Both Improved Following 5 Weeks of DHA-Rich Algae Oil Supplementation

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Abstract

Purpose Many athletes are deficient in long chain omega-3 polyunsaturated fatty acids (LC n-3 PUFA). A consequent low Omega-3 Index (O3I) and high arachidonic acid/eicosapentaenoic acid (AA/EPA) ratio increase cardiovascular disease risk and inflammation. Algae oil is a plant-based, sustainable source of LC n-3 PUFA, suitable for vegans and vegetarians. Effects of algae oil supplementation on whole blood fatty acids among athletes has not been previously reported. This study evaluated the effects of 5 weeks of DHA-rich algae oil supplementation on the whole blood fatty acid profile, O3I and AA/EPA ratio of omnivorous Division I American College Football (ACF) players. Methods: Data, including a spot blood sample, were collected at baseline for all participants ($n = 47$), then for a subset of players ($n = 22$) following a 5-week control period (usual diet) and 5 weeks of algae oil supplementation (usual diet + 1575 mg docosahexaenoic acid (DHA) + eicosapentaenoic acid (EPA) 5 days/week; average 1125 mg/day). Results: Baseline O3I was 4.3% ± 0.1% and AA/EPA ratio was 45.6 ± 23.8. After 5 weeks of algae oil supplementation, the O3I was 6.1% ± 1.0% and the AA/EPA ratio was 25.1 ± 11.6. The O3I was significantly higher and the AA/EPA ratio was significantly lower ($P < 0.0001$ for both) compared with both baseline and the end of the control period. The increase in O3I from baseline was correlated with calculated DHA + EPA dose per unit body mass ($R = 0.641$, $P = 0.001$). Conclusions: Algae oil supplementation for 5 weeks improved both the low baseline O3I and high AA/EPA ratio among ACF players, with body mass specific dose effects.

Keywords Omega-3 · Algae oil · Collegiate athletes · Vegetarian · Vegan

Introduction

Many athletes [13, 42], including National Collegiate Athletic Association (NCAA) athletes [20, 43] and American College Football (ACF) players [4, 19, 37], are deficient in long chain omega-3 polyunsaturated fatty acids (LC n-3 PUFA). With effects of increased cardiovascular and muscle function efficiency, and reduced exercise-induced oxidative stress and inflammation [35], the LC n-3 PUFA are pertinent to all athletes, including ACF players. The International

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Olympic Committee (IOC) classifies LC n-3 PUFA as "supplements that may assist [the high-performance athlete] with training capacity, recovery, muscle soreness and injury management" [30]. In 2019, the NCAA by-law 16.5.2.7 was amended to include omega-3 fatty acids as permissable nutritional supplements [8]. The Academy of Nutrition and Dietetics recommended dietary intake of at least 500 mg/day of the essential LC n-3 PUFA eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [41] can be accomplished by consuming 2 servings of fatty fish (8 oz; 225 g) per week. However, the majority of NCAA athletes [20, 43], including ACF players [1, 37] have inadequate dietary intake of fatty fish. LC n-3 PUFA supplementation with a plant-based algae oil rather than fish oil enables provision of the same supplement to all athletes in a team, whether omnivorous, vegetarian or vegan.

LC n-3 PUFA supplementation among athletes or physically fit individuals has positive effects on heart rate [27] and whole body oxygen consumption [21], reflecting enhanced cardiovascular efficiency. The Omega-3 Index (O3I), the percentage incorporation of DHA and EPA into red blood cell membranes, reflects the incorporation of LC n-3 PUFA into cardiac and skeletal muscle cell membranes [18] and is associated with cardiovascular disease risk. An O3I greater than 8% (desirable) is associated with the lowest risk of, and less than 4% (undesirable) with the greatest risk of, cardiovascular disease, including sudden cardiac death and myocardial infarction [18]. In three recent studies of ACF players, all participants had an O3I less than 8%, and one third had an O3I less than 4% [4, 19, 37].

