Section: Original Research

Article Title: n-3 Fatty Acid Supplementation During 4 Weeks of Training Leads to Improved Anaerobic Endurance Capacity, But Not Maximal Strength, Speed, or Power in Soccer Players

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ABSTRACT

Omega-3 fatty acid (n-3 FA) supplementation could promote adaptation to soccer-specific training. We examined the impact of a 4 wk period of n-3 FA supplementation during training on adaptations in 1RM knee extensor strength, 20m sprint speed, vertical jump power, and anaerobic endurance capacity (Yo-Yo test) in competitive soccer players. Twenty six soccer players were randomly assigned to one of two groups: n-3 FA supplementation (n-3 FA; n=13) or placebo (n=13). Both groups performed two experimental trial days. Assessments of physical function and respiratory function were conducted pre (PRE) and post (POST) supplementation. Training session intensity, competitive games and nutritional intake were monitored during the 4 wk period. No differences were observed in respiratory measurements (FEV1, FVC) between groups. No main effect of treatment was observed for 1RM knee extensor strength, explosive leg power, or 20 m sprint performance, but strength improved as a result of the training period in both groups ($p<0.05$). Yo-Yo test distance improved with training in the n-3 FA group only ($p<0.01$). The mean difference (95% CI) in Yo-Yo test distance completed from PRE to POST was 203 (66 to 340) m for n-3 FA, and 62 (-94 to 217) m for placebo, with a moderate effect size (Cohen's d of 0.52). We conclude that 4 wk of n-3 FA supplementation does not improve strength, power or speed assessments in competitive soccer players. However, the increase in anaerobic endurance capacity evident only in the n-3 FA treatment group suggests an interaction that requires further study.

Keywords: Fish oils, lipids, exercise, adaptation.

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INTRODUCTION

Several studies have demonstrated potential health benefits from the consumption of fish or fish oil derived omega-3 polyunsaturated fatty acid (n-3 FA) supplementation. For example, fish consumption has been associated with a reduced risk of developing coronary heart disease (Whelton et al., 2004; Hu et al., 2002). These observations have been explained by a decrease in plasma triglycerides (Harris, 1997; Hartweg et al., 2008), moderate reduction in blood pressure (Geleijnse et al., 2002) and reduced blood platelet aggregation (Hornstra, 2001) with fish or n-3 FA ingestion. Within the context of exercise training adaptation, n-3 FA's have been reported to impact upon inflammation and immunity (Shei et al., 2014), and elicit beneficial effects on pulmonary function in wrestlers (Tartibian et al., 2010). Moreover, dietary supplementation of n-3 FA (~3 g/day) over 8 wk has been shown to alter muscle lipid composition and increase the rate of muscle protein synthesis in young, middle-aged (Smith et al., 2011b) and older adults (Smith et al., 2011a) under experimental hyperinsulinaemic–hyperaminoacidaemic clamp conditions. Hence, there is clear evidence that n-3 FA ingestion has the potential to modulate a wide range of physiological processes that could, theoretically, enhance adaptation to exercise training.

Recently, we demonstrated that as little as 2 wk of supplementation with n-3 FA (4.5 g/day) was sufficient to significantly alter muscle lipid composition, and induce changes in key regulatory signalling proteins such as focal adhesion kinase protein content, and protein kinase B activity (McGlory et al., 2014). Further examination of the acute muscle protein synthesis (MPS) response to an exercise stimulus and/or protein feeding revealed no statistical difference between n-3 FA supplemented or placebo groups, but interestingly a similar MPS response was observed concomitant with a suppressed activity of several key

anabolic signalling proteins such as p70 s6 kinase (McGlory et al., 2016). These data suggest that short periods of n-3 FA supplementation can alter muscle lipid composition and improve the efficiency of the signalling pathways involved in stimulating MPS. Other findings from animal and cell-based studies are consistent with some of these observations and have identified a potential action of n-3 FA on muscle protein breakdown (MPB, Kamolrat and Gray, 2013).

To date, studies in humans that directly investigated the link between fish oil derived n-3 FA supplementation and adaptations to exercise training have revealed somewhat conflicting, and arguably incomplete, findings. Studies in older adults have reported beneficial impacts of n-3 FA supplementation on reducing/reversing the loss of muscle mass and function over a 6 month intervention period (Smith et al., 2015), have demonstrated a modest improvement in muscle quality (torque per unit cross sectional area) in older females, or have demonstrated no benefit beyond training alone in older males (Da Boit et al., 2017). Other studies report no effect of n-3 FA intake on endurance exercise performance or recovery after 4-10 wk of training (Buckley et al., 2009; Guzman et al., 2011; Raastad et al., 1997; Walser & Stebbins, 2008), and no impact of n-3 FA intake on improvements in high intensity treadmill running capacity following 4 wk of training in recreationally active males and females (Huffman et al., 2004).

