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Increasing the Omega-3 Index of Endurance Trained Adults: A Pilot Study Comparing Microencapsulated Algae to Fish Powders Provided as Chewable Tablets

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Abstract

Long-chain omega-3 fatty acids (LCn-3PUFA), including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), support heart function during exercise and recovery by incorporating into the cardiac cell membranes. Traditionally sourced from fish and fish oil, algae-derived LCn-3PUFA have become alternative in supplemental forms. This study evaluated whether 12 weeks supplementation of a microencapsulated algal oil, delivered as chewable tablets, would perform equivalently to fish-derived LCn-3PUFA in boosting the Omega-3 Index (O3I; erythrocyte membrane EPA + DHA%) of endurance athletes. Sixteen endurance-training adults (13 males, 3 females) supplemented daily microencapsulated chewable tablets (6/d) with fish-oil (FO; 142 mg/d EPA + 631 mg/d DHA) or algal-oil (AO; 21 mg/d EPA + 595 mg/d DHA) for 12 weeks. Baseline body composition was assessed using bioelectrical impedance, and whole blood fatty acid profiles were evaluated before and after supplementation. Additionally, participants maintained a self-recorded weekly training diary. Fat mass (%) was equivalent between the groups (FO: 9.18 ± 4.78%; AO: 9.94 ± 5.09%; P > 0.05). Weekly training times were also comparable (FO: 568 ± 242 minutes; AO: 579 ± 208

minutes; P > 0.05), and initial O3I were comparable between groups. Both groups showed significant post-supplementation increases in O3I (FO: pre, 4.85 ± 0.42%, post, 6.48 ± 0.52%; AO: pre, 4.30 ± 0.54%, post, 6.06 ± 0.70%; P < 0.01 within each group) and there was no significant difference in post-supplementation O3I levels between the groups (P = 0.467). In the context of endurance exercise training, algal-derived LCn-3PUFA (~600 mg/d EPA + DHA delivered as a chewable tablet) were equally as effective as those derived from fish in terms of elevating the body's O3I over 3 months. Athletes following a plant-based diet may indeed consider an algal source of LCn-3PUFA as part of their whole diet quality and the attainment of EPA and DHA.

Keywords
Nutritional supplementation; endurance training; omega-3 bioavailability; plant-based diet

1. Introduction

Endurance athletes require optimal heart heath to support the cardiac output requires of arduous physical training and competition. The pre-eminent physiological stress on the cardiovascular system during endurance exercise can in some instances result is sudden cardiac death (SCD) [1]. In fact, endurance exercise transiently can impair myocardial function [2], and whilst typically self-resolving, studies show that endurance exercise can lead to short and long-term cardiac remodeling [3]. In the predisposed athlete, especially the 'training-naïve' over 35 years old or those with certain existing heart conditions, this significantly increases risks of infarction, arrest and death [4].

Dietary long-chain omega-3 fatty acids (LCn-3PUFA), including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are recognised for the cardiovascular protective roles. In fact, EPA and DHA have differential impacts for modifying cell membranes of tissues, such as skeletal muscle [5] and heart [6], resulting in tissue specific physiological adaptation to these contractile cells. In physiological terms this increased dietary intake of DHA is reported to lower submaximal exercise heart rate in trained athletes [7, 8] and improve heart rate recovery [9]. In the case of heart health and cardiac function, it is well-established that dietary intake of preformed omega-3 EPA and DHA from fish and fish oil supplements is an effective way of increasing the Omega-3 Index (O3I; erythrocyte membrane EPA + DHA%), even at dietary achievable doses of EPA + DHA (500-1000 mg/d) [10], and can have significant impact on the omega-3 status of an individual [11]. In fact, it was with this cardiovascular strain in mind, that a cohort of 106 German elite winter-endurance athletes were first studied for their habitual O3I [12]. In that original screening study of athletes, only one participant was above the cardioprotective threshold O3I of >8% (summed value of EPA% and DHA% in the erythrocyte membrane [13]), thus observing the theoretical basis for providing supplementary LCn-3PUFA to support the cardiac and vascular cells, in order to support life-long coronary artery health in combination with exercise. More recent studies in athletic populations not only demonstrate the O3I is often low, in line with the general population, but also confirms inconsistent intake of LCn-3PUFA supplements. For example, only 15% of North American college athletes self-report regular usage of LCn-3PUFA supplements, reflective of their low O3I levels [14]

and comparison via sex reveals that only 33% of male and 20% of female college athletes self-report using supplements, with both groups also having low O3I levels [15].