LC n-3 PUFA supplementation can also attenuate skeletal muscle disuse atrophy [31], reduce muscle fatigue post-exercise [6, 26, 36], attenuate increases in neuroaxonal injury [19], and reduce eccentric exercise-induced delayed onset muscle soreness (DOMS) and inflammation [3]. These effects may be due to the associated changes in the PUFA n-6/n-3 ratio and inflammatory profile [22]. Metabolites of EPA and DHA are mostly anti-inflammatory, in contrast to the predominantly pro-inflammatory metabolites of the LC n-6 PUFA arachidonic acid (AA). An elevated AA/EPA ratio is a marker of chronic inflammation [33]. There are currently no established recommended levels for the AA/EPA ratio, and there is limited research on baseline AA/EPA ratios, particularly among athletes. Of the six publications to-date reporting the O3I among athletes, three did not report any n-6 PUFA data [13, 37, 43], and only two included the AA/EPA (or EPA/AA) ratio as a distinct measure [19, 42]. Among NCAA cross-country athletes, inflammation was the most and second-most common cause of injuries over a 4 year period for females and males, respectively [23]. Among healthy males, a higher baseline AA/EPA ratio was associated with greater loss of lean body mass after 5 weeks of inactivity [12]. Therefore, it is valuable to include the AA/EPA ratio alongside the O3I in LC n-3 PUFA supplementation studies among athletes.

DHA rather than EPA is selectively incorporated into skeletal muscle cell membranes [28] and associated with protection against concussion injury [34] and prevention of arrhythmias [32]. The latter is significant since arrhythmia is often a cause of sudden cardiac death among athletes [39]. Algae oil is a rich source of DHA, and algae oil supplementation is effective in improving a low or undesirable O3I in vegetarian and vegan adults [9]. Moreover, with inadequate fish sources to meet recommended LC n-3 PUFA intakes across the globe, algae, the main source of DHA in the marine food chain, is a viable and more sustainable source of LC n-3 PUFA [38].

The aim of this study was to evaluate the whole blood fatty acid profile, erythrocyte O3I, and AA/EPA ratio among Division I ACF players at baseline (consuming their usual omnivorous diets), after a 5-week control period during which subjects consumed their usual diet, and following 5 weeks of their usual diet plus supplementation with a DHA-rich algae oil. We hypothesised that ACF players would have a low baseline O3I and a high baseline AA/EPA ratio, and that 5 weeks DHA-rich algae oil supplementation would begin to ameliorate these.

Methods

Ethics Approval and Participants

Ethics approval was granted by the University of Wollongong Human Research Ethics Committee (Approval number: 2017/041) and the University of Oregon Institutional Review Board. Participants (all males) were recruited from the same National Collegiate Athletic Association (NCAA) Division I football team at the University of Oregon, prior to commencement of the 2017–2018 season. All participants provided informed signed consent prior to any data collection. The only exclusion criterion was current supplementation with LC n-3 PUFA, including fish and algae oil.

Study Design

Initially, cross-sectional data was collected during the first week of the pre-season period to gather baseline measures more broadly across the ACF team. A subset of these players volunteered to then complete a longitudinal study to determine the effects of 5 weeks supplementation with a plant based DHA-rich algae oil (Fig. 1). These players were age, weight and field-position matched with the remainder of the team, and each participant acted as their own control.

The longitudinal component of the study was 10 weeks in duration (the final 3 weeks of pre-season and 7 weeks of