Variations in the dose and composition of n-3 FA supplements, sample populations, and different performance-related endpoints assessed, makes it difficult to compare findings and provide firm conclusions about whether to recommend n-3 FA supplementation to sport teams. For example, ingestion of 2g/day of DHA and 3g/day of EPA over 6 wk was observed to enhance stroke volume and cardiac output during dynamic exercise (Walser & Stebbins,

2008), while ingestion of 3.5g/day of DHA rich fish oil over 4 wk improved complex reaction time in female soccer players (Guzman et al., 2011). However, other studies have not observed positive outcomes from n-3 FA supplementation (1.9g DHA rich oil, 4g EPA rich oil or 2.6g EPA/DHA balanced dose) when assessing endurance performance (Buckley et al., 2009; Huffman et al., 2004; Raastad et al., 1997), respectively. Similarly, it is clear that previous training studies in young active participants with n-3 FA supplementation have focussed on cardiovascular function outcome measurements with none assessing muscle-related changes in strength, speed or power, which could be crucial for improving performance outcomes in team sport players. Given the potential for n-3 FA supplementation to have an influence on muscle mass or function either through MPS, MPB or changes in muscle quality it could be hypothesized that n-3 FA could impact upon strength, speed or power.

The present study was therefore designed to examine a 4 wk intervention of high dose fish oil derived n-3 FA during a 4 wk period of monitored soccer training. Since a higher dose of n-3 FA supplementation over 4 wk (McGlory et al., 2014) leads to changes in the lipid composition of the skeletal muscle lipid pool comparable to a lower dose ingested over a more prolonged period (Smith et al., 2011b), we chose to supplement with a high dose of 0.1g/kg/day. We aimed to clarify the potential impact of n-3 FA supplementation on adaptations in strength, speed, power and anaerobic endurance capacity after a 4 wk training period in competitive soccer players. We hypothesized that n-3 FA supplementation at a dose of 0.1g/kg/day would enhance adaptation of physical function measures over a 4 wk training period.

METHODS

Participants

Twenty-six (24 ± 5 y) competitive soccer players volunteered for the study (n=19 males and n=7 females). Soccer players were recruited from International squads, local soccer academies, and the University soccer teams. All players were informed about the study and the potential risks involved. Smokers, those with a cardiovascular, metabolic, or neuromuscular disorder, or those with a history of n-3 FA supplementation were excluded. Volunteers eligible for the study provided their signed informed consent for participation. The study was approved by the local Ethics Committee.

Study design

Participants attended an initial familiarisation session in which a baseline blood sample was drawn to determine blood n-3 FA status and participants also performed a range of functional tests (respiratory and physical function tests as described below) that would be assessed on trial days. Following laboratory analysis of the baseline blood samples, participants were then pair matched (within their respective teams), based on baseline total n-3 FA (expressed at % of total lipids in the blood), into either the n-3 FA group (n=13, 9 males, 4 females) or Placebo group (n=13, 10 males, 3 females). While we did not directly assess fish consumption, the lipid profiles obtained pre-supplementation in both groups were consistent with participants who had not eaten large quantities of oily fish in the period immediately preceding the study. All participants were required to visit the laboratory on two further occasions for experimental trial days (one pre supplementation trial, and one post supplementation trial) where blood was drawn and participants were asked to complete a set of respiratory and physical function tests. Participant's dietary intakes were logged using a

three day weighed food diary prior to the pre-supplementation testing day. Food intake was then replicated over the three days prior to the post-supplementation testing day. Participants also recorded a training diary, logging intensity and duration of training sessions and competitive games played before the pre-supplementation testing and this was replicated throughout the supplementation period. Training intensity was evaluated on a 1-5 zone rating scale (where 1 indicates a very light intensity and 5 a very hard intensity). Training duration was noted and a total training impulse (TRIMP) score was calculated by multiplying duration by intensity of sessions (Foster et al., 1996). All participants were asked to avoid intense exercise, and alcohol intake for 48 h leading up to each of the testing days and were required to be fasted (>10 h) prior to testing.

Supplementation period

Participants in both n-3 FA and placebo groups were asked to ingest the equivalent of 0.1g/kg of supplement in capsules per day (mean intake: 7 ± 2 capsules per day). As well as wishing to provide a high absolute dose a mass adjusted dose was used as we anticipated that the body mass range of participants might be quite large due to inclusion of males and females in the study design. Capsules were ingested with meals throughout the day (2-3 capsules at each meal time). Each 1000 mg n-3 FA capsule contained eicosapentanoic acid (EPA, 70%), docosahexanoic acid (DHA, 20%), docosapentanoic acid (2%) and vitamin E (0.02mg). An EPA-rich supplement was chosen given that EPA rather than DHA is known to be biologically active in skeletal muscle (Kamolrat and Gray, 2013). The placebo group ingested 1000 mg capsules containing caprylic acid 8:0 (7%), capric acid 10:0 (92%) lauric acid 12:0 (0.9%) and palmitic acid 16:0 (0.3%)). In an attempt to isolate an effect of n-3 FA, short chain saturated fats were included in the placebo condition to avoid manipulation of the n-6:n-3

ratio in both directions with supplementation. Both sets of capsules were supplied through Ideal Omega (Glasgow Health Solutions, UK). All capsules were identical in colour and size and were distributed in a double blind manner.

