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Effects of 12 Wk of Omega-3 Fatty Acid Supplementation in Long-Distance Runners

MAJA TOMCZYK[1], ZBIGNIEW JOST[1], MACIEJ CHROBOCZEK[2], ROBERT URBAŃSKI[3], PHILIP C. CALDER[4,5], HELENA L. FISK[4], MATEUSZ SPRENGEL[6,7], and JĘDRZEJ ANTOSIEWICZ[6]

[1]Department of Biochemistry, Gdansk University of Physical Education and Sport, Gdansk, POLAND; [2]Department of Physiology, Gdansk University of Physical Education and Sport, Gdansk, POLAND; [3]Department of Biomechanics and Sports Engineering, Gdansk University of Physical Education and Sport, Gdansk, POLAND; [4]School of Human Development and Health, Faculty of Medicine, University of Southampton, Southampton, UNITED KINGDOM; [5]NIHR Southampton Biomedical Research Centre, University Hospital Southampton NHS Foundation Trust and University of Southampton, Southampton, UNITED KINGDOM; [6]Department of Bioenergetics and Exercise Physiology, Medical University of Gdansk, Gdansk, POLAND; and [7]Institute of Dietetics, University of Business and Health Sciences, Łódź, POLAND

BASIC SCIENCES

ABSTRACT

TOMCZYK, M., Z. JOST, M. CHROBOCZEK, R. URBAŃSKI, P. C. CALDER, H. L. FISK, M. SPRENGEL, and J. ANTOSIEWICZ. Effects of 12 Wk of Omega-3 Fatty Acid Supplementation in Long-Distance Runners. *Med. Sci. Sports Exerc.*, Vol. 55, No. 2, pp. 216–224, 2023. **Purpose:** This study aimed to investigate the effects of 12 wk of omega-3 fatty acid supplementation during endurance training on omega-3 index (O3I) and indicators of running performance in amateur long-distance runners. **Methods:** Twenty-six amateur male long-distance runners ≥29 yr old supplemented omega-3 fatty acid capsules (OMEGA group, n = 14; 2234 mg of eicosapentaenoic acid and 916 mg of docosahexaenoic acid daily) or medium-chain triglycerides capsules as placebo (medium-chain triglyceride [MCT] group, n = 12; 4000 mg of MCT daily) during 12 wk of endurance training. Before and after intervention, blood samples were collected for O3I assessment, and an incremental test to exhaustion and a 1500-m run trial were performed. **Results:** O3I was significantly increased in the OMEGA group (from 5.8% to 11.6%, $P < 0.0001$). A significant increase in $\dot{V}O_{2peak}$ was observed in the OMEGA group (from 53.6 ± 4.4 to 56.0 ± 3.7 $mL \cdot kg^{-1} \cdot min^{-1}$, $P = 0.0219$) without such change in MCT group (from 54.7 ± 6.8 to 56.4 ± 5.9 $mL \cdot kg^{-1} \cdot min^{-1}$, $P = 0.1308$). A positive correlation between the change in O3I and the change in running economy was observed when data of participants from both groups were combined (−0.1808 ± 1.917, $P = 0.0020$), without such an effect in OMEGA group alone ($P = 0.1741$). No effect of omega-3 supplementation on 1500-m run results was observed. **Conclusions:** Twelve weeks of omega-3 fatty acid supplementation at a dose of 2234 mg of eicosapentaenoic acid and 916 mg of docosahexaenoic acid daily during endurance training resulted in the improvement of O3I and running economy and increased $\dot{V}O_{2peak}$ without improvement in the 1500-m run trial time in amateur runners. **Key Words:** OMEGA-3 INDEX (O3I), POLYUNSATURATED FATTY ACIDS, RUNNING PERFORMANCE, ENDURANCE TRAINING, RUNNING ECONOMY

Omega-3 fatty acids include α-linolenic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), characterized by the first double bond on the third carbon atom from the methyl end of the fatty acyl chain. There is growing evidence that synthesis *de novo* of EPA and, in particular, DHA is limited in the human body, and sources of preformed EPA and DHA, e.g., seafood, especially fatty fish or supplements should be consumed (1,2). Despite this, athlete's intake of sources of omega-3 fatty acids is often inadequate (3,4). Harris and von Schacky (5) proposed the so-called omega-3 index (O3I) as a valid indicator of omega-3 PUFA status, reflecting both intake of these fatty acids and their biological and health effects. O3I is the sum of EPA and DHA expressed as a percent of total fatty acids in erythrocytes. It is proposed that values >8% are associated with the greatest cardioprotection, whereas values <4% are associated with the least (5). O3I has been recognized as the best marker of omega-3 PUFA status associated with many health indicators and outcomes in the general population (6); however, its relation with physical performance indicators in athletes is poorly understood. Observations on amateur and competitive athletes confirm low O3I values. For example, in 106 German elite winter endurance athletes, only one had an

Address for correspondence: Maja Tomczyk, Ph.D., Department of Biochemistry, Gdansk University of Physical Education and Sport, 80-336 Gdansk, Poland; E-mail: maja.tomczyk@awf.gda.pl.
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O3I in the target range of >8%, and the average O3I value of the others was 4.97% ± 1.19% (7). Analysis conducted on collegiate athletes, professional basketball players, and trained but not professional endurance athletes confirm low values of the O3I and its increase after supplementation with omega-3 PUFA (4,8,9). A recent systematic review summarizing randomized placebo-controlled trials in athletes revealed that omega-3 PUFA supplementation improved cognitive function (e.g., reduction of reaction time and improvement of mood state), promoted skeletal muscle recovery, and attenuated proinflammatory cell responses (10).