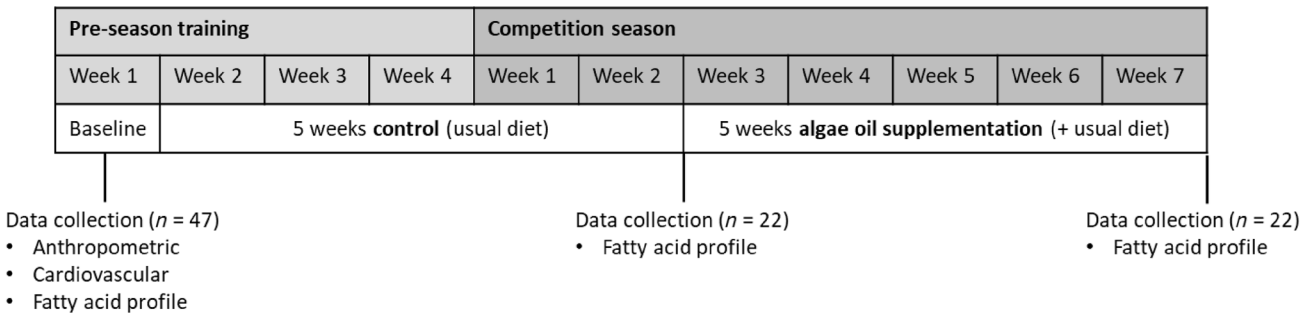

Fig. 1 Study design with cross-sectional and longitudinal components

competition season), with each participant acting as their own control. This included a 5-week control period, during which subjects consumed their usual diet, followed by 5 weeks of their usual diet plus algae oil supplementation (Brain Amour, Inc). During the supplementation period, three soft gel capsules, providing a total of 1050 mg DHA and 525 mg EPA, were consumed with breakfast for the 5 weekdays of each week. This equated to an intake of 5.25 g DHA and 2.625 g EPA per week (equivalent to 750 mg of DHA and 375 mg EPA per day). A member of the research team provided each player with the algae oil capsules and supervised consumption to ensure compliance. During the entire 10-week study period, players were provided with all meals and snacks at the training facility as part of their college football scholarship, which ensured consistency in the usual diet across the control and supplementation periods.

The control period incorporated 3 weeks of pre-season training and the first 2 weeks of competition (Fig. 1). The pre-season training included 6 football practice sessions and 2 strength sessions per week. During the competition season, there was 1 game, 2 high intensity football practice sessions, 2 light football practices, an off day and 2 strength sessions per week. Throughout both the pre-season and competition seasons, players participated in approximately 12–15 h per week of physical activity.

Data and Sample Collection and Analysis

At baseline, age (years) was recorded, body mass (kg and pounds) and height (cm) were measured, and BMI was calculated. Systolic and diastolic blood pressure (mmHg) and heart rate (beats/min) were recorded in triplicate, while seated. Each participant's BMI was classified as normal ($BMI < 25$ kg/m^2), overweight (25 kg/m$^2 \leq BMI < 30$ kg/m^2), or obese ($BMI \geq 30$ kg/m^2). Blood pressure was categorised as elevated (previously termed pre-hypertension) for systolic blood pressure 120–129 mmHg, and as hypertension for systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 80 mmHg. A spot blood sample was collected at baseline for the whole cohort, and also at the end of each of the 5-week control and supplementation periods for those in the longitudinal study, for analysis of whole blood fatty acid profile, including percentage of relevant fatty acids, and calculated O3I and AA/EPA ratio.

The blood sample collection was performed using the dried blood spot method from each participant's finger (OmegaQuant, South Dakota, United States). A lancet containing a spring-loaded needle was used to collect the blood spot on filter paper that was pre-treated with an antioxidant cocktail (Fatty Acid Preservative Solution, FAPS™), then allowed to dry at room temperature for 15 min. Samples were stored at − 80 °C and then fatty acid analysis was conducted using Gas Chromatography (Shimadzu Corporation, Columbia, MD) with comparison with a standard mixture of fatty acids characteristic of red blood cells (GLC OQ-A, NuCheck Prep, Elysian, MN). Whole blood fatty acid composition was expressed as a percent of total identified fatty acids. The O3I is defined as the sum of 20:5n-3 (EPA) and 22:6n-3 (DHA) adjusted by a regression equation ($r = 0.97$) to predict the O3I in the red blood cell [18].

Statistical Analyses

Statistical analyses were conducted using 'Statistical Package for the Social Sciences' (SPSS, Version 23). Values are reported with means and standard deviations. Comparisons between the subset of players who participated in the longitudinal study and the remaining players were made using one-way ANOVA with repeated measures and between groups analysis for anthropometric and fatty acid data, and with a Chi-square test for distribution of linemen versus non-linemen. For the longitudinal study, differences across time points (baseline, control and supplementation) were analysed using one-way ANOVA with repeated measures and post-hoc analysis with Bonferroni correction. Correlations are reported with Pearson's correlation coefficients.

Results

Baseline Whole Blood Fatty Acid Profile

Baseline anthropometric and cardiovascular data are presented in Table 1. The mean age of the cohort was 20.8 ± 1.3 years. The majority of players were categorized as overweight (58%) or obese (33%) and had elevated blood pressure (45%) or hypertension (30%). Among the cohort of 47 players, the mean baseline O3I was 4.3% ± 0.1% and showed normal distribution (D(47) = 0.104, $P = 0.200$, Kolmogorov–Smirnov). All players had an O3I less than 8.0%, and one third (34%) had an O3I less than 4.0%.

