

Effects of 12 weeks of omega-3 fatty acid supplementation in long-distance runners

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Purpose

To investigate the effects of 12 weeks 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

26 amateur male long-distance runners aged ≥ 29 years supplemented omega-3 fatty acid capsules (OMEGA group, n=14; 2234 mg of EPA and 916 mg of DHA daily) or medium chain triglycerides capsules as placebo (MCT group, n=12; 4000 mg of MCT daily) during 12-week of endurance training. Before and after intervention, blood samples were collected for O3I assessment and an incremental test to exhaustion and 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 VO_{2peak} was observed in the OMEGA group (from $53.6 \pm 4.4 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ to $56.0 \pm 3.7 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $P = 0.0219$) without such change in MCT group (from $54.7 \pm 6.8 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ to $56.4 \pm 5.9 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $P = 0.1308$). A positive correlation between the change in O3I and 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

12 weeks of omega-3 fatty acid supplementation at a dose of 2234 mg of EPA and 916 mg of DHA daily during endurance training resulted in improvement of O3I and running economy and increased VO_{2peak} without improvement in the 1500-m run trial time in amateur runners.

Clinical registry

The study was registered at <https://www.clinicaltrials.gov/> with identifier NCT04780451

Runing title

Omega-3 index and physical performance indicators in amateur runners

Key words

Omega-3 index (O3I), polyunsaturated fatty acids, running performance, endurance training, running economy

Abbreviations

AA, arachidonic acid

ALA, α -linolenic acid

CRP, C-reactive protein

DHA, docosahexaenoic acid

EPA, eicosapentaenoic acid

HR, heart rate

HRR, heart rate reserve

LT, lactate threshold

MCT, medium-chain triglycerides

O3I, omega-3 index

PUFA, polyunsaturated fatty acids

RE, running economy

RER, respiratory exchange ratio

V_e , pulmonary ventilation

VO_2 , volume of oxygen uptake

VCO_2 , volume of carbon dioxide

VO_{2peak} , peak oxygen uptake

VT1, first ventilatory threshold

VAT, ventilatory anaerobic threshold

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INTRODUCTION

Omega-3 fatty acids include α -linolenic acid (ALA), 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 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 (5). 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 it's 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 O3I in the target range of >8% and the average O3I value of the others was $4.97 \pm 1.19\%$ (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 PUFAs (8,4,9). 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).

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28 The effect of omega-3 fatty acid supplementation on exercise performance is unclear, although
29 several studies show positive effects on oxygen kinetics: cycling efficiency or maximal oxygen
30 uptake (10). To date, the longest study where physical performance parameters were analyzed
31 lasted 10 weeks with the applied dose of 1.60 g of EPA and 1.04 g of DHA daily (11). The
32 length and dose of omega-3 fatty acid supplementation seem to be crucial due to the
33 incorporation of EPA + DHA into target tissues, which would be reflected in erythrocyte
34 membranes and O3I. Maximal incorporation of EPA and DHA into erythrocytes is related to
35 erythrocyte turnover: in a twelve-month controlled intervention trial conducted on healthy
36 individuals, Browning and co-authors revealed that it takes 55 and 136 days for EPA and DHA
37 respectively, to achieve peak incorporation into erythrocytes in the case of a supplementation
38 dose of 3.27 g of EPA + DHA for 4 days a week (12).

39 Given the paucity of long-term studies using omega-3 fatty acid supplements in athletes
40 showing relation between O3I values and physical performance indicators, there is a need for
41 further work in this area. Accordingly, we determined the effects of 12 weeks of EPA+DHA
42 supplementation (2234 mg and 916 mg a day respectively) compared with medium-chain
43 triglycerides (MCT) as placebo in dose 4000 mg a day during endurance training on O3I and
44 physical performance indicators in amateur runners. We hypothesize that this duration and
45 dosage of omega-3 PUFAs will result in significant incorporation of EPA and DHA into
46 erythrocytes membranes and increase O3I to values considered as a target range (i.e. > 8%).
47 Moreover, using the longest duration and the highest dose of supplementation of the studies
48 conducted so far, we hypothesize that this will increase VO_{2peak} and improved running economy
49 (RE) to a degree that will translate into better running performance.