The effect of omega-3 fatty acid supplementation on exercise performance is unclear, although several studies show positive effects on oxygen kinetics: cycling efficiency or maximal oxygen uptake (10). To date, the longest study where physical performance parameters were analyzed lasted 10 wk with the applied dose of 1.60 g of EPA and 1.04 g of DHA daily (11). The length and the dose of omega-3 fatty acid supplementation seem to be crucial because of the incorporation of EPA + DHA into target tissues, which would be reflected in erythrocyte membranes and O3I. Maximal incorporation of EPA and DHA into erythrocytes is related to erythrocyte turnover: in a 12-month controlled intervention trial conducted on healthy individuals, Browning and coauthors (12) revealed that it takes 55 and 136 d for EPA and DHA, respectively, to achieve peak incorporation into erythrocytes in the case of a supplementation dose of 3.27 g of EPA + DHA for 4 $d \cdot wk^{-1}$.

Given the paucity of long-term studies using omega-3 fatty acid supplements in athletes showing relation between O3I values and physical performance indicators, there is a need for further work in this area. Accordingly, we determined the effects of 12 wk of EPA + DHA supplementation (2234 mg and 916 $mg \cdot d^{-1}$, respectively) compared with medium-chain triglycerides (MCT) as placebo in dose 4000 $mg \cdot d^{-1}$ during endurance training on O3I and physical performance indicators in amateur runners. We hypothesize that this duration and dosage of omega-3 PUFA will result in significant incorporation of EPA and DHA into erythrocytes membranes and increase O3I to values considered as a target range (i.e., >8%). Moreover, using the longest duration and the highest dose of supplementation of the studies conducted so far, we hypothesize that this will increase $\dot{V}O_{2peak}$ and improve running economy (RE) to a degree that will translate into better running performance.

METHODS

Ethical approval. The study was approved by the Bioethical Committee of Regional Medical Society in Gdańsk (NKBBN/628/2019) and conducted according to the Declaration of Helsinki. After comprehensive details of the study protocol were explained orally and in writing, all participants provided their written informed consent.

Participants. Forty amateur male long-distance runners were recruited through advertisements on the Internet. Inclusion criteria included age between 29 and 42 yr and completion of an official 10 km race over the 2016 and 2020 time period with a time result between 37 and 57 min. The exclusion criteria included chronic diseases, cigarette smoking, or use of prescribed medications or dietary supplements, including omega-3 fatty acids. On the day of familiarization with the laboratory conditions and the treadmill test, participants were allocated sequential numbers that were then used as the identifiable characteristic. Assignment to each group (OMEGA or MCT) using an online randomizer (http://www.randomizer.org) took place on the first day of the actual exercise tests. All participants agreed to carry out only the training courses included in the program and were instructed to continue with their habitual dietary patterns for the duration of the intervention.

Overview of study design. The trial was conducted in the Laboratory of Physical Exercise and Department of Biochemistry of the Academy of Physical Education and Sport in Gdansk. After inclusion, participants were randomly assigned to one of the two groups: OMEGA or MCT providing either omega-3 fatty acids or MCT. All participants completed a progressive endurance training supervised by a track and field coach. The parallel randomized trial consisted of three 4-wk phases, for a total of 12 wk together with simultaneous supplementation. A graded exercise test to exhaustion with assessment of $\dot{V}O_{2peak}$, RE, and a 1500-m run trial was carried out before and after completion of the exercise training program. Each test was preceded by a standardized breakfast for all participants consumed 1 h before the test began. Blood collection and weight assessment were performed when participants were in a fasting state. Figure 1 outlines the experimental protocol.

Omega-3 PUFA supplementation. Throughout the study, all participants took four identical-looking capsules each day (two in the morning and two in the evening) containing either omega-3 fatty acids or MCT. The omega-3 capsules provided 2234 mg of EPA and 916 mg of DHA daily (Omega-3 double plus, NAMED SPORT, Italy), whereas the MCT capsules contained 4000 mg of MCT (MCT Oil; Now Foods, Bloomingdale, IL). The dose of omega-3 fatty acids is consistent with the dosage applied in the study of Browning and coauthors (12). To maintain certainty of the amount of each fatty acid and the general quality of the supplements containing omega-3 fatty acids, a product certified by the International Fish Oil Standard was selected. The International Fish Oil Standard program verifies the amount of each fatty acid and the content of heavy metals, dioxins, and rate of oxidation. A publicly available batch report of the supplements used in the study indicated that the amounts of individual acids were in accordance with the manufacturer's claims, and content of heavy metals, dioxins, and rate of oxidation did not exceed accepted standards. Moreover, both supplements were certified by the informed-sport program, under which products are tested for substances banned by the World Anti-Doping Agency. To avoid a potential recognition of supplements, participants were informed that they were all taking omega-3 fatty acids in one of two chemical forms. On the day of arrival at the laboratory, 1 h before the graded exercise test and the 1500-m run trial, participants consumed the same standardized breakfast. Breakfast was a

