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ORIGINAL ARTICLE

Effect of docosahexaenoic acid supplementation on differences of endurance exercise performance in competitive and non-competitive male cyclists

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ABSTRACT

BACKGROUND: An open-label, single-center study was designed to assess whether there were differences in endurance exercise performance between competitive and non-competitive cyclist after consumption of a docosahexaenoic acid (DHA)-rich dietary supplement (2.1 g/day) for 3 months.
METHODS: Before and after DHA supplementation, participants performed two maximal incremental tests on a cycle, an initial speed load of 70 W, with a 25 W load increase every minute. Ergospirometric assessment included maximum capacity to transport and use oxygen (VO_2max), peak heart rate (HR), maximum test time, VO_2 at second ventilatory threshold (VT2), power output to reach VT2, respiratory exchange ratio (RER) at VT2, HR and RER at 2000 mL/min oxygen uptake, and power output to reach VO_2 at 2000 mL/min.
RESULTS: The analysis of variance for repeated measures was used. Thirteen participants completed the study. All of them were healthy male volunteers aged 18 years or more without history of clinically recognized chronic disease. In the overall study population, VO_2 at VT2 and power output to reach VT2 increased significantly after 3-month treatment with DHA. The competitive level, however, had no effect in the between-group comparisons of competitive and non-competitive cyclists.
CONCLUSIONS: In this preliminary study, high-dose DHA supplementation for 3 months showed a beneficial effect on VO_2 at VT2 and power output to reach VT2 regardless the competitive level of cyclists. These data should be further confirmed in a large study sample.

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KEY WORDS: Exercise tolerance; Fatty acids, omega-3; Dietary supplements; Bicycling.

Prolonged and intense muscular exercise has been associated with an increased production of free radicals and other forms of reactive oxygen species (ROS) that lead to protein oxidation and lipid peroxidation. Low levels of ROS have been shown to have an important function for muscle contraction.[1-3] However, increased ROS concentration in the muscle during exercise might contribute to both more rapid development of muscular fatigue and post-exercise muscle injury.[4-6] In fact, a dose- and time-dependent relationship between ROS concentration and muscle fatigue has been reported.[3, 4]

Muscle fibers contain both enzymatic and non enzymatic antioxidants that work as a complex unit to regulate ROS. It has been suggested

that increasing intracellular levels of antioxidants may reduce the risk of oxidative injury and reduce fatigue since antioxidant supplementation provides protection against ROS generated during exercise.[7, 8] However, a few studies have shown a direct positive effect of antioxidant supplementation on exercise performance.[9-11]

The fish-derived omega-3 (n-3) long chain polyunsaturated fatty acids (n-3 PUFAs) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have shown to decrease the production of inflammatory molecules (eicosanoids, cytokines) and ROS. Moreover, EPA and DHA have immunomodulatory effects and attenuate inflammatory diseases.[12-14] Studies assessing the impact of n-3 PUFA supplementation on physical activity have shown benefits on red blood cell deformability, muscle damage, inflammation, oxidative stress, and metabolism during exercise.[15-17] Moreover, it has been observed that DHA supplementation induces gene expression of the components of the endogenous antioxidant defence system.[18-20]

Elite and recreational athletes, who participate in various types of physical activity and sports, consume fish oils and conjugated linoleic acid supplements to improve their performance, increase training effects, reduce body fat, increase lean body mass, and reduce muscle damage and inflammatory responses.[21] However, the effect on exercise-induced inflammation and physical performance remains to be clearly established.[22-25]In studies that have examined whether fish oil supplementation during training enhances endurance adaptations, differences in the level of training of the study participants may account for controversial results.[21] In elite or well-trained athletes, the margin of improvement is so inappreciable that it would not be a surprise if small differences in performance parameters were observed; while in sedentary subjects, starting a training program, the improvement in performance is considerable, making a possible small enhancement in performance induced by food supplementation undetectable. In fact, the most reliable results have been observed in trained subjects, who already show adaptations for that specific type of exercise, although still improving their performance.

As far as we are aware, studies of n-3 PUFA supplementation after exercise used a combination of EPA and DHA in a ratio of approximately 2:1, so that it is unknown whether supplementation mostly based on a high concentration of DHA would exert the same effects. The present study was designed to assess whether there were differences in endurance exercise performance between competitive and non-competitive cyclist after consumption of a docosahexaenoic acid (DHA)-rich dietary supplement (2.1 g/day) for 3 months. Maximal capacity of the body to transport and use oxygen (VO_{2max}) and other ergospirometry parameters have shown its relevance to endurance exercise performance and are predictors of exercise capacity. Hence, we analyzed ergonomic parameters resulting from an endurance test before and after 3 months of DHA supplementation.