Experimental trial days

Participants arrived at the laboratory in a fasted state at ~07:00 and were seated in a comfortable environment for 15 minutes prior to a blood sample being drawn. Participants then undertook pre-exercise respiratory function assessments before completing a range of physical function tests designed to measure maximal strength, power, speed and anaerobic endurance during intermittent exercise, in that order. Finally, a post-exercise respiratory function assessment was conducted. The total time period to conduct all assessments was approximately 3 hours.

Respiratory function

Two respiratory function tests were performed immediately before and ~10 minutes after the physical function tests: 1) forced expiratory volume in one second (FEV1) and, 2) forced vital capacity (FVC). Both measures of respiratory function were repeated a minimum of three times each to meet the European Respiratory Society standards (Miller et al., 2005). Peak expiratory flow (PEF) values also were recorded from the outcome of these tests.

Physical function

Single leg 1RM on a leg extension machine was conducted on each leg separately to assess muscle strength. After the completion of 3 warm up sets of two repetitions each (at 40%, 60% and 80% of the 1RM load achieved in the familiarisation session). Participants were then allowed 3 attempts at increasing loads (determined by the stack increments) to lift their 1RM (with 1 min rest between lifts). Successful lifts were only accepted when the participant's

leg reached full extension, as verified visually by the experimenter. After a 5 min rest period participants then completed a vertical squat jump test (Takei 5406 vertical jump meter) to assess explosive power in the legs. This test involved 3 practice jumps followed by 3 attempts to reach maximal jump height using a countermovement jump technique (knee flex to 90°, without the use of arms). After a further 5 min rest period participants completed a 5 min warm-up prior to assessment of sprint time over 20 m to assess speed. Sprint warm-up consisted of 3 runs at ~50%, 3 at ~70%, 2 at ~80% and 1 at ~90% of their maximal sprint speed over 20 m (with a 2 min active recovery period between each sprint). Participants then completed 5 x 20 m maximal sprints with 2 minute recoveries. In all tests of strength, power and speed the best effort was recorded. Participant's then completed the intermittent recovery Yo-Yo level 1 test (Yo-Yo IR-1) (Bangsbo et al., 2008) to assess anaerobic endurance capacity.

Blood lipid analysis

Samples of whole blood were placed onto two circular collection spots on Whatman 903 blood collection cards (GE Healthcare Ltd, Forest Farm Industrial Estate, Cardiff, CF 14 7YT, UK). The cards were left open and allowed to dry for 3 h after which the dried whole blood sample was detached from the collection device using forceps and placed into a screw-cap vial containing 1 mL of methylating solution (1.25M methanol/HCl). The vials were placed in a hot block at 70°C for 1 h. The vials were allowed to cool to room temperature before 2 mL of distilled water and 2 mL of saturated KCl solution were added. Fatty acid methyl esters (FAME) were then extracted using 1 × 2 mL of iso-hexane + BHT followed by a second extraction using 2 mL of isohexane alone. This extraction method has been previously validated as a reliable measure of whole blood fatty acid composition in our own laboratories

(Bell et al 2011). FAME were then separated and quantified by gas-liquid chromatography (ThermoFisher Trace, Hemel Hempstead, England) using a 60 m x 0.32 mm x 0.25 µm film thickness capillary column (ZB Wax, Phenomenex, Macclesfield, UK). Hydrogen was used as carrier gas at a flow rate of 4.0 mL.min-1 and the temperature program was from 50 to 150°C at 40°C·min-1 then to 195°C at 2°C·min-1 and finally to 215°C at 0.5°C·min-1. Individual FAME were identified compared to well-characterised in house standards as well as commercial FAME mixtures (Supelco™ 37 FAME mix, Sigma-Aldrich Ltd., Gillingham, England).

Data presentation and statistical analysis

All data are presented as means ± standard deviation (SD) in the text and Tables and as mean ± standard error of the mean (SEM) in Figures, unless otherwise stated. Mean difference and (95% confidence interval) was calculated where appropriate and Cohen's d (Cohen, 1988) effect size used to indicate magnitude based inferential statistics. Statistical analysis was performed using SPSS (IBM statistics, version 21) software. A repeated measures ANOVA was used to detect changes from pre supplementation to post supplementation for the blood fatty acid profile, respiratory function, and physical function tests. Main effects of time (pre to post training/supplementation period), treatment (n-3 FA *vs*. Placebo), and treatment by time interaction effects were examined. Post-hoc analyses were paired or independent T-tests as appropriate. ANOVA was used to assess differences in dietary intake and training information between groups. Tests were considered significant at $p \leq 0.05$.