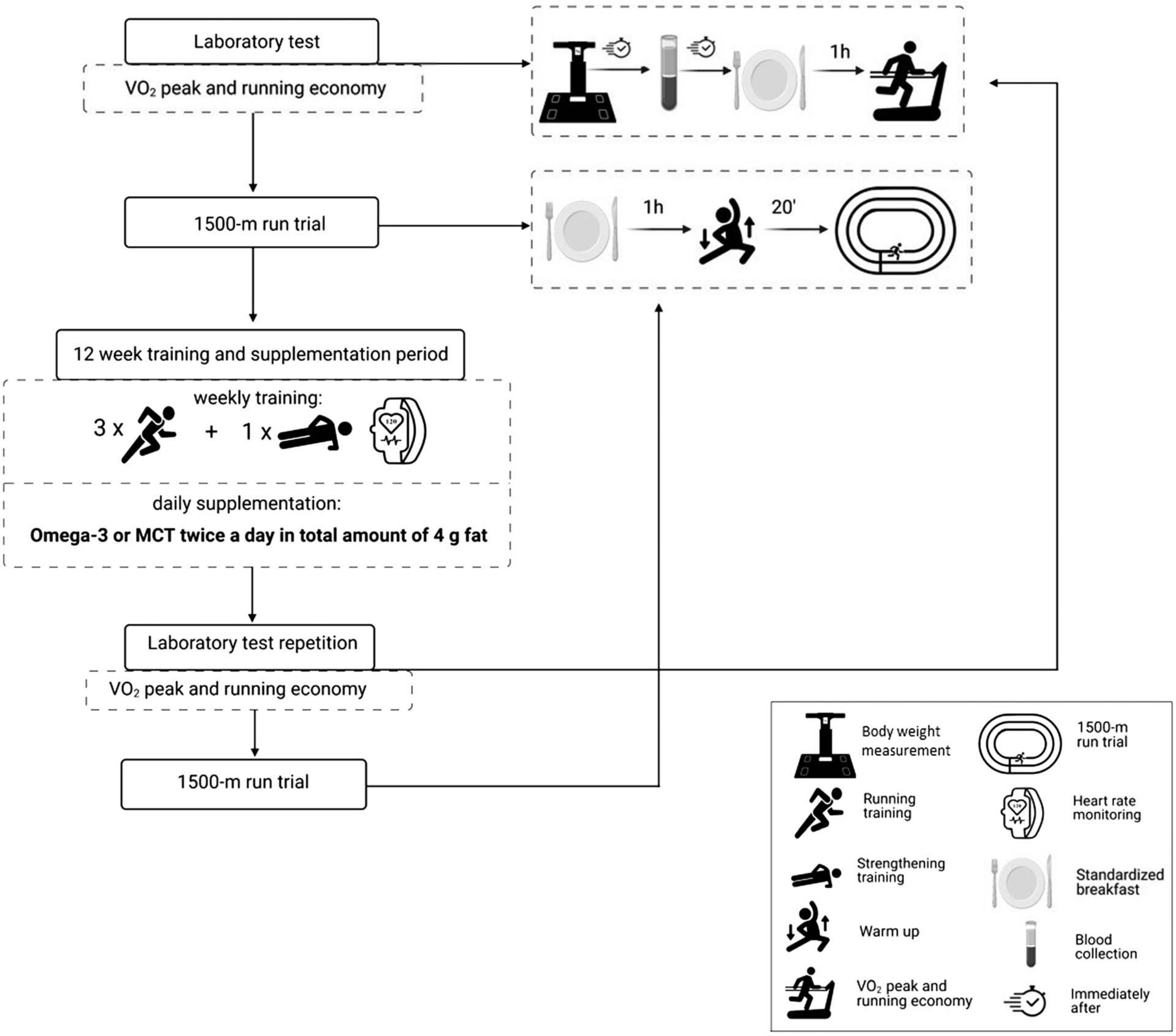

FIGURE 1—General experimental design.

replication of a typical prestart meal and consisted of wheat roll with butter and jam and half a banana.

Total energy value and amount of carbohydrate, protein, and fat was 290 kcal, 49 g, 5 g, and 8 g, respectively. Dietary intake over 3 d (2 d from week and 1 d from weekend) was recorded in the first and the last week of the program. Participants used the MyFitnessPal mobile application to record the meals they consumed. Before using the app for the first time, the basic functions were demonstrated to all participants. Moreover, the Web site ilewazy.pl was presented to participants, so they could more easily estimate the portions they consumed when kitchen scales were not available. If recorded meals were not precise, participants were asked to clarify the information. Collected dietary records where then analyzed using nutrition analysis software (Kcalmar.pro, Poland). Every food item in meals, with the consumed amount, was entered to the nutrition analysis software, and total dietary energy, carbohydrate, protein, and fat content were calculated.

Exercise testing. Before (week 0) and after completion (week 13) of the exercise training program, participants were submitted to a graded exercise test to exhaustion on a motorized treadmill (h/p Cosmos, Saturn, Germany) to determine whether omega-3 fatty acids combined with endurance training might positively affect the endurance potential of runners. Before the intervention, the participant's body weight and height were measured (analyzer InBody 720 and stadiometer Seca 213, respectively), then they were familiarized with the laboratory conditions and the treadmill test.

First, participants stood on the treadmill for 2 min to make sure the measuring equipment was ready and to measure the resting values. Thereafter, runners walked for 5 min at 5 $km \cdot h^{-1}$ speed and with a 1.5% inclination as a warm-up before starting the test. Every next stage lasted 3 min aimed to reach steady-state $\dot{V}O_2$ (13), and the treadmill belt was accelerated starting from 8×1 $km \cdot h^{-1}$ per stage up to 12 $km \cdot h^{-1}$. Then the inclination of the treadmill was increased to 5%, 10%, and 15% at 12 $km \cdot h^{-1}$

speed until volitional exhaustion. During both tests, heart rate (HR) was monitored (Polar RS400; Polar Electro Oy, Kempele, Finland) to define the highest value (HR_{max}) during each test. Minute ventilation ($\dot{V}_E$), oxygen uptake ($\dot{V}O_2$), carbon dioxide output ($\dot{V}CO_2$), and RER were continuously measured using a breath-by-breath analyzer (Oxycon Pro, Jaeger, Germany), which was calibrated before each test following the manufacturer's recommendations. Measurements were averaged in 10-s intervals. $\dot{V}O_{2peak}$ was obtained as the highest 30-s mean value recorded during the test. Running economy was measured as an oxygen cost from the last 50 s of each stage to 12 km·h^{-1} speed and was expressed as milliliters per kilogram per minute (14), and RE analysis was performed up to RER <1. All measurements were performed at similar time of day ±2 h and constant environmental conditions (18°C–20°C and humidity 40%–45%). Additionally, participants were informed to avoid strenuous exercise for 24 h before and caffeine and alcohol consumption for 12 h before laboratory tests. One week after the graded exercise test, participants took part in a 1500-m run time trial on an indoor 200-m track. The time was recorded with a handheld stopwatch to the nearest 0.1 s. During both tests, participants received strong verbal encouragement.