Preformed omega-3 EPA + DHA are predominantly sourced from dietary fatty fish such as salmon and tuna or from commercial dietary supplements mainly made from fish oil. Plant oils such as chia and flaxseed also contain omega-3 as alpha-linolenic acid (ALA), though, the body's conversion of ALA to form EPA and DHA is generally inefficient, favouring direct intake of preformed omega-3 EPA and DHA from marine sources. The rising demand for highly purified EPA + DHA fish oil products strain global supply and raises ecological and sustainability concerns regarding fish farming [16]. Furthermore, vegan athletes (or those with a plant-based diet) face challenges in achieving sufficient EPA and DHA levels without including marine animal-based sources in their diet [17]. Non-animal marine based sources, like microalgae offer preformed LCn-3PUFA EPA and DHA, but studies on algal oil are still limited, with large variation in both dose and duration of LCn-3PUFA. For example, studies that have included vegetarian healthy adults have demonstrated elevated EPA and DHA presence in plasma, serum, platelet and red blood cell fractions following inclusion of agal food products [18]. Certainly, algal oil potentially offers an alternative option to traditional fish supplies for human food chain LCn-3PUFA production, noting its differential environmental footprint [19]. So, expanding algal oil applications to contexts, such as athletic populations, will enhance our understanding of algae's potential role and contribution in the human diet.

Marine algae are neither plant nor animal and instead categorised in the protist kingdom. As a marine source of food, microalgae contain preformed LCn-3PUFA, however studies utilizing algal oil as a source of DHA, for example, are limited by short duration, typically <28 days [20-22], or a DHA-dose far exceeding (>2-3,000 mg/d) intakes that couldn't be replicated through whole food sources [23, 24]. Notwithstanding, when algal oil has been compared to whole fish intake in healthy adults, at a comparable intake of 600 mg/day DHA, the bioavailability for both plasma and erythrocyte uptake has been reported to equivalent [25]. Yet, LCn-3PUFA uptake remains to be evaluated, between fish and algae sources, for athletes who are engaged in arduous physical exercise training.

Therefore, this study aimed to determine if 12 weeks supplementation of a novel microencapsulated chewable tablet containing omega-3 EPA + DHA sourced from algae could perform as effectively as fish-derived LCn-3PUFA to increase the O3I in endurance training athletes during an extended period of their training.

2. Materials and Methods

2.1 Study Design and Participants

The study was designed as a translational pilot study where endurance athletes were supplemented for 12 weeks with two novel forms of preformed omega-3 EPA + DHA, comprising fish-derived and algal-derived chewable tablets. Whole blood fatty acid profiles and erythrocyte O3I was measured at baseline and after 12 weeks of supplementation. Participants were recruited from local athletic groups (cycling and running) around the Illawarra region of New South Wales, Australia. Participants were eligible for the study if they (i) were not previously consuming LCn-3PUFA dietary supplements, (ii) did not consume more than one fatty fish meal per week, (iii) engaged in at least 7 hours of training per week, (iv) have maintained their usual training load over the prior 6 months and (v) were able to maintain their usual training load during the 12 weeks study period. Participants

were required to maintain their usual training and eating behaviours throughout the intervention. Nineteen endurance training athletes met inclusion criteria and were recruited for the study.

2.2 Dietary Supplement

A proprietary microencapsulation technology was used to create a chewable tablet derived from either anchovy oil (Driphorm® HA HiDHA 300®) or algal oil (Driphorm® HA DHA-S® 200). All microencapsulated powders were prepared by Nu-Mega Ingredients, Brisbane, Australia. Once prepared, the microencapsulated powder was sent to Fakoteck Packaging., Quebec, Canada where it was compressed into chewable tablet form and packed into blister packs. Each tablet was circular with dimensions of approximately 17.7 mm in diameter and 5.9 mm in height. Samples from each completed batch of microencapsulated tablets were sent to an independent National Association of Testing Authorities (NATA) accredited laboratory (Hasta.org.au) to undergo supplement drug screening for World Anti-Doping Agency (WADA) prohibited substances.