RESULTS

Anthropometric, training and dietary intake data

No differences were observed between groups for age, body mass and height, or mean duration and intensity of training (**Table 1**). There also were no significant differences

between n-3 FA and placebo groups in dietary intakes from the recorded 3 day food diaries (**Table 1**).

Blood fatty acid profile

Pre-supplementation blood n-3 FA content was not different between treatment groups but there was a significant time effect and interaction between time and group on blood fatty acid content (**Table 2**). Following the 4 wk supplementation period there was a decrease in the proportion of total n-6 fatty acids, and a decrease in the arachidonic acid to EPA ratio (20:4n-6/20:5n-3) in the n-3 FA group. There also were increases in the proportion of total n-3 FA ($p<0.01$) and in the %n-3 highly unsaturated fatty acids (HUFA) / Total HUFA ratio ($p<0.01$) in the n-3 FA group. The proportion of monounsaturated fat was significantly higher pre and post supplementation in the placebo group.

Respiratory function

There were no treatment, time or treatment by time interactions for respiratory function measures. (**Table 3**).

Physical function

No significant treatment effect or treatment by time interaction was observed for leg strength (**Figure 1**). However, a significant time effect was evident with an increase in leg strength (left and right legs) observed in both groups (both $p<0.05$). No treatment, time or treatment by time effects were observed for explosive power in the legs assessed by vertical jump score (**Figure 2A**), or for 20 m sprint times (**Figure 2B**). There was a significant time effect for maximal anaerobic endurance capacity (**Figure 3**, $p<0.05$) but no treatment or treatment by time interaction was observed. Follow-up post hoc analysis revealed that the significant increase over time was only evident in the n-3 FA group ($p<0.01$). The mean difference (95%

CI) in Yo-Yo distance completed from pre to post supplementation was 203 (66 to 340) m in the n-3 FA group, and 62 (-94 to 217) m in the placebo group. The calculated effect size (Cohen's d) for the difference between groups was 0.52 (a moderate positive effect).

DISCUSSION

In the present study, we have demonstrated a potential impact on anaerobic endurance capacity after training only in the n-3 FA supplementation group. However, our data indicate no impact of n-3 FA supplementation on training-induced adaptations in muscle strength, power, and speed assessments. We also demonstrate that 4 wk of n-3 FA supplementation during training does not impact resting or post-exercise FEV1, FVC or PEF.

The diverse physiological requirements of soccer (Bangsbo et al., 1991; Spencer et al., 2005) dictate that studies designed to investigate the influence of a dietary intervention on soccer-specific measurements of physical function require a multi-dimensional approach. Accordingly, a battery of soccer-specific physical function tests was performed, including leg strength, explosive power, sprint speed, and anaerobic endurance capacity. Although improvements in strength were observed in both groups over the 4 wk training period, no significant differences were observed between n-3 FA and placebo groups for any measurement of strength, power or speed. Interestingly, whereas sprint speed times recorded in the present study were similar to previous literature (Gore, 2000), strength scores were, on average, lower than those reported previously in French elite division players (Cometti et al., 2001). This discrepancy in strength scores was likely due to the inclusion of female soccer players in the present study. The absence of any differences between groups in strength, speed or power tests could lend support to our previous observations in which we demonstrated no impact of n-3 FA supplementation on the acute response of MPS to

resistance exercise and protein ingestion in resistance-trained young men (McGlory et al., 2016), although it should be noted that acute post-exercise measures of MPS do not necessarily, at least quantitatively, reflect chronic changes in muscle mass (Mitchell et al., 2014).

An interesting observation from the present study is the potential influence of n-3 FA supplementation in promoting anaerobic endurance capacity after 4 wk of regular soccer training. Although there was no significant interaction effect between treatment group and time in the statistical analysis, we observed a significant time effect which revealed an improvement in distance covered on the Yo-Yo test which was evident in the n-3 FA group only. The test-retest reliability of the Yo-Yo IR1 test is reported to range between 4.9% and 8.1% (Bangsbo et al., 1991). Whereas 8 out of 13 participants exceeded the CV of the test method in the n-3 FA group, only 3 of 13 exceeded the CV in the placebo group. A sample size estimate indicates that, for 80% power, with a mean difference of 141 m in the Yo-Yo test score, and a pooled standard deviation of 270 m, 29 participants per group would be required. However, the magnitude of difference, alongside the moderate effect size (0.52) observed, suggests that the combination of high dose n-3 FA supplementation during training might lead to a positive impact on a soccer-specific measurement of anaerobic endurance capacity. However, the absence of a significant interaction means that further work in a larger sample is required to clarify this potential effect.