Training protocol. The training protocol lasted 12 wk and was built based on undulatory load manipulation 3:1, which was suggested to be effective to prevent overtraining and stress due to oscillations between volume/intensity according to Costa et al. (15) with slight modifications. Hence, participants performed endurance training 3 times per week. One additional training per week aimed to strengthen core muscles to reduce the risk of lower extremity injuries was also included in protocol (16). Training intensity was prescribed according to the first ventilatory threshold and ventilatory anaerobic threshold (VT1 and VAT), respectively, and their associated HR values were obtained during the laboratory testing. The threshold-based method was described as better than the HR reserve–based method to design more individualized exercise prescriptions that will enhance training efficacy and limit training unresponsiveness (17). Consequently, participants trained in three HR zones: [Z1: ≤HR@VT1 + 5 bpm; Z2: (>HR@VT1 + 5 bpm) to (≤HR@VAT-5 bpm); Z3: >HR@VAT-5 bpm], and their average training times spent in every mesocycle were (~80%–15%–5%) in zones (Z1–Z2–Z3), respectively, accordingly to previous authors (18) with slight modifications. On the last (12th) week, the tapering procedure was performed, whereby the training load was reduced to 70% from the volume obtained in the 11th week to reduce accumulated fatigue. Participant's training activity (training volume, intensity, and energy expenditure) was monitored by a Polar M430 wristwatch and an H9 HR chest sensor. All running tests and training procedures were supervised by a track and field coach.

Erythrocyte fatty acid analysis. Fasting blood samples were collected from participants by a nurse into 4-mL sodium citrate vacutainer tubes (BD Vacutainer®, Franklin Lakes, NJ) and centrifuged at 4°C (4000*g* for 10 min). After centrifugation, erythrocytes were collected with a disposable pasteur pipette and transferred into eppendorfs, which were stored in a − 80°C freezer until further analysis. Erythrocyte EPA and DHA were assessed using gas chromatography as described elsewhere (19). Briefly, erythrocyte lipids were extracted into chloroform–methanol, and fatty acid methyl esters (representing the erythrocyte fatty acids) were formed by heating the lipid extract with methanolic sulfuric acid. The fatty acid methyl esters were separated by gas chromatography on a Hewlett Packard 6890 gas chromatograph fitted with a BPX-70 column using the settings and run conditions described elsewhere (19). Fatty acid methyl esters were identified by comparison with run times of authentic standards. Data are expressed as weight % of total fatty acids. O3I was calculated by summing the percentages of EPA and DHA according to Harris and von Schacky (5).

Statistical analysis. The sample size calculation was based on changes in oxygen consumption during graded exercise test to exhaustion assessed as $\dot{V}O_{2peak}$, as this was the primary outcome of the study. A typical value for $\dot{V}O_{2peak}$ in population of recreational long-distance runners is about 54 mL·kg^{-1}·min^{-1} with an SD of about 5 (20).

It is considered that an 8% increase in $\dot{V}O_{2peak}$ is meaningful in amateur runners (21). A sample size of 18 participants per group (i.e., 36 participants in total) would give 70% power to detect this difference as significant with alpha = 0.05. In order to account for a dropout rate of 10%, 40 participants were recruited. Statistical analysis was performed using the tools of GraphPad Prism 7. Arithmetic means, SD, and significance levels of differences between means were calculated. A two-way repeated-measures ANOVA was used to investigate the significance of differences between groups and time. Significant main effects were further analyzed using the Bonferroni corrected *post hoc* test. Changes (Δ) in both groups were compared using an independent samples *t*-test. Correlations between variables were evaluated using the Pearson correlation coefficient. All analyses used a significance level of $P < 0.05$.

RESULTS

Participant flow through the study. Participants excluded from the final analysis completed insufficient (<80%) training sessions ($n = 3$) or withdrew from the study for health ($n = 9$) or personal reasons ($n = 1$). Moreover, one participant from MCT group increase intake of omega-3 fatty acids during study; therefore, he was also excluded from statistics. Participant flow through the study is presented in Figure 2. From the 40 participants enrolled, 26 completed the entire study and their characteristic is shown in Table 1.

Erythrocyte EPA, DHA, and O3I. The percentage values of erythrocyte EPA, DHA, and O3I pre- and postintervention in the OMEGA and MCT groups are presented in Figures 3 and 4. There was no difference in baseline values of either omega-3 PUFA or O3I between the groups (OMEGA group: 1.1% EPA, 4.7% DHA, 5.8% O3I; MCT group: 1.2% EPA, 4.4% DHA, 5.6% O3I; all $P > 0.9999$). Twelve weeks of omega-3 fatty acid supplementation during endurance training