Materials and methods

Study design

A single-center open label study was performed in a randomly selected group of male amateur cyclists, including competitive (bicycle racing) and non-competitive sportsmen. The objectives of the study were as follows: 1) to determine whether DHA supplementation over 3 months modified aerobic condition differently in subjects who performed competitive physical exercise and in those who performed physical exercise as a leisure activity; and 2) to assess modifications of the aerobic physical condition in each study group (competitive *versus* non-competitive cyclists). The study was approved by the Ethics Committee of the Catholic University of San Antonio of Murcia, Spain, and the study protocol met all requirements of the Declaration of Helsinki for research involving human subjects. All participants were fully informed of the aims of the study and gave written informed consent.

Participants

The participants were healthy male volunteers aged 18 years or more without history of clinically recognized chronic disease. Subjects were excluded if they were current smokers or regular alcohol users, as well as if they had consumed any food product enriched with omega 3 fatty acids in the previous 30 days or had been exposed to ionizing

radiation for accidental, diagnostic or therapeutic reasons in the previous 30 days. Participants treated with any medication that in the opinion of the physician in charge could influence physiological responses to the endurance test were also excluded as were participants with American College of Sports Medicine (ACSM) absolute or relative indications for termination of an endurance test.

Study procedures

All participants had a full medical history, physical examination, and a baseline electrocardiogram (ECG). The study consisted of 1-week run-in period followed by a 13-weeks intervention period, in which participants received 3500 mg of Algatrium® daily (Brudy Lab S.L., Barcelona, Spain) (containing 2.1 g DHA) in three divided doses for 3 months. Two endurance tests were performed, at baseline before DHA supplementation and after 3 months. Competitive cyclist underwent endurance tests during the season in which they were engaged in racings where the objective was maintenance of physical condition. During the study period and 2 weeks before measurements, competitive cyclists neither made changes in their training programs nor were allowed to participate in bicycle competitions. Forty-eight hours before each endurance test on a cycle ergometer participant (both competitive and non-competitive cyclists) did not make any intense physical or physiological effort. Non-competitive cyclists were allowed to continue with their leisure cycling activities during the study.

Participants were fasted for 120 minutes before each test. At the facilities at which endurance tests were performed, environmental conditions were controlled and kept constant in both tests. The maximal incremental endurance test was performed with a cycle ergometer Cyclus2®. The bicycle was adapted to each subject. Participants cycled until exhaustion with a continuous increase of intensity based on an initial load of 70 W, with a 25 W load increase every minute. During the test, cyclists were asked to maintain a cadence (pedaling rate) between 60 and 100 revolutions per minute. The possible oscillations of the pedaling rate did not affect the effort made to the extent that the cycle ergometer allows the control of the power output and increases the resistance when the cyclist slow down. The duration of the test differed for each subject.

At the end of each endurance test, the following ergospirometric parameters were analyzed: maximum capacity to transport and use oxygen (VO_{2max}); peak heart rate (HR) defined as the highest HR attained during the endurance test; maximum test peak power output; VO_2 at ventilatory threshold 2 (VT2); respiratory exchange ratio (RER); and power output to reach VT2. Also, HR and RER were measured when cyclists reached an oxygen uptake of 2000 mL/min. Power output to reach that level of oxygen consumption was also determined. The anaerobic threshold was measured using the same expiratory volume. Inflection points over time were used to determine thresholds of the VE/VCO_2, VE/VO_2, and the VE. The second increase in VE with a concomitant rapid increase in VE/VO_2 and VE/VCO_2 was defined as VT2. All measurements of VT were made by visual inspection of graphs by two experienced exercise physiologists independently and in a blinded fashion. If the determinations of VT were not within a 3% agreement between investigators, a third trained researcher independently analyzed the same data to adjudicate the determination of VT. The adjudicated VT value was then compared with those of the initial determinations and averaged using the value within 3% of the initial measurement. Expired air was collected via a face mask and analyzed using a SensorMedics Vmax 29c Series Auto Respiratory Analyzer. Heart rate was measured using a HR monitor.