While we cannot determine the mechanism(s) responsible for any improvement in anaerobic endurance capacity with n-3 FA supplementation, it could be speculated that n-3 FA may have led to improvements in blood flow to contracting skeletal muscle during exercise, which subsequently delayed the onset of fatigue. Zebrowska et al., (2015) reported

an increase in circulating nitric oxide (NO), following a 3 wk period of supplementation with n-3 FA, which was evident at rest and during exercise. The elevated NO also was associated with a greater improvement in VO_{2max} in their n-3 FA group. If a similar effect of n-3 FA intake on NO was evident in our study then this may have improved Yo-Yo test performance in our cohort of competitive soccer players. However, further work is required to verify the outcome we have observed by conducting larger scale intervention studies and also acute mechanistic studies to directly address mechanisms.

Sex differences in response to training with n-3 FA supplementation have been recently reported by Da Boit et al. (2017). Their observations suggest that muscle quality (torque per unit cross sectional area) was improved with n-3 FA supplementation compared with placebo in females, whereas no impact of n-3 FA supplementation was evident in males. Due to using a mixed sex sample in the present study it is possible that this may have impacted upon our observed outcomes. However, for the key variables of interest (e.g. anaerobic endurance, strength, speed and power) there was no apparent sex-difference in response to n-3 FA supplementation between the 19 males and 7 females.

Whereas it has previously been documented that n-3 FA supplementation improves respiratory function in athletes, the majority of these studies recruited athletes diagnosed as suffering from asthma and exercise induced bronchoconstriction (Gore, 2000; Spencer et al., 2005; Mickelborough et al., 2003 & 2006). In our study, baseline measurements of FEV1, FVC and PEF confirmed that none of the study volunteers experienced any respiratory limitation. We observed no influence of n-3 FA supplementation on three measurements of respiratory function in healthy players. Hence, in the absence of impaired respiratory function, the

influence of n-3 FA supplementation on respiratory function appears to be absent in healthy individuals.

A common methodological consideration with any medium- to long-term human study is compliance to the intervention. In the present study, dietary supplementation of n-3 FA significantly reduced % of total n-6 lipids, and significantly increased the % of n-3 lipids in blood. The ratio of n-3 HUFA:total HUFA (%) in blood of the n-3 FA group increased by 80% with no change in the placebo group. Similarly, the ratio of arachidonic acid to EPA (an inflammation index) decreased by more than 80% in the n-3 FA group but remained unchanged in the placebo group. Significant treatment differences are expected in blood lipid composition with EPA showing increases of 175% and DHA increases of 40% following 10 wk of omega-3 supplementation (Raastad et al., 1997). In the present study, EPA content increased by more than 600% and DHA content increased by 37% following the n-3 FA supplementation period. From our previous work we have demonstrated that such changes in the EPA and DHA content of blood are quickly reflected in tissue lipid changes (McGlory et al., 2014). These changes in blood fatty acid profile, combined with data gathered from the pill count strategy, provide both direct and indirect evidence that all soccer players complied with the dietary intervention during the study period. On this basis, and given that no apparent general differences in habitual diet or training volume existed between groups, we are confident that n-3 FA supplementation played a role in improving anaerobic endurance capacity.

NOVELTY AND PRACTICAL APPLICATION STATEMENTS

This study reveals a significant increase in anaerobic endurance capacity during intermittent exercise in soccer players following 4 wk of structured training while

supplementing with n-3 FA. However, further work is required in larger studies to confirm our observation, and to explore the mechanism(s) involved. We also can conclude that 4 wk of n-3 FA supplementation did not improve strength, power or speed assessments of physical function, nor tests of respiratory function.

The dose of n-3 FA administered in this study was a high dose provided on a body mass adjusted basis to maximise incorporation of n-3 FA into cell membranes, and this ingestion is unlikely to be feasible for all soccer players. Future studies should investigate the dose-response effect of n-3 FA supplementation on training induced outcomes, or examine whether a loading dose followed by a maintenance dose can sustain the lipid changes observed.

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SDRG and FFB planned the study. LG, FFB, LA, OCW and SDRG assisted in data collection. JD, GB, LG, LA, FFB and SDRG completed sample and data analysis. LG and SDRG drafted the manuscript. All other authors contributed to editing the content. Glasgow Health Solutions Ltd funded the study and supplied all n-3 FA and placebo supplements.

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Figure 1. Mean (SEM) 1RM Left (panel A) and 1 RM Right (panel B) strength scores for leg extension exercise before (Pre) and after (Post) 4 weeks of n-3 FA or placebo supplementation. * indicates that there was a main effect of time ($p<0.05$) for Pre to Post measures in both groups. No treatment or time by treatment interaction was observed.