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53 **METHODS**

54 **Ethical approval**

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

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60 **Participants**

61 40 amateur male long-distance runners were recruited through advertisements on the internet.
62 Inclusion criteria included age between 29 and 42 years, and completion of an official 10 km
63 race over the 2016 and 2020 time period with a time result between 37 and 57 min. The
64 exclusion criteria included chronic diseases, cigarette smoking, or use of prescribed
65 medications or dietary supplements, including omega-3 fatty acids. On the day of
66 familiarization with the laboratory conditions and the treadmill test, participants were allocated
67 sequential numbers that were then used as the identifiable characteristic. Assignment to each
68 group (OMEGA or MCT) using an online randomizer (<http://www.randomizer.org>) took place
69 on the first day of the actual exercise tests. All participants agreed to carry out only the training
70 courses included in the programme and were instructed to continue with their habitual dietary
71 patterns for the duration of the intervention.

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75 **Overview of study design**

76 The trial was conducted in the Laboratory of Physical Exercise and Department of Biochemistry
77 of the Academy of Physical Education and Sport in Gdansk. After inclusion, participants were
78 randomly assigned to one of the two groups: OMEGA or MCT providing either omega-3 fatty
79 acids or MCTs. All participants completed a progressive endurance training supervised by a
80 track and field coach. The parallel, randomized trial consisted of 3 four-week phases, for a total
81 of 12 weeks together with simultaneous supplementation. A graded exercise test to exhaustion
82 with assessment of $VO_{2\text{ peak}}$, running economy and a 1500-m run trial were carried out before
83 and after completion of the exercise training programme. Each test was preceded by a
84 standardized breakfast for all participants consumed 1 hour before the test began. Blood
85 collection and weight assessment were performed when participants were in a fasting state.
86 Fig. 1 outlines the experimental protocol.

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Omega-3 PUFA supplementation

Throughout the study all participants took 4 identical-looking capsules each day (2 in the morning and 2 in the evening) containing either omega-3 fatty acids or MCTs. 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 MCTs (MCT Oil, Now Foods, USA). The dose of omega-3 fatty acids is consistent with the dosage applied in the study of Browning and co-authors (12). To maintain certainty of the amount of each fatty acid and the general quality of the supplements containing omega-3 fatty acids, an International Fish Oil Standard (IFOS) certified product was selected. The IFOS programme 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 programme, 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 hour prior to the graded exercise test and the 1500-m run trial, participants consumed the same standardized breakfast. Breakfast was a replication of a typical pre-start meal and consisted of wheat roll with butter and jam and half a banana.

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116 Total energy value and amount of carbohydrate, protein and fat was 290 kcal, 49 g, 5 g and
117 8 g, respectively. Dietary intake over 3 days (2 days from week and 1 day from weekend) was
118 recorded in the first and the last week of the programme. Participants used the
119 MyFitnessPal mobile application to record the meals they consumed. Before using the app for
120 the first time, the basic functions were demonstrated to all participants. Moreover, the
121 website ilewazy.pl was presented to participants, so they could more easily estimate the
122 portions they consumed when kitchen scales were not available. If recorded meals were not
123 precise, participants were asked to clarify the information. Collected dietary records were then
124 analysed using nutrition analysis software (Kcalmar.pro, Poland). Every food item in meals,
125 with the consumed amount, was entered to the nutrition analysis software and total dietary
126 energy, carbohydrate, protein and fat content was calculated.

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128 **Exercise testing**

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

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140 First, participants stood on the treadmill for 2 minutes to make sure the measuring equipment
141 was ready and to measure the resting values. Thereafter, runners walked for 5 min at 5 km/h
142 speed and with a 1.5% inclination as a warm-up prior to starting the test. Every next stage lasted
143 3 min aimed to reach steady-state VO_2 (13), and the treadmill belt was accelerated starting from
144 8 km/h by 1 km/h per stage up to 12 km/h. Then, the inclination of the treadmill was increased
145 to 5%, 10% and 15% at 12 km/h speed until volitional exhaustion. During both tests, heart rate
146 (HR) was monitored (Polar RS400 Kempele, Finland) to define the highest value (HRmax)
147 during each test. Pulmonary ventilation (V_e), oxygen uptake (VO_2), carbon dioxide output
148 (VCO_2) and respiratory exchange ratio (RER) were continuously measured using a breath-by-
149 breath analyzer (Oxycon Pro, Jaeger, Germany) which was calibrated before each test following
150 the manufacturer's recommendations. Measurements were averaged in 10-second intervals.
151 $\text{VO}_{2\text{peak}}$ was obtained as the highest 30 s mean value recorded during the test. Running economy
152 was measured as an oxygen cost from last 50 seconds of each stage to 12 km/h speed and was
153 expressed as $\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (14), and RE analysis was performed up to $\text{RER} < 1$. All
154 measurements were performed at similar time of day ± 2 h and constant environmental
155 conditions (18-20°C and humidity 40-45%). Additionally, participants were informed to avoid
156 strenuous exercise for 24 h before and caffeine and alcohol consumption for 12 h before
157 laboratory tests. 1 week after the graded exercise test, participants took part in a 1500-m run
158 time trial on an indoor 200-m track. The time was recorded with a handheld stopwatch to the
159 nearest 0.1 s. During both tests, participants received strong verbal encouragement.