Statistical analysis

Results are expressed as frequencies and percentages for categorical variables and as mean and standard deviation (SD) for continuous variables. Analysis of variance (ANOVA) for repeated measures was used to compare ergospirometry parameters obtained in the endurance performance tests carried out at baseline and after DHA supplementation for 3 months. Competitive level (non-competitive and competitive cyclists) was included as an inter-subject factor in the analysis as it is known to be a possible confounder related to endurance exercise performance. Statistical significance was set at $P<0.05$. Statistical analy-

sis was performed with the SPSS version 15.0 (Statistical Package for Social Sciences, SPSS, Inc., Chicago, IL, USA).

Results

Six competitive male cyclists who regularly competed in road cycle racings (mean [standard deviation, SD] 31.8 [±5.4] years, peak power output 415 [±15] W, VO_{2max}/peak ratio 61.7 [±7] mL/kg·min, > 5 training sessions per week, > 500 km per week, > 5 years of cycling experience) and seven non-competitive male cyclists (41.1 [4.7) years, peak power output 355 [±40] W, VO_{2max}/peak ratio 44.8 [±1.6] mL/kg·min, > 4 training sessions per week, > 250 km per week, > 5 years of cycling experience) volunteered to participate in this study.

Results of ergospirometric and cardiac parameters for the overall study population are shown in Table I. DHA supplementation for 3 months resulted in a significant increase in VO_2 at VT2 from a mean (SD) of 2681.0 (495.4) mL/min at baseline to 2881.0 (606.2) mL/min at the end of the study (P=0.019). Also, power output to reach VT2 also increased significantly from 290 (45) to 308 (47.5) W (P=0.032). Other differences in VO_{2max}, peak HR, maximum test peak power output, HR at VT2, RER at VT2, power output to reach VO_2 2000 mL/min, and HR and RER at VO_2 2000 mL/min between baseline and after 3 months of DHA administration were not found.

Table II shows the comparison of results obtained after the first and second endurance tests in both competitive and non-competitive cyclists. Among competitive cyclists, within-group comparison showed significant differences in maximum peak power output, HR at VT2, RER at VT2, and RER at VO_2 2000 mL/min. In non-com-

TABLE I.—*Results of ergospirometry at baseline and after 3 months of DHA supplementation in the overall study population.*

Parameters	Baseline	After 3 months DHA intake	P value
VO_2 max, mL/kg·min	52.3 (10.7)	52.3 (9.7)	0.823
Peak HR, bpm	179.3 (6.0)	180.4 (4.8)	0.461
Maximum peak power output, W	383 (43)	387 (38)	0.649
VO_2 at VT2, mL/min	2681.0 (495.4)	2881.0 (606.2)	0.019
HR at VT2, bpm	152.4 (5.6)	155.6 (7.7)	0.082
Power output to reach VT2, W	290 (45)	308 (47,5)	0.032
RER at VT2	0.98 (0.04)	1.01 (0.07)	0.206
HR at VO_2 2000 mL/min, bpm	132.3 (14.2)	128.0 (14.2)	0.066
Power output to reach VO_2 2000 mL/min, W	225 (30)	225 (25)	0.806
RER at VO_2 2000 mL/min	0.92 (0.06)	0.93 (0.08)	0.693

VO2: oxygen uptake; VO2 max: maximal oxygen uptake; HR: heart rate; bpm: beats per minute; VT2: ventilatory threshold 2; RER: respiratory exchange ratio. Data as mean (standard deviation, SD).

TABLE II.—*Results of ergospirometry at baseline and after 3 months of DHA supplementation in the groups of competitive and non-competitive cyclists.*

Parameters	Competitive			Non-competitive			P value competitive level x time
	Baseline	After 3 months DHA supplement	P value	Baseline	After 3 months DHA supplement	P value	
VO2 max, mL/kg·min	61.7 (7.0)	59.5 (9.5)	0.18	44.8 (5.7)	46.5 (5.3)	0.23	0.086
Peak HR, bpm	177 (7)	179 (7)	0.41	181 (5)	181 (3)	0.85	0.619
Maximum peak power output, W	415 (15)	412 (35)	0.023	355 (40)	365 (28)	0.05	0.313
VO2 at VT2, mL/min	3077.3 (284.5)	3318.3 (481.5)	0.071	2364 (384.0)	2531.2 (465.8)	0.18	0.572
HR at VT2, bpm	153 (9)	161 (7)	0.017	152 (2)	151 (5)	0.68	0.036
Power output to reach VT2, W	330 (12.5)	350 (20)	0.002	255 (30)	270 (27)	0.15	0.999
RER at VT2	0.97 (0.03)	1.01 (0.10)	0.03	0.98 (0.04)	1.00 (0.03)	0.02	0.569
HR at VO2 2000 mL/min, bpm	121 (6)	119 (6)	0.49	141.4 (11)	135.2 (15)	0.04	0.302
Power output to reach VO2 2000 mL/min, W	235 (25)	230 (25)	0.43	215 (30)	218 (28)	0.60	0.359
RER at VO2 2000 mL/min	0.88 (0.03)	0.90 (0.06)	0.04	0.94 (0.06)	0.95 (0.04)	0.03	0.965