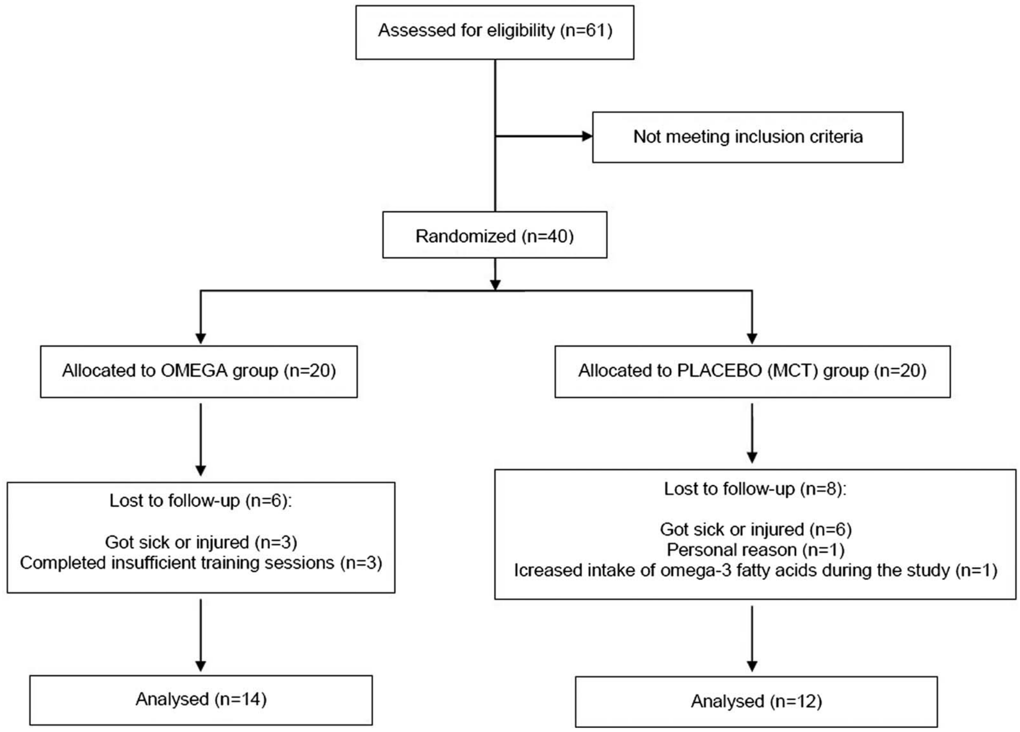

FIGURE 2—Flow of participants through the study.

increased both omega-3 PUFA and O3I in the OMEGA group (to 4.9% ± 1.1% EPA, 6.7% ± 0.8% DHA, 11.6% ± 1.7% O3I; all $P < 0.0001$) without significant changes in the MCT group (to 1.1% EPA, 4.5% DHA, 5.6% O3I; all $P > 0.9999$). At the end of the intervention period EPA, DHA and O3I were significantly higher in OMEGA group than in MCT group (all $P < 0.0001$).

$\dot{V}O_{2peak}$, RE, and 1500-m run trial. There was no significant difference between groups in change in $\dot{V}O_{2peak}$ over the 12-wk intervention period ($P = 0.6764$) (Fig. 5B). However, a significant increase in $\dot{V}O_{2peak}$ from pre- to postintervention in OMEGA group was observed (from 53.6 ± 4.4 to 56.0 ± 3.7 mL·kg^{-1}·min^{-1}, $P = 0.0219$) with no significant change in MCT group (from 54.7 ± 6.8 to 56.4 ± 5.9 mL·kg^{-1}·min^{-1}, $P = 0.1308$) (Fig. 5A). Increase in $\dot{V}O_{2peak}$ was seen in 13 (93%) out of 14 participants in the OMEGA group, whereas in the MCT group, improvements were visible in 9 (75%) out of 12 runners.

Moreover, oxygen uptake at 12 km·h^{-1} changed in both groups: the RE increased significantly in the OMEGA group (from 47.6 ± 1.8 to 46.5 ± 2.4 mL·kg^{-1}·min^{-1}, $P = 0.0295$), whereas it decreased in the MCT group (from 47.7 ± 3.3 to 48.7 ± 2.9 mL·kg^{-1}·min^{-1}, $P = 0.1127$) (Fig. 5C). The change in oxygen uptake over the 12-wk intervention period was significantly different between groups ($P = 0.0033$) (Fig. 5D). When results before and after the 12-wk intervention from all participants were combined, correlation highlighted the relationship between O3I and oxygen cost of submaximal running (Fig. 6A, $P = 0.0338$; Fig. 6B, $P = 0.0020$). There was significant improvement in completion of the 1500-m run trial in both groups from pre- to postintervention; however, results did not differ between groups over the study period (OMEGA group from 356.3 to 344.9 s, $P = 0.0002$; MCT group from 362.1 to 347.3 s, $P < 0.0001$; pre- to postintervention between groups, $P > 0.9999$).

Physiological and nutritional variables. Table 2 summarizes physiological and nutritional variables obtained from the participants at the beginning and after completing the intervention program. There was no difference in weekly training volume ($P = 0.7399$), energy expenditure ($P = 0.1828$), and HR_{max} ($P = 0.4624$) between the groups. However, in both groups, there was a significant increase in HR_{max} at VAT (%) postintervention compared with preintervention (OMEGA group from 91.7 ± 2.6 to 93.9 ± 2.8, $P = 0.0331$; MCT group from 90.8 ± 3.9 to 95.2 ± 3.7, $P = 0.0001$). Total energy (kcal·d^{-1}), carbohydrate, and protein (g·kg^{-1}·d^{-1}) intake did not differ pre- to postintervention within either group (OMEGA group $P > 0.9999$, $P = 0.5442$, $P = 0.5777$; MCT group $P = 0.1973$, $P > 0.9999$, $P = 0.7721$, respectively).

There was a statistically significant difference in fat intake between the two groups with a significantly higher fat intake in the OMEGA group (from 83.4 ± 25.9 to 91.9 ± 25.9 g,

TABLE 1. Characteristics of participants who completed the study.

Variable	Omega (n = 14)	MCT (n = 12)
Age (yr)	37 ± 3	37 ± 4
Body mass (kg)	76.3 ± 11	78.0 ± 8
Height (cm)	181 ± 7	180 ± 4
$\dot{V}O_2$ peak (mL·kg^{-1}·min^{-1})	53.6 ± 4	54.7 ± 7
Personal best in 10-km run between 2016 and 2020 (min)	45 ± 4	46 ± 5

Data are presented as mean ± SD.