Participants were randomized into either the fish oil (FO; 142 mg/d EPA + 631 mg/d DHA) or algal oil (AO; 21 mg/d EPA + 595 mg/d DHA) tablets, unless they were vegan or vegetarian, in which case they were allocated to the AO group. Participants were instructed to chew three tablets, twice per day (total of six chewable tablets/day) with food for 12 weeks. To encourage adherence and compliance, the participants were instructed to consume three tablets with breakfast and three tablets with dinner. In addition, the tablets were blister packed into rows according to each day and week. The sheets were arranged into a personalised box which visually acted as a reminder to the participant, each week. Table 1 shows the daily content of the primary fatty acids (as triglycerides) of interest for the present study for the fish and algal groups.

Table 1 Fatty acid composition of dietary supplements as provided via the fish or algal microencapsulated powders. Fatty acids were delivered in the form of triglycerides.

	Fish oil (mg/d)	**Algal oil (mg/d)**
Total fat	1,079	1,673
AA	8.6	4.2
EPA	141.8	20.7
DHA	631.1	594.6
EPA + DHA	772.9	615.3

Abbreviations: AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid. Amount based on serving size of 6 tablets per day.

2.3 Blood Sampling

Each athlete provided a blood sample (non-fasted) using the finger prick method at baseline and after 12 weeks of supplementation. A drop of blood was spotted onto the commercially available collection card for independent analysis (Fatty Acid Labs, Victoria, Australia). The sample card was immediately sent to Fatty Acid Labs for determination of whole-blood fatty acid profiles, O3I and AA: EPA ratio. Each fatty acid in the whole blood was individually identified using high quality standards and then described as a relative percentage (%) of all the fatty acids. The O3I, a marker

of heart, skeletal muscle and brain membrane incorporation, was then calculated according to a validated algorithm (r = 0.96) [19].

2.4 Anthropometry

At baseline, participants' body composition was measured by bioelectrical impedance analysis (BIA) (TANITA DC 360, Tokyo, Japan). Body composition measurement using BIA was acceptable in this context for describing the cohort prior to supplementation [26]. Height was measured with a stadiometer (SECA217 Stable Stadiometer, SECA). Participants were lightly clothed and without shoes during body composition and height measurements.

2.5 Training Load Assessment

To determine if participants met physical activity eligibility prior to commencing the study, the International Physical Activity Questionnaire (IPAQ-SF) was used to assess self-reported moderate and vigorous physical activity within the prior 7 days. Throughout the duration of the 12 weeks intervention period, participants maintained a weekly training diary where they tracked the number of training sessions completed and total training time.

2.6 Statistical Analysis

To assess the main objective of this randomized study, the change in O3I was interrogated as a repeated measures analysis of variance (ANOVA) in the context of an intention to treat design. Exclusion of data only occurred where the participant did not return at week 12 (withdrawal) or self-report non-compliance, and therefore did not provide a post-supplement blood sample. The type of diet supplement (FO, AO) was used as the group factor, with the primary effects being the time points (baseline and 12 weeks post-treatment) and the interaction between the supplement and time. Where significant difference was established, a *post hoc* Tukey test was conducted for comparisons of individual means. Baseline variables including anthropometry and training load were compared using an unpaired *t*-test between FO and AO groups. Collected data were analysed using Graphpad Prism software package (Version 9.5.1 for Mac, Graphpad Software, Boston, Massachusetts USA). The data collected were expressed as mean (standard deviation). Alpha was set at $p < 0.05$.

2.7 Ethics Statement

The study was reviewed and approved by the University of Wollongong Human Research Ethics Committee (HE_2021/404) and was conducted in accordance with the Declaration of Helsinki. Accordingly, each participant provided informed written consent.

