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**ORIGINAL ARTICLE**

# Omega-3 fatty acids supplementation improves endothelial function and maximal oxygen uptake in endurance-trained athletes

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**Abstract**
The study aimed to evaluate the effects of a 3-week n-3 polyunsaturated fatty acids (n-3 PUFA) supplementation on serum nitric oxide (NO), asymmetric dimethyloarginine (ADMA), ultrasound indices of endothelial function and maximal oxygen uptake ($\dot{V}O_{2max}$) of elite cyclists. The effects of dietary supplementation (n-3 PUFA at a dose of 1.3 g twice daily for 3 weeks) and placebo administration on flow-mediated dilatation (FMD), pulse wave velocity, serum markers (NO, ADMA), lipid profile, and $\Delta\dot{V}O_{2max}$ were analysed in 13 cyclists both before and after dietary protocols. Significant differences between pre- and post-intervention baseline NO levels were observed after n-3 PUFA dietary protocol (13.9 ± 4.2 vs. 23.5 ± 3.6 μmol·l$^{-1}$; $P < 0.001$). Higher post-intervention baseline NO level was observed after n-3 PUFA diet compared with placebo (23.5 ± 3.6 vs. 15.3 ± 3.0 μmol·l $^{-1}$; $P < 0.01$, respectively). The n-3 PUFA increased baseline NO concentration (ΔNO) by 6.7 ± 3.8 μmol·l$^{-1}$ and placebo by 1.6 ± 4.4 μmol·l$^{-1}$. The positive correlation was observed between baseline post-intervention NO concentration and maximal oxygen uptake ($r = 0.72$; $P < 0.01$) and also between ΔNO and $\Delta\dot{V}O_{2max}$ ($r = 0.54$; $P < 0.05$) in response to omega-3 fatty acids supplementation. There was an association between a 5.25% higher FMD ($P < 0.05$) and higher $\dot{V}O_{2max}$ ($P < 0.001$) after n-3 PUFA diet compared with lower values of placebo ($r = 0.68$; $P < 0.05$). These findings suggest that an increase in NO release in response to n-3 PUFA supplementation may play a central role in cardiovascular adaptive mechanisms and enhanced exercise performance in cyclists.

**Keywords:** *Omega-3 fatty acids, nitric oxide, flow-mediated dilatation, physical exercise*

## Introduction

Endurance-trained athletes, including those with high aerobic capacity, can improve exercise performance with training. This improvement is the result of adaptive changes in skeletal muscle, such as increase in oxidative fibres, and ability to use oxygen to generate energy for muscle work. Exercise training leads to beneficial changes in cardiovascular system including an improvement in left ventricular function, a decrease in myocardial oxygen demand for the same level of external work performed as well as metabolic shift towards an increased lipid metabolism (Bell, 2008; Jensen, Bangsbo, & Hellsten, 2004).

In a recent study, arterial structural and functional remodelling and the existence of an "athlete's artery" was suggested in humans following regular training (Green, Spence, Rowley, Thijssen, & Naylor, 2012). These functional responses in arteries are influenced by exercise-induced endocrine and/or neural mechanisms and are probably affected by athletic status (Prior, Yang, & Terjung, 2004). Physical exercise leads to progressive increase in heart rate (HR), which, in turn, increases blood flow and vascular shear stress. The potential mechanisms through which vascular control may be beneficially modified in response to repeated exposure to shear stress include increased endothelium-dependent dilator capacity (Celec & Yonemitsu, 2004), higher production of endothelial nitric oxide (NO) and endothelial NO synthase (eNOS) expression (Bailey, Vanhatalo, Winyard, & Jones, 2012; Jensen et al., 2004). Several studies support that functional responses in arteries are influenced by mechanism that impacts arterial vasomotor tone and can be affected by type of exercise training and/or athletic

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status (Bell, 2008; Calbet et al., 2007; Green et al., 2012). It is important to note that power athletes have larger brachial arteries and greater vasodilatory responses; however, in endurance-trained athletes, lower and not higher flow-mediated dilatation (FMD)'s values have been observed in response to larger artery size (Green et al., 2012). It has been hypothesized that higher oxygen extraction fraction in the exercising muscle could be associated with peripheral vascular adaptations in skeletal muscle consisting of longer blood transit time, more homogenous perfusion and enhanced vascular flow capacity (Kalliokoski et al., 2001).