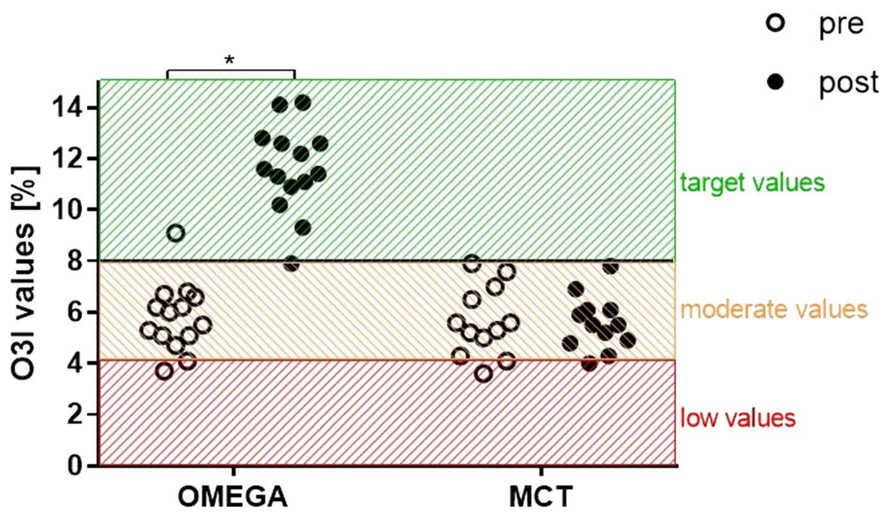

FIGURE 3—Effect of supplementation with omega-3 PUFA or MCT on individual values of O3I before and after the 12-wk intervention. **P* < 0.0001.

P = 0.0321) and lower; however, not significant fat intake in the MCT group (*P* = 0.0943).

DISCUSSION

The main finding of the study is that 12 wk of supplementation with omega-3 fatty acids at a dose of 2234 mg of EPA and 916 mg of DHA daily shifts erythrocyte O3I to values considered as a target range for cardiovascular health. Moreover, this duration and dose of supplementation during endurance training increased $\dot{V}O_{2peak}$ and improved RE at velocity 12 km·h^{-1} with no effect on 1500-m run trial results. Insufficient values of O3I in active individuals are well described. In a study including vegan and omnivorous endurance athletes, Cradock et al. (8) showed suboptimal O3I in both groups: 4.13% in vegans and 5.40% in omnivores, respectively. Similarly, O3I below the desirable values was demonstrated in German national elite winter endurance athletes (4.97% ± 1.19%), professional basketball players from the NBAG League (5.02% ± 1.19%), and collegiate athletes, representing diverse disciplines throughout the United States (4.33% ± 0.81%) (4,7,22). Our observations are in agreement with these reports, indicating that amateur runners had mean baseline O3I of around 5.7% (5.8% and 5.6% in OMEGA and MCT groups, respectively).

Twelve weeks with omega-3 fatty acid supplementation at a dose of 2234 mg of EPA and 916 mg of DHA daily during

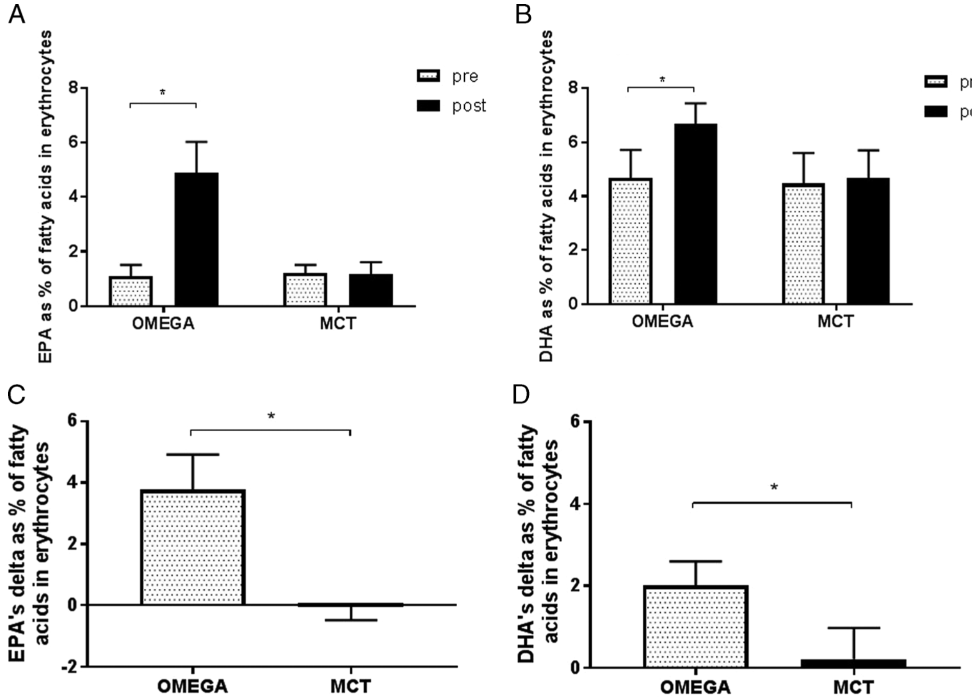

FIGURE 4—Effect of supplementation with omega-3 PUFA or MCT on erythrocyte EPA (A) and DHA (B) before and after the 12-wk intervention and change from baseline in EPA (C) and DHA (D) compared between the two groups. Data are expressed as mean. Error bars indicate ± SD, **P* < 0.0001.