3. Results

3.1 Participants

Of the 19 participants who enrolled in the study, two participants withdrew due to minor gastrointestinal discomfort and one participant was excluded due to self-reported non-compliance. A total of 16 participants (FO: 7 males, 1 female; AO: 6 males, 2 females) met inclusion in the analysis

and completed the 12 weeks study (Table 2). Supplement duration was 82 ± 4 days for the FO group and 81 ± 6 days for the AO group. As assessed by the weekly training dairy completed throughout the 12 weeks intervention period, there was no difference between groups for the average number of training sessions per week (FO: 7 ± 2 days; AO: 7 ± 3 days; P > 0.05) or the average training minutes per week (FO: 568 ± 242 minutes; AO: 579 ± 208 minutes; P > 0.05). The baseline anthropometry measurements, including fat mass percentage, showed no significant differences between the groups, indicating they were equally matched lean endurance training groups (Table 2).

Table 2 Participant characteristics for fish and algal oil groups at baseline.

	Fish oil (n = 8)	**Algal oil** (n = 8)	**P-Value**
Age (y)	38 (9)	33 (11)	0.370
Body mass (kg)	74.64 (6.63)	71.75 (7.10)	0.415
Height (m)	1.79 (0.09)	1.80 (0.07)	0.810
BMI (kg/m^2)	23.41 (1.91)	22.18 (1.50)	0.175
FM (%)	9.18 (4.78)	9.94 (5.09)	0.762
FM (kg)	6.74 (3.09)	6.93 (2.97)	0.903
FFM (kg)	67.90 (7.65)	64.83 (8.93)	0.472
Muscle mass (kg)	64.54 (7.29)	61.60 (8.53)	0.471
Bone mass (kg)	3.36 (0.36)	3.23 (0.40)	0.483
BMR (Kcal)	1949.38 (221.69)	1876.13 (240.52)	0.537

Data expressed as mean (SD) with comparison between Fish oil and Algal oil groups completed using an unpaired *t*-test. Abbreviations: BMI, body mass index; FM, fat mass; FFM, fat free mass; BMR, basal metabolic rate.

3.2 Baseline Whole Blood Fatty Acid Profile and Omega-3 Index

Prior to supplementation there was no difference in any component of the whole blood fatty acid profile between groups (Table 3). The mean sum of n-6 PUFA comprised of one third of the relative proportion of all fatty acids where AA contributed close to 8-9% on average for each group (Table 3). The mean n-6/n-3 ratio was greater than 5 and the AA/EPA ratio was greater than 11 in both groups. When participants were rank ordered according to baseline O3I, the four vegan athletes grouped under the 25th percentile in comparison to omnivore athletes (Figure 1). The usual intake of dietary fatty acids, including omega-3 EPA + DHA, in the cohort of endurance training runners revealed none (0 out of 13) of the runners had an O3I above 8% (AO mean O3I: 4.30 ± 0.54%; FO mean O3I: 4.85 ± 0.42%; Figure 2).

Table 3 Whole blood relative fatty acid profile (%) of endurance runners before and after 12 weeks LCn-3PUFA supplementation sourced from either fish or algal oil.

	Fish oil (n = 8)		Algal oil (n = 8)		ANOVA (*p*-values)		
Fatty acid	Pre Mean (SD)	Post Mean (SD)	Pre Mean (SD)	Post Mean (SD)	Time	Group	Int.
Σ SFA	34.90 (1.89)	35.70 (1.53)	34.99 (1.97)	35.20 (1.27)	0.27	0.81	0.64
Σ MUFA	24.04 (3.64)	23.19 (2.67)	24.71 (2.78)	23.06 (2.47)	0.10	0.84	0.53
Σ n-6 PUFA	35.19 (2.12)	33.59 (2.72)	35.12 (2.62)	34.96 (2.41)	0.30	0.50	0.14
LA (18:2n6)	23.32 (1.97)	22.69 (2.87)	22.83 (2.56)	23.35 (1.57)	0.95	0.94	0.35
AA (20:4n6)	8.51 (0.92)	7.86 (1.07)	8.55 (1.43)	7.95 (1.06)	0.007	0.90	0.92
Σ n-3 PUFA	5.19 (0.52)	6.64 (0.70)*	4.71 (0.63)	6.07 (0.58)*	<0.001	0.16	0.82
ALA (18:3n3)	0.62 (0.30)	0.72 (0.18)	0.66 (0.39)	0.77 (0.22)	0.16	0.71	0.88
EPA (20:5n3)	0.69 (0.23)	0.90 (0.16)	0.61 (0.16)	0.65 (0.16)	0.009	0.07	0.25
DHA (22:6n3)	2.41 (0.24)	3.64 (0.50)*	2.01 (0.49)	3.51 (0.56)*	<0.001	0.29	0.35
Ratios							
n-6/n-3	6.83 (0.68)	5.10 (0.56)*	7.57 (1.10)	5.81 (0.66)*	<0.001	0.11	0.83
AA/EPA	13.45 (4.53)	9.07 (2.34)	14.74 (3.93)	12.93 (4.00)	<0.001	0.17	0.33