The physiological consequence of intense physical exercise is peripheral vasoconstriction induced by activation of the arterial baroreflex, which results in an increase in sympathetic outflow and blood pressure (BP; Calbet et al., 2007). In athletes, peripheral vasoconstriction during maximal exercise is prevented by improved endothelium-dependent dilator capacity (as indicated by plasma levels of nitrite oxide), which may increase skeletal muscle oxidative capacity during exercise (Newcomer, Leuenberger, Hogeman, Handly, & Proctor, 2004). Apart from these molecular mechanisms, dietary factors also seem to play an important role in optimizing the effects of training (Fahs et al., 2010). There is strong evidence suggesting that dietary supplementation with omega-3 polyunsaturated fatty acids (n-3 PUFA) had a positive impact on vascular function in healthy subjects (Anderson et al., 2010; Khan et al., 2003) and patients with metabolic (Juturu, 2008) and cardiovascular disease (Lovegrove & Griffin, 2013; Wang et al., 2012). The potential mechanisms by which omega-3 fatty acids could improve cardiovascular function are currently under investigation, but their action may be related to the improvement in vascular endothelial function (e.g. increased availability of NO; Juturu, 2008; Wu & Meininger, 2002) and lipid metabolism (Chen, Montagnani, Funahashi, Shimomura, & Quon, 2003), enhancement of vascular reactivity, production of endogenous antioxidant enzymes and anti-inflammatory effects (e.g. decreased interleukin-6, monocyte chemotactic protein-1, tumour necrosis factor; Mickleborough, 2013; Wu & Meininger, 2002). Several mechanisms have been reported that may be responsible for the omega-3 fatty acids effect on the bioavailability of NO, including the effect of eicosapentaenoic acid (EPA) on NO release and endothelial function via adenosine monophosphate-activated protein kinase activation in response to depletion of intracellular adenosine triphosphate (Nishimura et al., 2000; Wu et al., 2012). Asymmetric dimethyloarginine (ADMA), an endogenous competitive inhibitor of NO synthase, is another factor that regulates NO availability to target cells (Eid et al., 2006). Increased plasma levels of ADMA have been shown to antagonize the endothelium-dependent vasodilatation (Jobgen, Fried, Fu, Meininger, & Wu, 2006).

There are limited data regarding the effect of omega-3 acid supplementation on vascular endothelial function and optimization of physical performance of elite athletes. We hypothesized that n-3 PUFA might improve this function via the stimulation of NO production and release. To verify this, we examined the relationships between endothelial function, as measured by FMD, arterial stiffness (pulse wave velocity, PWV), NO concentrations and maximal oxygen uptake ($\dot{V}O_{2max}$) and omega-3 fatty supplementation in elite cyclists.

## Methods

### *Subjects*

Thirteen male cyclists (aged: 23.1 ± 5.4 years) who were endurance trained for about 7 years participated in the study. They were randomly assigned to both dietary protocols (i.e. placebo or the omega-3 fatty acids supplementation, placebo-controlled study). Then, after a 2-week break (i.e. washout period), members of each group were crossed over to the opposite treatment protocol and following brachial artery measurement, biochemical investigations and physical exercise test were repeated. All subjects participated in the study during the pre-season period. The training status of the subjects expressed as maximal oxygen consumption ($\dot{V}O_{2max}$) was 69.8 ± 4.9 $ml \cdot kg^{-1} \cdot min^{-1}$ and the mean individual monthly training volume was 655 ± 53 km. Age, height, body mass and body mass index of the participants (mean ± SD) are presented in Table I. Echocardiography (ECG) measurements of cyclists indicate that left ventricular structure and function were within normal range (Table I).