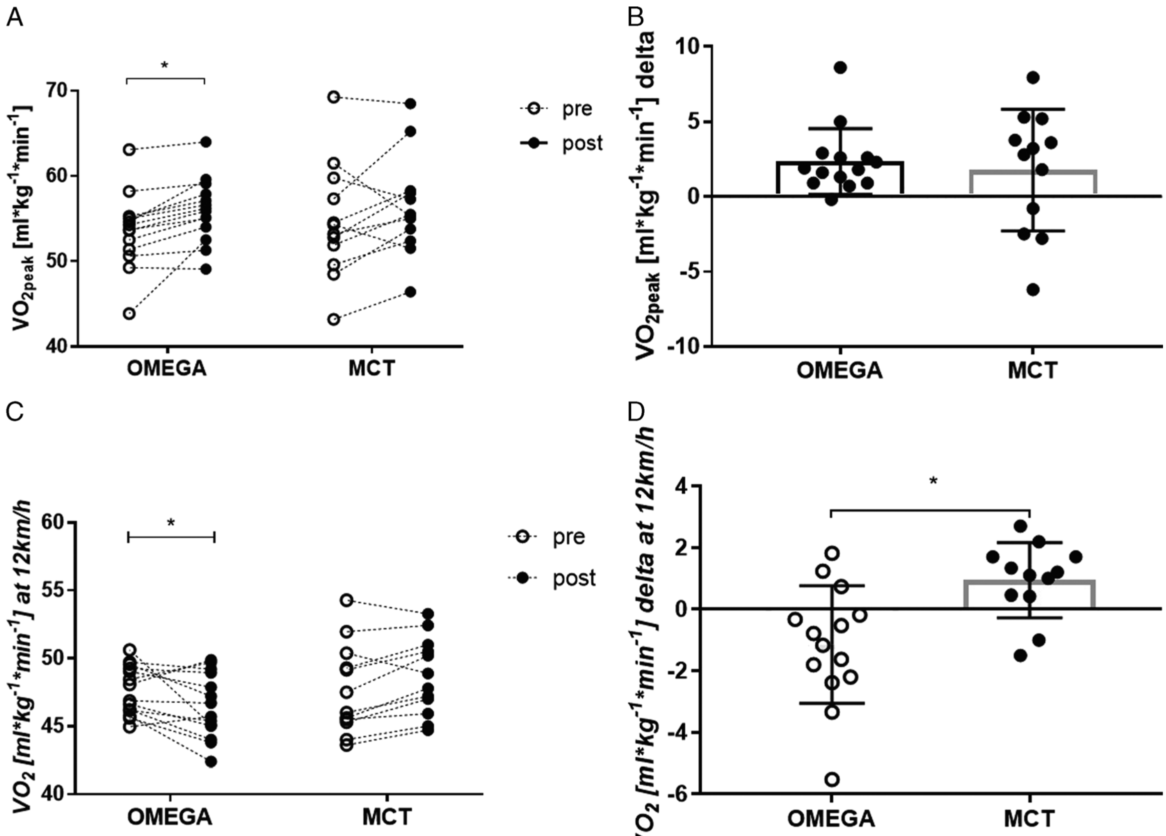

FIGURE 5—Effect of training and supplementation on peak oxygen consumption (A) (mL·kg^{-1}·min^{-1}) before and after the 12-wk intervention, change in peak oxygen consumption (B) (mL·kg^{-1}·min^{-1}) in the two groups over the 12-wk intervention, oxygen utilization (C) (mL·kg^{-1}·min^{-1}) during submaximal treadmill running at 12 km·h^{-1} before and after the 12-wk intervention, change in oxygen utilization (D) (mL·kg^{-1}·min^{-1}) in the two groups over the 12-wk intervention. Data are expressed as mean. Error bars indicate ± SD, *P < 0.05.

endurance training increased O3I in all but one participant in OMEGA group to mean of 11.4%, which is considered to be well within the O3I target range (5). Moreover, an increase in O3I correlated with an increase in RE at velocity 12 km·h^{-1} when results post- minus pre-12-wk intervention of participants from both groups were combined. Improvements in exercise economy as an effect of supplementation with omega-3 fatty acids have previously been shown in both amateur and competitive athletes (9,23,24). In an 8-wk double-blind, parallel design study in well-trained cyclists, Peoples et al. (23) showed that 3.2 g·d^{-1} of omega-3 fatty acids reduced whole-body O_2 consumption throughout 60 min of sustained submaximal cycling. Contrary to our observations, peak oxygen consumption in these cyclists was not changed, which may be related to their high level of training status or quite high compared with other data (above 9%) baseline O3I values (23). Improved economy of cycling during the physiologically demanding time trial in trained cyclists and runners was also revealed by Hingley et al. (9) after 8 wk of supplementation with a dose of 560 mg of DHA + 140 mg of EPA a day. Despite an elevation in O3I (from 4.7% ± 0.2% to 6.3% ± 0.3%), the values did not achieve the recommended O3I >8%, which may be related to the low dose of EPA + DHA used. A study conducted by Kawabata et al. (24) with recreational players of American football, rugby, baseball, and basketball is consistent with other observations in trained individuals: 8 wk of daily supplementation with 914 mg of EPA and

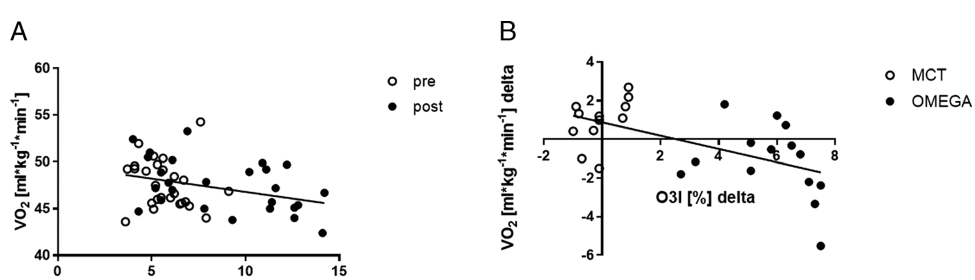

FIGURE 6—Correlation between O3I and oxygen cost of submaximal running when: OMEGA and MCT groups were combined before and after the 12-wk intervention (A). B. Results postintervention minus preintervention (Δ) in OMEGA and MCT groups were combined.