Fatty acid values expressed as a percentage of total identified fatty acids from a whole blood, dry sample. Abbreviations: Int., Interaction; SFA, Saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA polyunsaturated fatty acids; LA, Linoleic Acid; AA, Arachidonic Acid; ALA, alpha-Linolenic Acid; EPA, Eicosapentaenoic Acid; DHA, Docosahexaenoic Acid. * p < 0.05 within supplement group pre vs. post (corrected for multiple comparisons using the Bonferroni procedure).

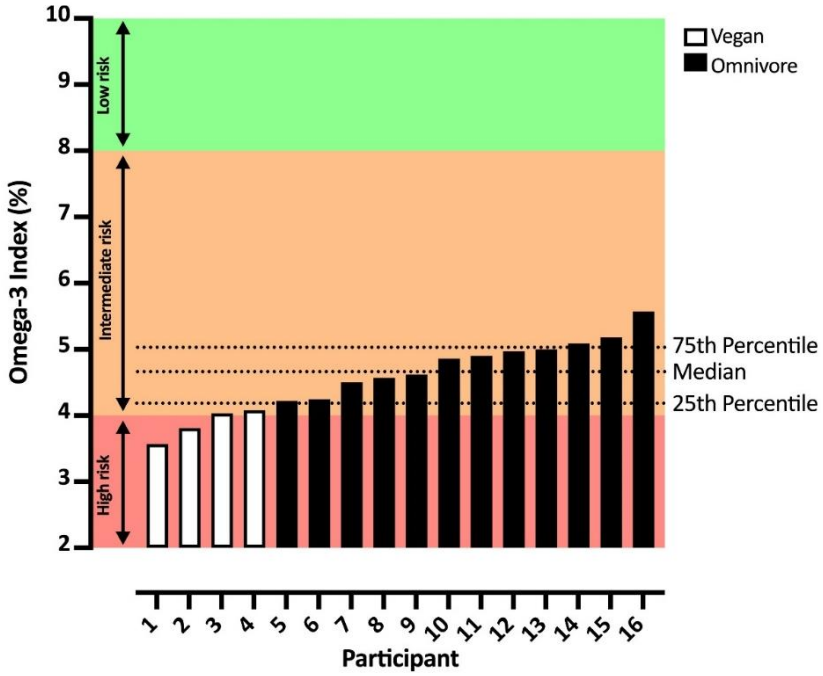

Figure 1 Rank-ordered individual participants' baseline Omega-3 Index (O3I). The O3I is expressed as the percentage of DHA + EPA of total identified fatty acids from a whole blood, dry sample.

3.3 Post-Supplementation Whole Blood Fatty Acid Profile and Omega-3 Index

Whole blood DHA and the O3I were significantly increased in both groups following supplementation, with all participants showing an elevation in O3I at week 12 ($P < 0.01$, Figure 2). Total omega-6 fatty acids remained unchanged following supplementation ($P > 0.05$), however there was a significant increase in total LCn-3PUFA fatty acids leading to a significant reduction in n-6: n-3 ratio in both groups (Table 3). There was an increase in whole blood EPA in the fish group and a decrease in AA in both groups, however, neither of these changes reached statistical significance (Table 3).