The exclusion criteria used in order to eliminate factors which might influence the vascular parameters were as follows: evidence of hemodynamic dysfunction, inflammatory diseases in the preceding 3 months and cigarette smoking. All subjects reported that they were not taking fish oil supplements or any medication that could affect cardiovascular function. They were instructed to abstain from strenuous exercise within 24 hours before the ultrasound measurements. No caffeine, antioxidants and alcohol were permitted during 48 hours before the experiment. Three weeks prior to the study, all participants were put on a mixed, isocaloric diet (2899 ± 1100 kcal/day) consisting of carbohydrates in the amount of 375.3 ± 162.6 g/day, proteins: 132.3 ± 48.5 g/day and fats: 104.9 ± 46.7 g/day (monounsaturated fats: 37.4 ± 16.6 g/day and

Table I. Anthropometric and ECG variables of the subjects (mean ± SD)

| Variables | Subjects ($n$ = 13) |
|---|---|
| Age (years) | 23.1 ± 5.4 |
| Body mass (kg) | 71.5 ± 5.9 |
| Body height (cm) | 178.5 ± 4.4 |
| BMI (kg/m$^2$) | 22.4 ± 1.3 |
| LVM (g) | 221.1 ± 52.3 |
| LVMI (g/m$^2$) | 120.6 ± 24.7 |
| IVSDd (mm) | 10.1 ± 1.7 |
| LVPWTd (mm) | 9.4 ± 1.3 |
| LVEF (%) | 63.3 ± 3.9 |
| SV (ml) | 83.3 ± 9.3 |

BMI, body mass index; LVM, left ventricular mass; LVMI, left ventricular mass index; IVSDd, intraventricular septum diameter during diastole; LVPWTd, left ventricular posterior wall thickness during diastole; LVEF, left ventricular ejection fraction; SV, stroke volume.

polyunsaturated fats: 8.7 ± 4.2 g/day). The isocaloric diet was continued with PUFA or placebo administration and during the washout period. We supplemented our subjects for 3 weeks and before each diet protocol the brachial artery and biochemical variables were analysed. The cyclists consumed the prescribed diet for a total of 11 weeks (3 weeks before, during the 3 weeks of both dietary regimens and 2-week break period). To make sure that subjects consumed a diet, they were asked to sign a statement of compliance with diet. Physiologist and cyclist's coach regularly reminded them about the need to comply with dietary regime.

The experiment was approved by the Ethics Committee of the Academy of Physical Education in Katowice and conformed to the standards set by the Declaration of Helsinki.

### *Supplementation procedure and training protocol*

All clinical data, including biochemical parameters and ultrasound examination, were obtained after an overnight fast. M-mode and two-dimensional Doppler-ECG were performed in all subjects using a Sonos 100 Hewlett-Packard Image Point HX ultrasound system (USA) with standard imaging transducers to determine the left ventricular muscle mass, interventricular septum diameter during diastole and left ventricular posterior wall thickness during diastole. The left ventricular mass index (LVMI) was calculated by correcting for body surface area (Table I). The brachial artery measures included: FMD of the brachial artery, brachial systolic BP (SBP) and diastolic BP (DBP), PWV, and central arterial stiffness. Following these measurements, blood samples were taken through a peripheral catheter inserted into the antecubital vein; each participant completed incremental ergometer exercise test. After initial testing, the omega-3 fatty acids supplemented group received 1.3 g (20 mg/kg) of n-3 PUFA (GOLD OMEGA 3, Olimp LABS) consisting of 660 mg EPA, 440 mg docosahexaenoic acid, 200 mg other acids and 13.4 mg vitamin E (gelatin capsules). The control group received a placebo in the form of gelatin capsules (1.3 g lactose monohydrate). Participants were instructed to take the capsules with meals twice daily for a total of 3 weeks. The participants returned to the laboratory after 3 weeks for post-testing. After a 2-week break, participants were crossed over to the opposite treatment protocol (placebo vs. n-3 PUFA diet).

### *Measurements and blood collection*

At the beginning of the study (pre-intervention) and at the end of each treatment period (post-intervention supplementation or placebo protocol), all subjects reported to the laboratory and had venous blood drawn for the determination of NO and ADMA concentrations and lipid analysis.

All investigated subjects underwent bioelectric impedance analysis (InBody Data Management System) under resting conditions to determine their body mass. The endothelial function was assessed by vascular ultrasonography techniques.