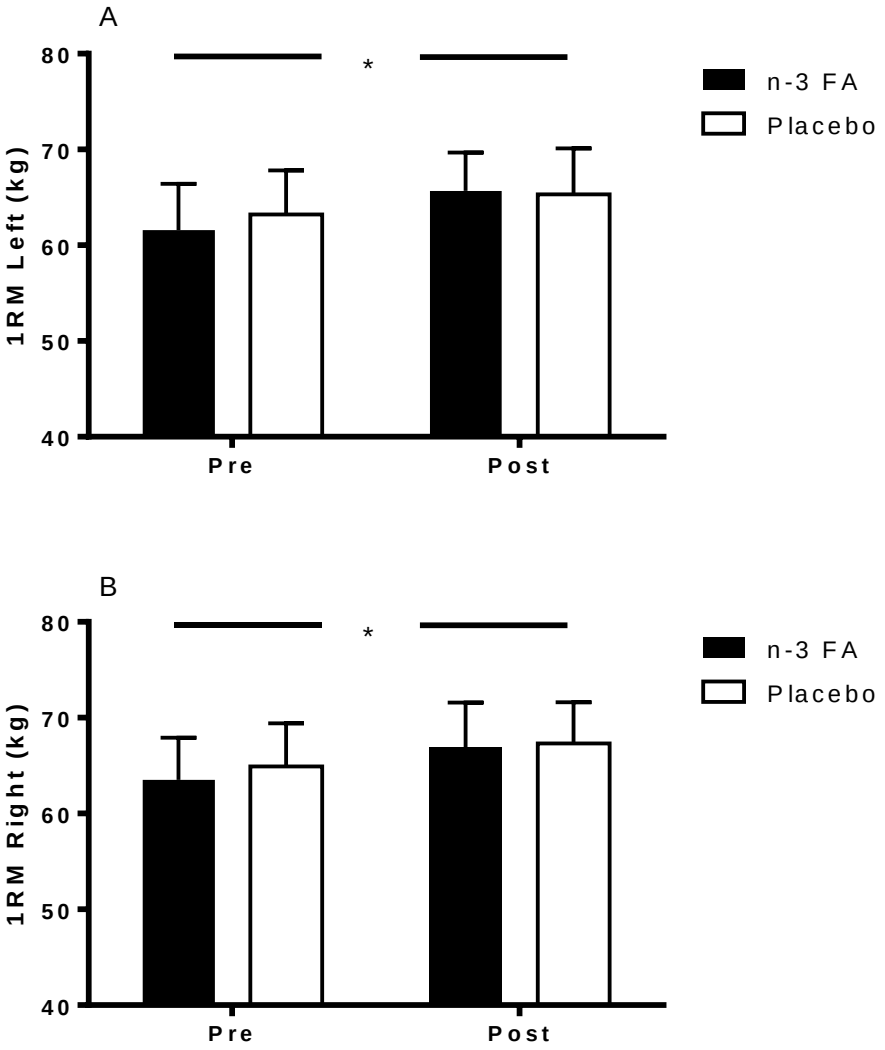

Figure 2. Mean (SEM) vertical jump height (panel A) and 20 m sprint time (panel B) before (Pre) and after (Post) 4 weeks of n-3 FA or placebo supplementation. No main effects of time, treatment or time by treatment interaction were observed.