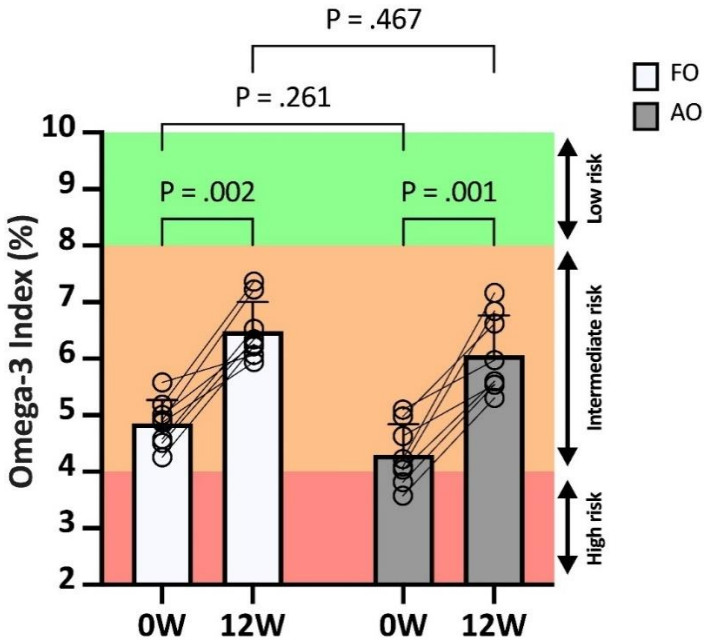

Figure 2 Omega-3 Index (O3I; erythrocyte membrane EPA + DHA%) for fish oil (FO; n = 8) and algal oil (AO; n = 8) groups, pre and post supplementation. Individual data shown via open circles with the group mean (SD) shown via column bar graph. A repeated measures analysis of variance (ANOVA) was used to compare the factors of diet (FO, AO), time (0 W, 12 W) and their interaction. Where significant difference was established, a *post hoc* Tukey test was conducted for comparisons of individual means.

4. Discussion

The primary aim of the study was to evaluate whether a microencapsulated chewable LCn-3PUFA supplement, derived from algae could perform as effectively as fish derived EPA and DHA to increase the O3I in endurance training athletes, whilst in a training period of high energy demands. At baseline the participants' mean relative fat mass was <10% and a fat-free mass <68 kg reflecting the that of typical endurance athletes completing 10 hours per week of training [27]. Across the course of the 3 months, O3I was increased in both groups from their baseline omega-3 status and there was an equivalent improvement in those athletes allocated the agal derived LCn-3PUFA source.

Prior to supplementation, the cohort had an average O3I of 4.58% (range from 3.57% to 5.57%) reflective of habitual consumption of a typical Western-style diet that is less than optimal in providing preformed omega-3 EPA + DHA [28], that can be implied from global erythrocyte O3I

monitoring [29]. This average O3I is comparable to previous published data in studies of athletes prior to fish oil supplementation or dietary intervention that have consistently demonstrated a mean O3I <5% in North American collegiate athletes [30-32], Australian rugby league athletes [33] and Canadian elite rugby 7 s players [34]. Furthermore, in the cohort of 106 German elite winter-endurance athletes consuming their usual training diet, the mean O3I was reported to be <5% and only one individual was >8% [12] which in the context of their very high cardiovascular load is intriguing. In fact, the low mean O3I values observed in this German study, consistent with previous research, strengthen the argument for routinely measuring baseline erythrocyte EPA + DHA concentrations as part of best practice in omega-3 study designs [35].