TABLE 2. Physiological and nutritional variables according to treatment group.

Variable		Omega		MCT
Weekly training volume (km)		30.95 ± 2.47		31.5 ± 5.51
Energy expenditure (kcal·d^{-1})		2515 ± 445		2748 ± 415
$\dot{V}O_{2peak}$ (mL·kg^{-1}·min^{-1})	Pre	53.61 ± 4.36	Pre	54.66 ± 6.76
	Post	55.96 ± 3.72*	Post	56.44 ± 5.89
HR_{max} (bpm)	Pre	190 ± 9	Pre	186 ± 9
	Post	189 ± 9	Post	184 ± 7
HR_{max} at VAT (%)	Pre	91.71 ± 2.65	Pre	90.81 ± 3.95
	Post	93.89 ± 2.77*	Post	95.20 ± 3.69**
Body mass (kg)	Pre	76.30 ± 10.96	Pre	78.03 ± 7.70
	Post	76.55 ± 11.32	Post	77.0 ± 7.35*
Energy and nutrient intake (per day)				
Energy (kcal)	Pre	2393 ± 453	Pre	2456 ± 587
	Post	2429 ± 420	Post	2338 ± 627
Carbohydrate (g)	Pre	301 ± 63	Pre	310 ± 111
	Post	289 ± 46	Post	302 ± 127
Protein (g)	Pre	98 ± 20	Pre	99 ± 20
	Post	102 ± 17	Post	95 ± 17
Fat (g)[a]	Pre	83 ± 27	Pre	86 ± 18
	Post	92 ± 27*	Post	79 ± 15

Data are presented as mean ± SD.
*$P < 0.05$ for post- vs preintervention value.
**$P < 0.01$ for post- vs preintervention value.
[a]Statistically significant difference in groups (Δ) with a trend of higher intake in the O3I group and lower intake in the MCT group.

399 mg of DHA increased exercise economy during a steady-state submaximal cycloergometer test. In one crossover study with trained cyclists, researchers observed an increase in $\dot{V}O_{2max}$ after 3 wk of supplementation with a daily dose of 660 mg of EPA and 440 mg of DHA (25).

In contrast to this report, an earlier study conducted by Raastad et al. (11) showed no changes in $\dot{V}O_{2max}$ and running performance in well-trained soccer players receiving 1.60 g of EPA and 1.04 g of DHA a day through 10-wk period. Exercise economy together with $\dot{V}O_{2max}$, lactate threshold, and critical power are all strongly related to endurance exercise performance (26). Therefore, studies showing increased exercise economy, $\dot{V}O_{2max}$, or $\dot{V}O_{2peak}$ provide a rationale to further explore this topic together with the potential underlying mechanisms. Supplementation with omega-3 fatty acids reduces exercise-induced inflammation in athletes through decreasing in proinflammatory omega-6 fatty acids (27) and AA/EPA ratio (28). Given the large cross-sectional study indicating that inverse relationship between $\dot{V}O_{2max}$ and C-reactive protein is modified by omega-3 fatty acid levels (29), this may be the case. Moreover, an increase in insulin sensitivity due to unsaturation of skeletal muscle membranes (30), improved calcium handling by skeletal muscle sarcoplasmic reticulum (23), and improved endothelial function via increase in NO release (25) should be taken into account in searching for potential mechanisms of action. Of note, in the present study, 13 out of 14 participants in the OMEGA group showed an improved $\dot{V}O_{2peak}$ compared with a variable response in the MCT group, in which only 9 out of 12 runners improved their results. This may suggest better adaptation to endurance training in response to omega-3 fatty acid supplementation, as has been observed with several other dietary supplements (31). Still, neither our nor previous reports support the hypothesis that long-term supplementation with omega-3 fatty acids enhances exercise performance. Duration and dose of omega-3 supplementation are crucial factors determining the amount of fatty acids incorporated into erythrocyte membranes, and more than 4 months is needed to reach the highest concentration of DHA in case of a supplementation dose of 1.5 g of EPA and 1.77 g of DHA for 4 d·wk^{-1} (12).

Compared with previous studies in which performance indicators were assessed, our supplementation protocol (2234 mg of EPA and 916 mg of DHA daily for 12 wk) was a higher dose over a longer supplementation period (9,23–25). However, what values of O3I are sufficient for amateur and competitive athletes to optimize athletic performance remains a question to be answered in future studies.

Our study has some limitations that must be highlighted. Running economy is typically determined by measuring the consumption of oxygen when the steady state of $\dot{V}O_2$ is observed (13). We recognized steady-state conditions when runners had RER <1 during treadmill running (13,32); however, the concentration of lactic acid was not assessed. Considering that lactate threshold (LT) is one of the indicators of disturbance in $\dot{V}O_2$ steady state (26,33), it should be included in future research. Animal studies showed that DHA is incorporated into the membranes of fast-oxidative glycolytic fibers (type IIA) of skeletal muscle (34). These muscle fibers have both a high oxidative and glycolytic capacity, and because of their increased activation during moments of high energy demand (35), we decided to perform a 1500-m run trial. Our participants typically perform distances from 10 km to a marathon; therefore, lack of experience and unfamiliarization at such a short distance as 1500-m may influence the outcome of the run trial, and this must be taken into consideration when interpreting our findings. Future studies with omega-3 supplementation should also consider prescreening, during which individuals with similar baseline O3I should be selected (36).

CONCLUSIONS

In conclusion, 12 wk of omega-3 fatty acid supplementation at a dose of 2234 mg of EPA and 916 mg of DHA daily during an endurance running program increased O3I to values currently considered as a target range. This duration and dose of supplementation combined with endurance training increased peak oxygen consumption and improved RE in amateur runners without affecting their performance.

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accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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