Longitudinal LC n-3 PUFA Supplementation Study

There were no significant differences between the 22 players who completed the longitudinal study and the remaining players for baseline anthropometric data, or whole blood fatty acid profile [one-way ANOVA with repeated measures and between groups analysis; $F(1) = 0$, $P = 0.992$] or for distribution of linemen versus non-linemen ($\chi^2 = 0.869$, $P = 0.351$); hence they could be considered representative of the wider team. All O3I data points for the full cohort ($n = 47$) and the longitudinal study subset ($n = 22$) at baseline, and for the longitudinal study participants after the control and algae oil supplementation periods, are presented in Fig. 2.

When individual whole blood fatty acids were compared between the three time points (at baseline, and the end of the 5-week control and algae oil supplementation periods) for the longitudinal study, there was a significant effect for all poly-unsaturated fatty acid measures except for total n-6 (Table 2). The O3I, n-6/n-3 ratio, AA/EPA ratio, total n-3 and DHA were each significantly different post-supplementation compared to both baseline and control time points ($P < 0.0001$ for all). EPA was significantly different between the control time point and following algae oil supplementation ($P = 0.019$).

After 5 weeks DHA-rich algae oil supplementation, the O3I significantly increased by one-third of the initial value and no participant had an undesirable O3I of less than 4%, with similar inter-individual variability to baseline and after the control period (Fig. 2). All participants except one (Fig. 3B) had an increase in their O3I after the 5-week supplementation period, with a mean magnitude of change of O3I of 1.4% ± 0.7%. Similarly, the AA/EPA ratio was significantly reduced by almost one-third of the baseline value after 5 weeks algae oil supplementation. The change in O3I was not correlated with baseline O3I ($R = -0.088$, $P = 0.698$); however, the n-6/n-3 ratio pre-supplementation was inversely correlated with the change in the n6/n3 ratio after supplementation ($R = -0.664$, $P = 0.001$).

When participants were classified into tertiles, based on body mass specific DHA + EPA dose (mg/kg body mass), there was a 1.5-fold difference between the lowest and highest tertiles. There was a greater than two-fold difference in the change in O3I with 5 weeks algae oil supplementation between the lowest and highest tertiles (Table 3). The body mass specific DHA + EPA dose was strongly correlated with both the O3I after 5 weeks supplementation ($R = 0.548$, $P = 0.008$; Fig. 3A) and the change in O3I from baseline

Table 1 Baseline characteristics, whole blood fatty acid profile and Omega-3 Index for American College Football Players ($n = 47$)

Variable		Values (mean ± SD)
Anthropometric data	Weight (pounds)	237.7 ± 46.9 (174.0–322.7)
	Weight (kg)	107.1 ± 3.0 (79.8–144.7)
	BMI (kg/m^2)	30.1 ± 4.9 (22.9–41.0)
Cardiovascular data	Systolic blood pressure (mmHg)	123.3 ± 1.3 (102–140)
	Diastolic blood pressure (mmHg)	72.3 ± 1.4 (56–90)
	Heart rate (beats/min)	65.9 ± 1.3 (48–85)
Fatty acid data[a]	Omega-3 Index (%)	4.3 ± 0.1 (3.1–6.4)
	AA/EPA ratio	45.6 ± 23.8 (17.6–141.6)
	DHA (%)	2.3 ± 0.08 (1.5–3.8)
	EPA (%)	0.3 ± 0.02 (0.1–0.7)
	AA (%)	11.7 ± 1.4 (8.0–15.9)
	Total n-3 (%)	4.3 ± 0.1 (3.1–6.0)
	Total n-6 (%)	38.4 ± 0.5 (32.4–43.5)
	n-6/n-3 ratio	9.0 ± 0.2 (6.1–11.8)
	Monounsaturated fat (%)	19.8 ± 0.4 (15.0–28.9)
	Saturated fat (%)	36.6 ± 0.2 (34.2–39.6)
	Trans fat (%)	0.9 ± 0.02 (0.6–1.2)

[a]Relative (%) whole blood fatty acids and calculated Omega-3 Index. Values are mean ± standard deviation, with range in parentheses