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163 **Training Protocol**

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

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186 **Erythrocyte fatty acid analysis**

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

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201 **Statistical analysis**

202 The sample size calculation was based on changes in oxygen consumption during graded
203 exercise test to exhaustion assessed as VO_{2peak} , as this was the primary outcome of the study.
204 A typical value for VO_{2peak} in population of recreational long-distance runners is about 54
205 $ml \cdot kg^{-1} \cdot min^{-1}$ with a SD of about 5 (20).

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207 It is considered that a 8% increase in VO_{2peak} is meaningful in amateur runners (21). A sample
208 size of 18 participants per group (i.e. 36 participants in total) would give 70% power to detect
209 this difference as significant with $\alpha = 0.05$. In order to account for a dropout rate of 10%,
210 40 participants were recruited. Statistical analysis was performed using the tools of GraphPad
211 Prism 7. Arithmetic means, standard deviation (SD), and significance levels of differences
212 between means were calculated. Two-way analysis of variance (ANOVA), with repeated
213 measures, was used to investigate the significance of differences between groups and time.
214 Significant main effects were further analyzed using the Bonferroni corrected post hoc test.
215 Changes (Δ) in both groups were compared using an independent samples t-test.
216 Correlations between variables were evaluated using the Pearson correlation coefficient. All
217 analyses used a significance level of $P < 0.05$.

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219 **RESULTS**

220 **Participant flow through the study**

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

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230 **Erythrocyte EPA, DHA and O3I**

231 The percentage values of erythrocyte EPA, DHA and O3I pre- and post-intervention in the
232 OMEGA and MCT groups are presented in Figs. 3 and 4. There was no difference in baseline
233 values of either omega-3 PUFA or O3I between the groups (OMEGA group: 1.1% EPA, 4.7%
234 DHA, 5.8% O3I; MCT group: 1.2% EPA, 4.4% DHA, 5.6% O3I, all $P > 0.9999$). 12 weeks of
235 omega-3 fatty acid supplementation during endurance training increased both omega-3 PUFAs
236 and O3I in the OMEGA group (to $4.9 \pm 1.1\%$ EPA, $6.7 \pm 0.8\%$ DHA, $11.6 \pm 1.7\%$ O3I, all $P <$
237 0.0001) without significant changes in the MCT group (to 1.1% EPA, 4.5% DHA, 5.6% O3I,
238 all $P > 0.9999$). At the end of the intervention period EPA, DHA and O3I were significantly
239 higher in OMEGA group than in MCT group (all $P < 0.0001$).

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241 **VO_{2peak}, running economy and 1500-m run trial**

242 There was no significant difference between groups in change in VO_{2peak} over the 12-week
243 intervention period ($P = 0.6764$) (Fig. 5B). However, a significant increase in VO_{2peak} from
244 pre- to post-intervention in OMEGA group was observed (from $53.6 \pm 4.4 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ to 56.0
245 $\pm 3.7 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $P = 0.0219$) with no significant change in MCT group (from 54.7 ± 6.8
246 $\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ to $56.4 \pm 5.9 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $P = 0.1308$) (Fig. 5A). Increase in VO_{2 peak} was seen
247 in 13 out of 14 (93%) participants in the OMEGA group while in the MCT group improvements
248 were visible in 9 out of 12 (75%) runners.