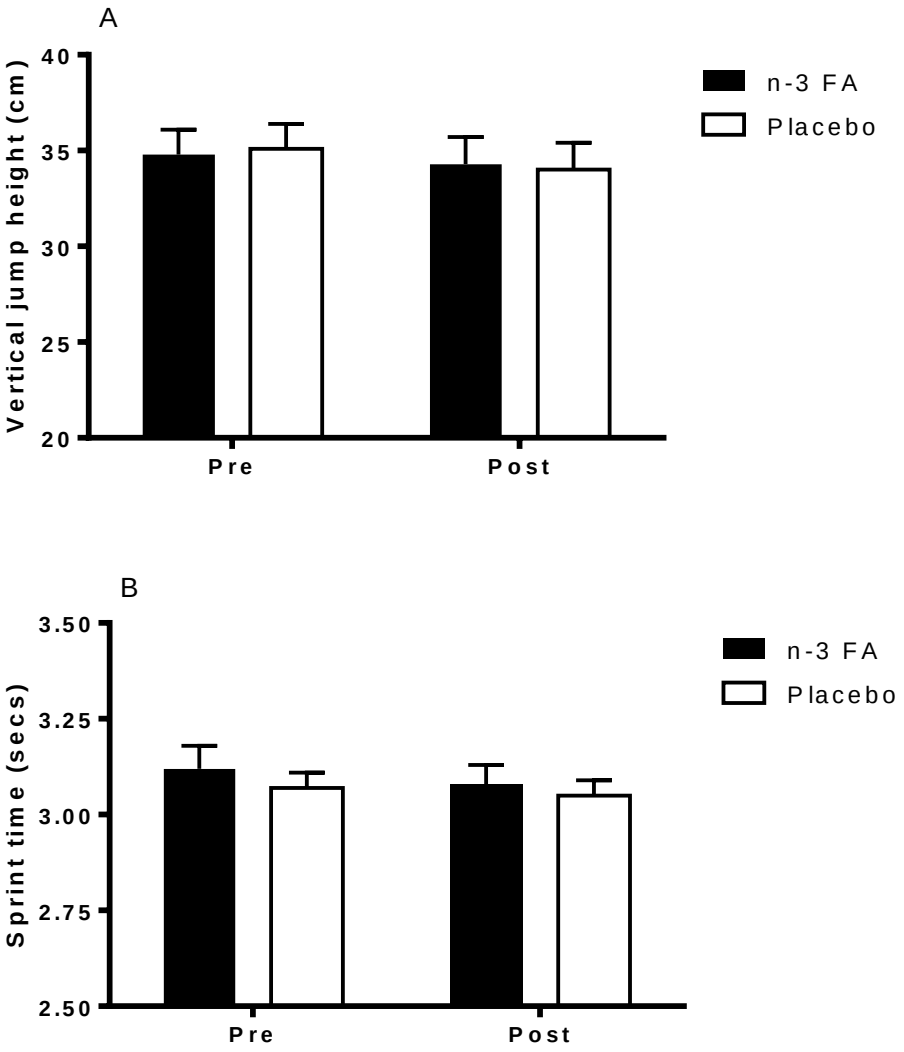

Figure 3. Panel A, mean (SEM) Yo-Yo test distance (m) covered before (Pre) and after (Post) 4 weeks of n-3 FA or placebo supplementation. ** indicates that there was a significant main effect of time ($p<0.01$) which was only evident in the n-3 FA group. Panel B, individual changes in Yo-Yo distance covered (with mean difference and 95%CI) from Pre to Post supplementation in n-3 FA and placebo groups. Dotted line in panel B represents twice the coefficient of variation of the test method. Grey shaded data points in panel B refer to female participants' data.

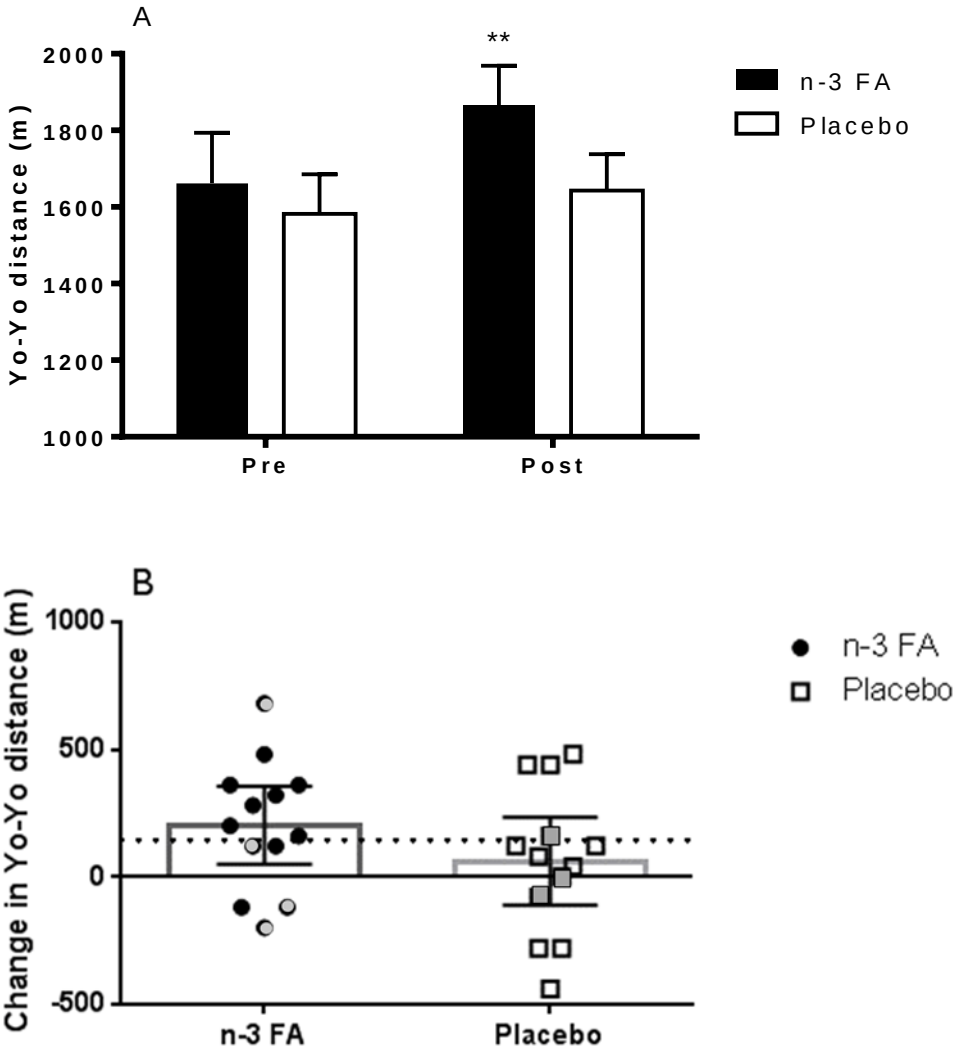

Table 1. Mean (±SD) physical characteristics, training data, and dietary intake of soccer players from n-3 FA (n=13) and Placebo (n=13) supplemented groups. No differences were observed between groups for any variable.