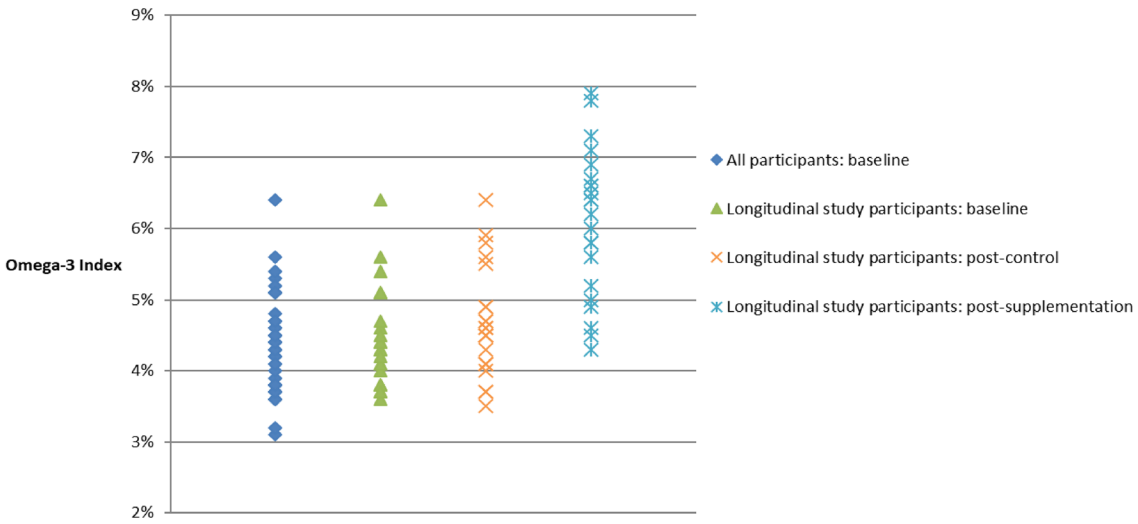

Fig. 2 Distribution of Omega-3 Index (%) for all ACF players ($n=47$) at baseline and for participants in the longitudinal supplementation study ($n=22$) also at baseline, and then after 5 weeks control and 5 weeks supplementation with 5.25 g DHA per week (averaging 750 mg per day). The Omega-3 Index was significantly different post-supplementation compared to both baseline and after the control period

Table 2 Whole blood fatty acid profile at baseline and after 5 weeks each of control and algae oil supplementation ($n=22$; data represented as mean ± standard deviation)

Measure	Baseline	Control	Supplementation	ANOVA[a]	
				$F(2, 20)$	P
Omega-3 Index (%)	4.4 ± 0.7	4.6 ± 0.7	6.1 ± 1.0	47.310	< 0.0001
AA/EPA ratio	33.5 ± 9.3	34.1 ± 11.3	25.1 ± 11.6	24.887	< 0.0001
DHA (%)	2.3 ± 0.5	2.5 ± 0.6	3.7 ± 0.8	42.572	< 0.0001
EPA (%)	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.2	4.435	0.026
AA (%)	11.5 ± 1.2	11.9 ± 1.2	10.4 ± 1.6	11.284	0.001
n-3 total (%)	4.4 ± 0.6	4.6 ± 0.8	5.8 ± 0.9	37.701	< 0.0001
n-6 total (%)	37.9 ± 0.4	37.9 ± 2.1	36.7 ± 2.7	2.026	0.158
n-6/n-3 ratio	8.7 ± 1.1	8.5 ± 1.4	6.5 ± 1.0	52.137	< 0.0001
Saturated fat (%)	36.8 ± 1.3	37.0 ± 1.1	36.5 ± 1.6	0.935	0.409
Monounsaturated fat (%)	20.0 ± 1.8	20.0 ± 2.0	20.0 ± 2.8	0.596	0.560

[a]Differences between time points: one-way ANOVA with repeated measures

Table 3 Effects of 5 weeks DHA-rich algae oil supplementation on Omega-3 Index (O3I) per Tertiles of Calculated Body Mass Specific DHA Dose (data represented as mean ± standard deviation)

Measure	Tertile 1 ($n=7$)	Tertile 2 ($n=8$)	Tertile 3 ($n=7$)
Weight pre-supplementation (kg)	133.7 ± 8.9	103.6 ± 8.3	87.1 ± 3.3
DHA dose (mg/kg body mass per week)	39.4 ± 2.6	51.0 ± 4.2	60.3 ± 2.3
O3I pre-supplementation (%)	4.5 ± 0.8	4.7 ± 0.8	4.8 ± 0.8
O3I post-supplementation (%)	5.4 ± 1.1	6.2 ± 0.8	6.7 ± 0.8

until the end of the 5 weeks supplementation ($R=0.641$, $P=0.001$; Fig. 3B).