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253 Moreover, oxygen uptake at 12 km/h changed in both groups: the running economy increased
254 significantly in the OMEGA group (from 47.6 ± 1.8 to 46.5 ± 2.4 ml*kg⁻¹*min⁻¹, $P = 0.0295$),
255 while it decreased in the MCT group (from 47.7 ± 3.3 to 48.7 ± 2.9 ml*kg⁻¹*min⁻¹, $P = 0.1127$)
256 (Fig. 5C). The change in oxygen uptake over the 12-week intervention period was significantly
257 different between groups ($P = 0.0033$) (Fig. 5D). When results pre- and post- 12-week
258 intervention from all participants were combined, correlation highlighted the relationship
259 between O3I and oxygen cost of submaximal running (Fig. 6A, $P = 0.0338$ and Fig. 6 B, $P =$
260 0.0020). There was significant improvement in completion of the 1500-m run trial in both
261 groups from pre- to post-intervention, however, results did not differ between groups over the
262 study period (OMEGA group from 356.3s to 344.9s, $P = 0.0002$ and MCT group from 362.1s
263 to 347.3s, $P < 0.0001$; post- to post- between groups, $P > 0.9999$).

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265 **Physiological and nutritional variables**

266 Table 2 summarises physiological and nutritional variables obtained from the participants at
267 the beginning and after completing the intervention programme. There was no difference in
268 weekly training volume ($P = 0.7399$), energy expenditure ($P = 0.1828$) and HRmax ($P =$
269 0.4624) between the groups. However, in both groups there was a significant increase in HR
270 max at VAT [%] post-intervention compared to pre-intervention (OMEGA group from $91.7 \pm$
271 2.6 to 93.9 ± 2.8 , $P = 0.0331$ and MCT group from 90.8 ± 3.9 to 95.2 ± 3.7 , $P = 0.0001$). Total
272 energy (kcal/d), carbohydrate and protein (g*kg⁻¹*day⁻¹) intake did not differ pre- to post-
273 intervention within either group (OMEGA group $P > 0.9999$, $P = 0.5442$, $P = 0.5777$; MCT
274 group $P = 0.1973$, $P > 0.9999$, $P = 0.7721$ respectively).

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276 There was a statistically significant difference in fat intake between the two groups with a
277 significantly higher fat intake in the OMEGA group (from 83.4 ± 25.9 to 91.9 ± 25.9 g, $P =$
278 0.0321) and lower, however not significant fat intake in the MCT group ($P = 0.0943$).
279 Moreover, a significant decrease in body mass during the study was observed in the MCT group
280 (-1.225 kg; $P = 0.0265$).

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282 **DISCUSSION**

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284 The main finding of the study is that 12 weeks of supplementation with omega-3 fatty acids at
285 a dose of 2234 mg of EPA and 916 mg of DHA daily shifts erythrocyte O3I to values considered
286 as a target range for cardiovascular health. Moreover, this duration and dose of supplementation
287 during endurance training increased VO_{2peak} and improved running economy at velocity
288 12 km/h with no effect on 1500-m run trial results. Insufficient values of O3I in active
289 individuals are well described. In a study including vegan and omnivorous endurance athletes,
290 Cradock et al. (8) showed suboptimal O3I in both groups: 4.13% in vegans and 5.40% in
291 omnivores respectively. Similarly, O3I below the desirable values was demonstrated in German
292 national elite winter endurance athletes ($4.97 \pm 1.19\%$), professional basketball players from
293 NBAG-League ($5.02 \pm 1.19\%$) and collegiate athletes, representing diverse disciplines
294 throughout the U.S. ($4.33 \pm 0.81\%$) (7,4,22). Our observations are in agreement with these
295 reports, indicating that amateur runners had mean baseline O3I of around 5.7% (5.8% and 5.6%
296 in OMEGA and MCT groups, respectively).