After 12 weeks of supplementation with either the fish-derived or algal-derived preformed omega-3 EPA + DHA supplements, both groups effectively raised the O3I (FO: +1.63%; AO: +1.76%), predominantly because of significantly increased whole blood DHA, with no significant differences in either the O3I or DHA levels observed between groups. These findings demonstrate that algal-derived LCn-3PUFA were equally as effective as those derived from fish in increasing the O3I, and further, endurance training did not negatively affect omega-3 EPA + DHA membrane incorporation. This finding of an improved O3I whilst training is contrary to previous reports that high exercise volumes might reduce O3I in runners [36] and American National Football League players [37] during peak training and competition seasons, which may likely be best explained by inadequate intakes. Our study reveals that endurance athletes who regularly consumed 6 tablets daily (~600 mg/day EPA + DHA) could in fact increase their O3I, irrespective of the source of LCn-3PUFA, highlighting the equivalent bioavailability from algae. This is consistent with evidence that exercise can enhance the incorporation of omega-3 EPA + DHA into skeletal muscle [38] as longs as the intake is sufficient. In fact, further observations that elite cyclists can also maintain an optimal O3I across a season of Grand Tours, as long as the provision of LCn-3PUFA is consistent [39] reinforces this principle of adequate intake.

Evaluating and advising on preformed omega-3 EPA + DHA intake is an effective strategy for optimising O3I levels, even amid intensive training. In fact, our study demonstrated an approximate 1.7% increase in the O3I on average for both groups, aligning with other research using similar daily doses of 450-700 mg/d EPA + DHA over at least 8 weeks in both trained [9] and untrained individuals [11]. The dosage used in the current study was chosen specifically because it corresponds to the approximate LCn-3PUFA intake from consuming two fatty fish meals per week and this represents a realistic dietary achievable rather than a therapeutic-dose. The dietary achievable dose used significantly raised the O3I above 6% in both supplement groups. However, this level did not reach the 8% O3I associated with the lowest risk of coronary heart disease [13]. To achieve an O3I of 8% or higher in a population with a baseline O3I of 4.6%, the dosage would likely need to be doubled to (and >1,200 mg of EPA + DHA daily) in line with LCn-3PUFA intake observed to sustain the O3I >8% in elite professional cyclists [39]. This increased intake should also be maintained over several months to effectively optimise body tissue levels of EPA and DHA across the body's membranes [40].

An optimised O3I of >8% is certainly the recommended population target especially as it relates to cardio-protection from DHA intake [11]. Arguably, endurance trained individuals also benefit from the exercise derived cardiovascular benefit of physical activity [41]. Yet, protracted endurance training can be arduous on both the heart and vascular system [42]. It was with this physiological strain in mind that the cohort of German elite winter-endurance athletes were first studied for their habitual O3I [12] observing the theoretical basis for providing LCn-3PUFA to the cardiac and vascular

cells in order to support life-long coronary artery health. In the time since the study of these winter endurance athletes, observations have been made in physically trained cohorts of LCn-3PUFA being attributed to reductions in submaximal heart rate [7, 8], improved heart rate recovery [9], modified vascular reactivity [43], cardiac output [44] and attenuated sympathetic drive [45]. Yet, although more challenging to reveal, the potential role of EPA and DHA for nutritional pre-conditioning the cardiovascular system across a life span of endurance training is theoretically possible, but as of yet, needs to be prospectively tested.

The innovative chewable tablets used in this study were produced using a proprietary microencapsulation technology (Nu-Mega Ingredients), resulting in two distinct types: one derived from algae (Driphorm® HA DHA-S® 200) and the other from anchovy (Driphorm® HA HiDHA 300®). The chewable tablets were generally well tolerated by the vast majority of the participants. As a pilot study, the participants were engaged in a brief discussion at the 12 weeks follow up. A common theme was they 'enjoyed the orange flavor'. However, it has to be acknowledged that the dose per tablet required consumption of six chewable tablets per day. One participant was excluded due to self-reported non-compliance when they contacted the research team (at approximately week 6) to declare they were not engaged with consistent/daily consumption and no longer interested in taking part in the study. It was a limitation of the pilot study that a tablet count was not conducted at week 12, and should be done so for future interventions using this method of delivery in the context of randomized control studies. Two participants withdrew from the study due to minor self-perceived gastrointestinal discomfort (within the first 14 days of commencing) the consumption. The gastrointestinal discomfort was likely related to the sugar alcohol content of the tablets, particularly sorbitol which has dose-dependent effects on symptoms relating to flatulence, abdominal discomfort, and laxative effects [46]. Therefore, the supplement delivery method used in the current study would likely be unsuitable for providing higher doses of EPA + DHA unless the concentration per tablet could be increased (requiring fewer total tablets to be consumed per day). In practice, the delivery of microencapsulated EPA + DHA does not need to be in the form of tablets. The technology of the powder is designed to be included as an 'ingredient' in whole foods. Specifically, the powders are designed to be included in a multitude of products including the sports nutrition market and thus offering choice to the consumer. When derived from algae, they provide a dietary option for the plant based individual.