Variable	**Placebo**	**n-3 FA**
Age (y)	21 ± 3	24 ± 5
Body mass (kg)	71.2 ± 12.7	71.2 ± 11.4
Height (cm)	174.8 ± 8.8	175.0 ± 11.5
Training duration (h/wk)	5.7 ± 0.6	5.6 ± 0.7
Training intensity (1-5)‡	3.4 ± 0.3	3.4 ± 0.4
TRIMP	1118 ± 84	1096 ± 68
Energy intake (kcal/d)	2188 ± 964	2126 ± 786
Carbohydrate (g/d)	304 ± 177	303 ± 149
Protein (g/d)	87 ± 33	81 ± 24
Fat (g/d)	75 ± 30	72 ± 21

‡ intensity score ranged from 1 (very light) to 5 (very hard).

Table 2. Mean (±SD) blood fatty acid profile (expressed as % of total lipids in the blood) of soccer players before (PRE-SUPPLEMENTATION) and after 4 weeks dietary supplementation (POST-SUPPLEMENTATION) with n-3 FA (n=13) or Placebo (n=13).

	PRE-SUPPLEMENTATION			**POST-SUPPLEMENTATION**		
	n-3 FA	Placebo	P value	n-3 FA	Placebo	P value
Saturated fats (%)	36.8 ± 2.0	35.8 ± 1.3	0.14	35.7 ± 1.3	35.9 ± 1.1	0.62
Monounsaturated fats (%)	20.1 ± 3.7	21.5 ± 1.1	0.23	19.5 ± 2.1[a]	20.9 ± 1.5[a]	0.07
Total n-6 (%)	33.3 ± 2.7	33.7 ± 1.7	0.69	30.8 ± 3.0*	34.2 ± 1.2	0.00
Total n-3 (%)	5.2 ± 0.8	5.3 ± 0.9	0.93	10.4 ± 2.5**	5.2 ± 0.7	0.00
20:4n-6/20:5n-3 (%)	17.7 ± 3.7	17.4 ± 4.8	0.82	3.0 ± 2.2**	17.5 ± 3.3	0.00
n-3 HUFA/Total HUFA (%)	25.2 ± 3.1	24.6 ± 3.1	0.60	45.3 ± 8.8**	24.5 ± 2.6	0.00

P value columns represent assessment of differences between treatment groups at pre-supplementation and post-supplementation time points, respectively. [a] indicates significant effect of treatment (placebo greater than n-3 FA at both pre and post supplementation time points) * indicates significant treatment by time interaction ($p<0.05$) and ** significant treatment by time interaction ($p<0.01$) with significant changes in n-3 FA group pre to post supplementation. HUFA – highly unsaturated fatty acids.

Table 3. Respiratory function of soccer players assessed pre- and post- n-3 FA (n=13) or placebo (n=13) supplementation. Data (mean ± SD) were obtained before (PRE-EX) and after (POST-EX) completion of strength, power, speed and endurance capacity tests, respectively.

		PRE-SUPPLEMENTATION			POST-SUPPLEMENTATION		
		n-3 FA	Placebo	P value	n-3 FA	Placebo	P value
FEV1 (L)	**PRE EX**	4.11 ± 1.31	4.17 ± 0.49	0.87	4.06 ± 1.21	4.19 ± 0.54	0.73
	POST EX	4.21 ± 1.0	4.22 ± 0.63	0.97	4.21 ± 1.09	4.13 ± 0.62	0.85
FVC (L)	**PRE EX**	4.73 ± 1.39	5.00 ± 0.94	0.57	4.67 ± 1.37	5.01 ± 0.95	0.46
	POST EX	4.85 ± 1.16	5.03 ± 0.99	0.66	4.72 ± 1.15	4.94 ± 1.03	0.62
PEF (L/min)	**PRE EX**	524 ± 115	508 ± 75	0.68	516 ± 109	496 ± 76	0.59
	POST EX	538 ± 94	517 ± 81	0.56	543 ± 100	510 ± 83	0.38

P value columns represent assessment of differences between treatment groups at pre-supplementation and post-supplementation time points, respectively. FEV1 – forced expiratory volume, FVC – forced vital capacity, PEF – peak expiratory flow.