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300 12 weeks with omega-3 fatty acid supplementation at a dose of 2234 mg of EPA and 916 mg
301 of DHA daily during endurance training increased O3I in all but one participant in OMEGA
302 group to mean of 11.4%, which is considered to be well within the O3I target range (5).
303 Moreover, an increase in O3I correlated with an increase in running economy at velocity 12
304 km/h when results post minus pre 12-week intervention of participants from both groups were
305 combined. Improvements in exercise economy as an effect of supplementation with omega-3
306 fatty acids have previously been shown in both amateur and competitive athletes (9,23,24). In
307 an 8-week double-blind, parallel design study in well trained cyclists, Peoples et al. (23) showed
308 that 3.2 g/day of omega-3 fatty acids reduced whole-body O₂ consumption throughout 60
309 minutes of sustained submaximal cycling. Contrary to our observations, peak oxygen
310 consumption in these cyclists was not changed, which may be related to their high level of
311 training status or quite high compared to other data (above 9%) baseline O3I values (23).
312 Improved economy of cycling during the physiologically demanding time-trial in trained
313 cyclists and runners was also revealed by Hingley et al. after 8 weeks of supplementation with
314 a dose of 560 mg of DHA + 140 mg of EPA a day. Despite an elevation in O3I (from 4.7±0.2
315 to 6.3±0.3%) the values did not achieve the recommended O3I > 8% (9), which may be related
316 to the low dose of EPA + DHA used. A study conducted by Kawabata et al. (24) with
317 recreational players of American football, rugby, baseball, and basketball is consistent with
318 other observations in trained individuals: 8-week of daily supplementation with 914 mg of EPA
319 and 399 mg of DHA increased exercise economy during a steady-state submaximal
320 cycloergometer test. In one cross-over study with trained cyclists, researchers observed an
321 increase in VO₂max after 3 weeks of supplementation with a daily dose of 660 mg of EPA and
322 440 mg of DHA (25).

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In contrast to this report, an earlier study conducted by Raastad et al. (11) showed no changes in VO_{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-week period. Exercise economy together with VO_{2max} , lactate threshold and critical power are all strongly related to endurance exercise performance (26). Therefore, studies showing increased exercise economy, VO_{2max} or VO_{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 pro-inflammatory omega-6 fatty acids (27) and AA:EPA ratio (28). Given the large cross-sectional study indicating that inverse relationship between VO_2 max and C-reactive protein (CRP) 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 VO_{2peak} compared to 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 are 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 days a week (12).

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Compared to previous studies in which performance indicators were assessed, our supplementation protocol (2234 mg of EPA and 916 mg of DHA daily for 12 weeks) 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 VO_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 VO_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 fibres (type IIA) of skeletal muscle (34). These muscle fibres have both a high oxidative and glycolytic capacity and due to 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 pre-screening, during which individuals with similar baseline omega-3 index should be selected (36).

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376 In conclusion, 12 weeks of omega-3 fatty acid supplementation at a dose of 2234 mg of
377 EPA and 916 mg of DHA daily during an endurance running programme increased O₂I to
378 values currently considered as a target range. This duration and dose of supplementation
379 combined with endurance training increased peak oxygen consumption and improved running
380 economy in amateur runners without affecting their performance.

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383 **Additional information**

384

385 **Competing interests**

386 None of the authors of this paper has a competing interest.

387

388 **Author contributions**

389 Conception and design of the experiments was undertaken by M.T, Z.J, P.C.C and J.A.
390 Collection, assembly, analysis and interpretation of data was undertaken by M.T, Z.J, M.C,
391 R.U., H.L.F, P.C.C, M.S, J.A. Drafting the work or revising it critically for important
392 intellectual content by M.T, Z.J, M.C, P.C.C, J.A. All authors have approved the final version
393 of the manuscript and agree to be accountable for all aspects of the work. All persons designated
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407 The authors declare the results of this unfunded study are presented clearly, honestly, and
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CAPTIONS FOR FIGURE

FIGURE 1. General experimental design.

FIGURE 2. Flow of participants through the study.

FIGURE 3. Effect of supplementation with omega-3 PUFAs or MCTs on individual values of O3I pre- and post-12-week intervention $*P < 0.0001$.

FIGURE 4. Effect of supplementation with omega-3 PUFAs or MCTs on erythrocyte *A*) EPA and *B*) DHA pre- and post-12-week intervention and change from baseline in *C*) EPA and *D*) DHA compared between the two groups. Data expressed as mean.

Error bars indicate \pm SD, $*P < 0.0001$.

FIGURE 5. Effect of training and supplementation on *A*) peak oxygen consumption ($\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) pre- and post-12-week intervention *B*) change in peak oxygen consumption ($\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) in the two groups over the 12-week intervention *C*) oxygen utilisation ($\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) during submaximal treadmill running at 12 km/h pre- and post-12-week intervention and *D*) change in oxygen utilisation ($\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) in the two groups over the 12-week intervention Data expressed as mean. Error bars indicate \pm SD, $*P < 0.05$.

FIGURE 6. Correlation between O3I and oxygen cost of submaximal running when:

A) OMEGA and MCT groups were combined pre- and post-12-week intervention

B) Results post minus pre (delta) in OMEGA and MCT groups were combined