Discussion

In this group of ACF players, just 5 weeks of supplementation with a plant-based DHA-rich algae oil resulted in

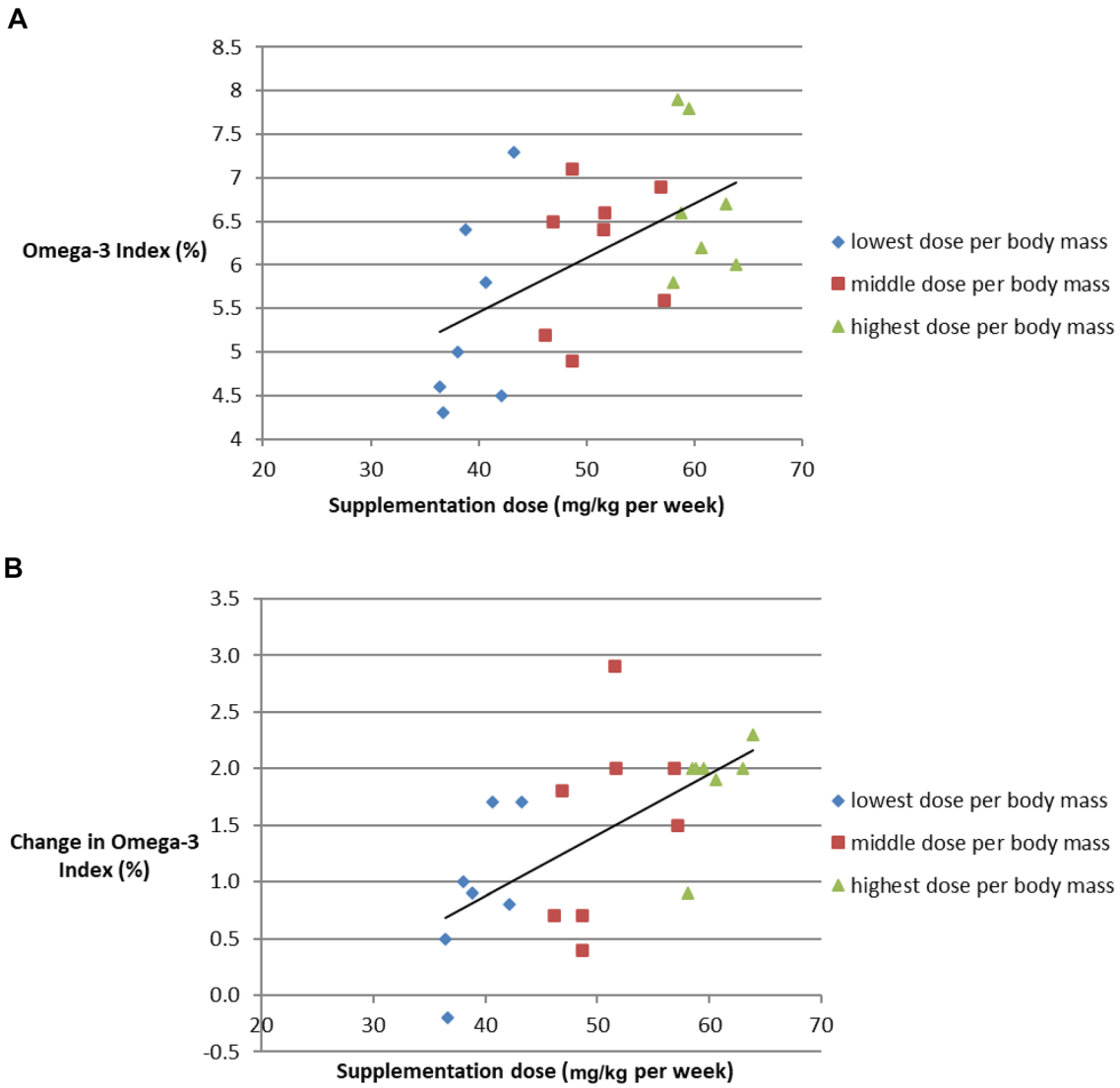

Fig. 3 Body mass specific supplementation dose was significantly correlated with: **A** Omega-3 Index after 5 weeks DHA-rich algae oil supplementation; **B** change in Omega-3 Index after 5 weeks DHA-rich algae oil supplementation

significant improvements in their low O3I and high AA/EPA ratio. The current results add to the few other studies that have reported on LC n-3 PUFA supplementation among ACF players, and demonstrate the effectiveness of supplementation with a plant based algae oil, providing DHA and EPA, in athletes. When body mass was taken into account, those with a higher supplementation dose relative to their body mass achieved the greatest changes in O3I. This DHA-rich algae oil supplementation, equivalent to approximately 2–3 fatty fish meals per week, was easily administered on training days (5 days per week).