The O3I increase in the AO group was equivalent and not significantly different to that achieved in the FO group, indicating that algae-derived preformed omega-3 EPA + DHA supplements are a feasible alternative for athletes who prefer vegan or vegetarian diets. Notably, the four vegan athletes in our study started with lower baseline O3I levels than their omnivorous counterparts, a trend consistent with previous studies showing lower O3I in vegans and vegetarians [17]. Humans show poor conversion of dietary ALA to LCn-3PUFA like DHA, with conversion rates of only 0-3% [47]. Accordingly, algal-derived supplements, which contain preformed omega-3 EPA + DHA, offer vegans and vegetarians the most effective way to enhance their O3I and related whole blood fatty acid profile markers. Minor differences were observed in individual fatty acid responses post-supplementation. Specifically, the FO group demonstrated a slight increase in whole blood EPA levels compared to the AO group (EPA increase, FO: 0.21 ± 0.22%; AO: 0.04 ± 0.20%, P = 0.1381), leading to a near-significant decrease in the AA:EPA ratio in the FO group but not in the AO group. Whilst most algal strains contain a higher DHA content relative to EPA, compositional differences

do exist across strains which can be used to tailor the EPA + DHA dose of the final product depending on the desired outcome [48].

Growing awareness of the nutritional benefits of preformed LCn-3PUFA is boosting demand for EPA + DHA-rich fish oil, straining global supply chains and raising ecological concerns [16]. Furthermore, substituting red meat with aquatic animal-source food or forage fish like anchovies, herrings, and sardines has been suggested as a way to reduce diet-related non-communicable diseases, especially in low- and middle-income countries [49]. However, only 26% of forage fish are consumed directly by humans; the rest is processed into fishmeal and fish oil for aquaculture and high-income markets [50]. This study has demonstrated that algal-derived microencapsulated EPA and DHA are as effective as fish-based LCn-3PUFA in boosting the O3I to support physiological health outcomes. Algae-sourced LCn-3PUFA offers an alternative food and differing environmental impact with its potential to be farmed away from the coastline. It also supports carbon capture, aligning with the United Nation Sustainable Development Goals 12 (Responsible Consumption and Production) and 13 (Climate Action). Notwithstanding, technological advancement is necessary to address the life cycle analysis of EPA + DHA production from microalgae and fish biomass, ready for inclusion in the human diet [51]. This advancement must include microencapsulation techniques, such as the powder used in this current study, to enhance the product stability.

5. Conclusions

This study demonstrated that algal derived microencapsulated EPA and DHA are equally as effective as fish sources for increasing the O3I in endurance athletes taking part in usual training. With a daily dosage equivalent to consuming approximately two fatty fish meals per week, over 12 weeks, participants demonstrated an average O3I increase of 1.7%, validating the efficacy of ~600 mg EPA + DHA daily in increasing omega-3 status in male and female participants. Overall, this study underscores the potential for algal-derived preformed omega-3 EPA + DHA supplementation strategies to support cardiovascular health of training athletes, while also promoting the use of non-animal marine source of LCn-3PUFA. As a pilot study, these results provide a strong basis to delivering EPA and DHA via a microencapsulated form (powder) for future randomised controlled trials, using food products rather than oil capsules.

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Author Contributions

The conceptualisation was carried out by G.E.P and M.J.M, while R.A was responsible for data curation. R.A conducted the investigation and formal analysis. The collaborative research agreement (*in kind* provision) was managed by G.E.P. R.A was in charge of project administration. Supervision was handled by G.E.P, M.J.M. The original draft was written by R.A, and the review and editing of the writing were done by all authors.

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Competing Interests

The authors have declared that no competing interests exist.

Data Availability Statement

De-identified data generated or analysed during this study are available from the corresponding author upon reasonable request.

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