petitive cyclists similar results were observed but HR at VT2 was not significant. However, when between-group differences were assessed, statistically significant differences were only observed in HR at VT2. For the remaining ergospirometric parameters, no significant effect of the competitive level was documented (Table II).

Discussion

The present findings of an increase in VO_2 at VT2 and power output to reach VT2 after 3 months of high-rich DNA supplementation indicates a beneficial effect of DHA consumption on endurance exercise performance in male amateur cyclists. Moreover, these favourable effects were independent of the competitive level of the sportsmen. Likewise, the results suggest that HR at an oxygen uptake of 2000 mL/min might decrease after DHA consumption, whereas an impact on other parameters, such as VO_{2max}, peak HR, maximum peak power output, HR and RER at VT2, power output to reach VO_2 at 2000 mL/min and HR and RER at VO_2 at 2000 mL/min were not observed.

Although omega-3 fatty acids supplementation seems to be associated with an improvement in performance in laboratory animals and migrating birds, the evidence in humans is limited.[16] However, this study adds positive results regarding the benefits of DHA supplementation in exercise performance under some conditions. So far, the parameters that have shown positive results after omega-3 fatty acids intake reported in other studies were different. In 13 athletes of both genders who took 4 g/day of fish oil (300 mg EPA and 200 mg DHA) for 4 weeks, a trend for improvement in exercise time to volitional fatigue after jogging for 75 min at 60% VO_{2max} was found.[26] In another study, supplementing 24 female elite soccer players with 3.5 g/day of DHA-rich fish oil for 4 weeks produced perceptual-motor benefits (*i.e.*, improvements in complex reaction time and efficiency). The authors of this study concluded that DHA could be a beneficial supplement in sports where decision making and reaction time efficiency are of importance.[27]

In our study, the lack of changes of VO_{2max} after DHA consumption could be expected as the maximal capacity of the body to transport and use oxygen does not change after PUFAs supplementation. Fish oil supplementation (4 g/day) was investigated in 32 sedentary males (aged 19-34) and a slight non-significant improvement in VO_{2max} and ventilatory anaerobic threshold was found in the fish group as compared to controls, exercise, and exercise and fish groups.[28] In addition, 10 weeks of omega-3 supplementation (1.6 g/day of EPA and 1.04 g/day of DHA among trained soccer players had no effect on aerobic power or running performance.[22] In another study that tested the effect of omega-3 supplementation (0.36 g/day of EPA and 1.56 g/day of DHA) on endurance exercise performance among Australian Rules football players, an improvement in endurance performance or recovery was not observed, although an improvement in cardiovascular function was observed which may enhance the sustained performance of submaximal exercise.[29] A more recent study evaluating the influence of omega-3 fatty acids on exercise performance in athletes who received 0.4 g/day of EPA and 2 g/day of DHA, no changes in exercise performance was observed.[30]

Limitations of the study

Our findings should be interpreted taking into account the limitations of the study, which should be considered preliminary and obtained in a non-controlled and small group of participants. Although further data in a larger study population are needed, the present results indicate that high-rich DHA supplementation for 3 months increased power output to reach VT2 and VO_2 at VT2 in male cyclists. This observation, which was independent of the competitive level, is relevant because of the potential influence of DHA intake to improve endurance exercise performance.

Conclusions

This study provides evidence that daily supplementation with high-dose DHA for 3 months was associated with an improvement of aerobic condition in both cyclists performing competitive physical and non-competitive cyclists who trained regularly. Importantly, no differences in the improvement of the anaerobic condition according to levels of cycling performance were observed.

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Conflicts of interest.—The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Authors' contributions.—The study was designed by Francisco J. López-Román and José A. Villegas-García; data were collected and analyzed by Francisco J. López-Román, Carlos J. Contreras-Fernánde, Antonio J. Luque-Rubia; data interpretation and manuscript preparation were undertaken by Francisco J. López-Román and José A. Villegas-García. All authors approved the final version of the paper.

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