All 47 ACF players in this study had a baseline O3I less than 8%, in the range associated with increased overall mortality, cardiovascular disease risk and depression [18]. The mean O3I was 4.3% ± 0.1%, and one third of players had an O3I less than 4% (undesirable), placing them in the highest cardiovascular disease risk category. The same mean and distribution were recently reported among three other cohorts of ACF athletes [4, 19, 37], and are similar to other athlete groups [13, 42, 43] and to the general USA population [40]. All players also had a high AA/EPA ratio, representative of a pro-inflammatory state. Although there are no established recommended levels for the AA/EPA ratio, the AA/EPA ratio of the current cohort of 45.6 ± 23.8 is well above the desirable range of 2.5–11 (OmegaQuant) and higher than other reports of: 20 in a large ($n = 160{,}000$) population of USA adults across the age-span [17]; 20–25 among athletes or physically fit males consuming a usual diet [27, 42]; and 30 in an ACF cohort [4]. Taken together, these results indicate that athletes, and ACF players even more so,

have a high AA/EPA ratio, indicating their pro-inflammatory state and elevated risk of inflammatory diseases.

Among the subset of ACF players in the longitudinal aspect of the current study, 5 weeks supplementation with algae oil providing 5.25 g of DHA and 2.625 g EPA per week (equivalent to 750 mg of DHA and 375 mg EPA per day): significantly increased the low baseline O3I by approximately one-third, from 4.4% ± 0.6% to 5.8% ± 0.9%; and significantly reduced the AA/EPA ratio by almost one-third, from 34.1 ± 11.3 to 25.1 ± 11.6. This resulted in all participants achieving an O3I corresponding to a lesser risk of cardiovascular disease [18], albeit none were within the desirable range (O3I > 8%). This increase in O3I is of similar magnitude and end-point to previous research that also reported associated improvements in cardiovascular efficiency during exercise [21]. Achieving these physiologically relevant increases in O3I after only 5 weeks of supplementation is convenient with respect to the potential to improve any identified LC n-3 PUFA deficiencies in ACF players prior to the competition season. Interestingly, in the current cohort, the resultant O3I of almost 6% from a low baseline O3I (4.4%) after 5 weeks supplementation with approximately 1 g LC n-3 PUFA per day reflects the theoretical threshold effect for this DHA + EPA dose and baseline O3I [14]. This also suggests that the bioavailability of the LC n-3 PUFA provided by an algae oil is comparable to purified EPA + DHA and fish oil, since these supplementations were the basis for these theoretical projections [14]. Considering the low baseline O3I of athletes in the current study and the literature more broadly, initial supplementation with a higher daily dose of more than 2 g LC n-3 PUFA, before switching to a maintenance low dose (up to 1 g LC n-3 PUFA per day) akin to the current study seems practical, but will need to be followed up in subsequent studies.

Although the dose of LC n-3 PUFA in the current study can be obtained by consuming approximately 2 servings of oily fish per week, student-athletes with diets restricted to food service options on campus [24], and those with vegetarian or vegan dietary patterns [10] will require supplements to ensure adequate LC n-3 PUFA intake. Indeed, a recent study revealed that although 39% of 1528 NCAA athletes consumed the recommended amount of dietary fish per week, only 6% met the requirement for EPA + DHA intake [37], indicating that the fish sources were not adequate in terms of their LC n-3 PUFA composition. Similarly, the high AA/EPA ratios are indicative of consumption of a "Western" diet, high in red meat, dairy and eggs, and low in fatty fish. Further, the O3I of National Football League players decreased over the competition season [7], reinforcing the need for supplementation during the season.

Of relevance is that the effects of 5 weeks DHA-rich algae oil supplementation on the O3I post-supplementation and the change in O3I were dependent on LC n-3 PUFA dose per kg body mass, which was previously noted as a strong predictor of change in O3I [15]. The diverse body mass range of the current study participants, with an almost two-fold weight difference between the lightest and heaviest participants, is consistent with other ACF cohorts [16]. Extrapolation from the equation of the line for O3I post-supplementation plotted against LC n-3 PUFA dose per kg body mass (Fig. 3) suggests a minimum dose of 120 mg DHA + EPA/kg per week is necessary to achieve an O3I of 8% (desirable) within this time period of 5 weeks. This equates to a daily DHA + EPA dose of 1.37 g for an 80 kg player and 2.485 g for a player weighing 145 kg. Taking into account an individual's weight is particularly important among cohorts of athletes with a large body mass range.

The low O3I and high AA/EPA ratio are particularly concerning because many of the ACF players in this cohort had additional cardiovascular disease risk factors: 91% had an overweight or obese BMI, and 75% had elevated blood pressure or hypertension. Without specific data on muscle mass versus adipose tissue, the use of BMI in this population is limited. Nevertheless, these results are in line with previous research reporting high BMI and body fat percentage [2], elevated blood pressure [11], and poor dietary habits [1] among ACF players. In addition, ACF student-athletes also experience mental stresses due to competing academic and sporting pressures, and there are concerns about the long-term mental health of athletes [29]. Low LC n-3 PUFA levels are associated with anxiety, depression and poorer quality of life measures [25]. Therefore, this represents a population who would benefit from LC n-3 PUFA supplementation to reduce modifiable risk factors for multiple diseases. Supplementation with LC n-3 PUFA aligns well with the focus areas for the well-being of collegiate athletes including "keeping hearts healthy", "managing mental health", and "fuelling performance" [5], with beneficial effects across all these domains.

The current study was limited in terms of a small sample size, a lack of physiological performance measures, and only omnivorous, male athletes being included. Inclusion of functional measures of cardiovascular and muscle performance, such as whole body oxygen consumption and muscle soreness, would increase the application of this research in athletic groups. Further, having BMI as the only measure of body composition without any data on the proportions of adipose and muscle mass to this measure is a limitation, particularly in an AFC cohort of diverse body fat and muscle percentage. Since adipose tissue is a storage site for fatty acids [15], it would be useful to assess its contribution to the body mass specific dose effect on the O3I. Inclusion of waist circumference as an estimate of central adiposity would also be beneficial. Further, it would be beneficial to assess the efficacy of algae oil supplementation among a more diverse athletic team,

also including females, and vegetarians and vegans, and to gather data with respect to any side effects and acceptability of algae oil as a supplement.

Overall the results of this study demonstrated that a low and in some cases undesirable baseline O3I and high AA/EPA ratio in ACF players can be improved with 5 weeks supplementation of a DHA-rich algae oil. There was an influence of body mass specific dose on the effects of LC n-3 PUFA supplementation on both the resulting O3I and the change in O3I from baseline. This should be considered with respect to recommendations for LC n-3 PUFA supplementation to achieve a healthy O3I, which do not currently account for body mass specific dose effects or inter-individual weight variation. The provision of LC n-3 PUFA via an algae oil facilitates an opportunity to improve cardiovascular disease risk factors, cardiovascular and muscle physiology, and inflammatory status, in athlete groups who follow any of omnivorous, vegetarian or vegan dietary patterns. Such interventions are directly aligned with the focus of the NCAA and other athletic governing bodies on the health, wellbeing and nutritional status of these student-athletes.

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Author Contributions The study was conceptualised by JS, AM, BM, CP, NL and TL. Data were collected by BM and JD and analyzed by TL and JD. Statistical analyses and data interpretation were undertaken by TL. Manuscript preparation was undertaken by TL, GP and JS. All authors reviewed and edited the manuscript and approved the final version of the paper. JS was responsible for funding acquisition and oversight of the research.

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Data Availability Any/all data is available to reviewers and readers on request.

Declarations

Conflict of Interest Charles Pedlar is seconded to the position of Chief Science and Research Advisor, and Nathan Lewis is an employee, of Orreco Ltd. Orreco provided the OmegaQuant kits and analysed blood spot samples for this study. However, all samples were anonymised and the technicians who analysed the samples were never in contacted with any of the authors, were providing routine blood sample analyses only and were not aware of this study at all.

Ethical Approval This study was performed in line with the principles of the Declaration of Helsinki. Ethics approval was granted by the University of Wollongong Human Research Ethics Committee (Approval number: 2017/041) and the University of Oregon Institutional Review Board.

Consent to Participate Informed consent was obtained from all individual participants included in the study.

Consent to Publish No individual data is included in this manuscript. All participants gave consent to have the aggregated results of the study published.

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