### *Flow-mediated dilatation*

The examination was performed between 08.00 and 10.00 hours in a temperature-controlled room (20–22°C) with subjects in the supine position. Conduit-vessel endothelial function was assessed using the ultrasound measurement of right brachial artery diameter (BAD) during changes in brachial artery flow (7–12 MHz linear array transducer, Logic 7, GE). A 5-cm segment of the right brachial artery was imaged just above the antecubital fossa in the longitudinal plane with the optimal probe site marked on the skin. Baseline images of BAD and Doppler velocities from the centre of the vessel were recorded. A proximal occluding forearm cuff placed above the antecubital fossa was inflated to 50 mm Hg above systolic pressure for 3 minutes. BAD and blood flow were obtained during 50–60 seconds after the cuff's deflation. Blood flow was measured from the pulsed Doppler signal and arterial diameters were taken from the anterior to the posterior "M" line at the end diastole. Images were acquired with an ECG gating with the end of the diastole corresponding to the onset of the R wave. After 10-minute rest, sublingual nitroglycerine (0.5 mg) was administrated to determine the maximum obtainable exogenous vasodilatatory response. The response of the vessel diameter to reactive hyperemia (FMD) was calculated and expressed as the percentage

change relative to the diameter determined immediately before cuff inflation. The nitroglycerin-induced diameter change was expressed as the percentage change relative to the recovery scan (nitroglycerin-mediated dilatation, NMD; Corretti et al., 2002).

### *Brachial BP*

Resting SBP and DBP was measured in the supine position using an automated oscillometric cuff (HEM-907 XL, Omron Corporation, Japan). The BP measurements were made in duplicate and the average of the two values was recorded.

### *Pulse wave velocity*

The SphygmoCor device (AtCor Medical, Australia) was used to assess PWV. The pulse waveforms were recorded in the supine position from the right radial artery at the wrist and at the base of the neck for the right common carotid artery using applanation tonometry. The measurements were obtained by a one-experienced operator who had passed SphygmoCor measurements training. After the waveform was stabilized, the 10-second signal was recorded with a sampling rate of 128 Hz. About 10 recurrences per subjects were used in the analysis of the pulse waveform. Recordings with an operator index above 85 (in the scale of 0–100) were used only.

Transit time was measured automatically as the time between the foot of the pulse wave and the foot of the R wave. Time delay ($t$) was calculated as the difference between these two transit times. The distance ($d$) travelled by the pulse wave was measured over the body surface as the distance between two recording sites. PWV was calculated automatically as PWV = $d$ (meters)/$t$ (seconds; Asmar et al., 1995).

### *Exercise test*

Before and after 3 weeks of each treatment protocol (supplementation or placebo), all subjects performed a standard cycling exercise test (Lode Excalibur Sport Ergometer Bicycle, Groningen, Netherlands) to analyse whether n-3 fatty acids might positively affect exercise performance of cyclists. The test started with a 3-minute warm-up; the intensity was then increased by 40 W every 3 minutes up to maximal exercise intensity. Pulmonary ventilation, oxygen uptake ($\dot{V}O_2$) and carbon dioxide output ($CO_2$) were measured continuously from the sixth minute prior to exercising and throughout each stage of the exercise load using the Oxycon Apparatus (Jaeger, Germany). HR was continuously monitored (PE-3000 Sport-Tester, Polar Inc. Finland) and BP (SBP/DBP) was measured in duplicate with a sphygmomanometer before and immediately after exercise.

### *Biochemical analyses*

For biochemical analysis, antecubital venous blood samples were drawn always at the same time of day, with the subject in a seated position. Venous blood samples were collected 5 time points (baseline, pre- and post-exercise with placebo and pre- and post-exercise with n-3 PUFA intervention). Blood was allowed to clot at room temperature and then centrifuged. The resulting serum was aliquoted and frozen at −80°C for later analyses.

The measurements of ADMA and total NO and nitrite/nitrate were performed using enzyme-linked immunosorbent assay (R&D System, Inc., Minneapolis, USA). The sensitivity of total NO/nitrite/nitrate assay was 0.25 $\mu mol \cdot l^{-1}$. Intra- and interassay coefficients of variation for total NO/nitrite/nitrate were <2.5% and <4.6%, respectively. ADMA test disclosed values as low as 0.05 $\mu mol \cdot l^{-1}$. The intra-assay coefficient of variation was <9.8%, and the interassay coefficient of variation was <7.5%. Total serum cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol (CHOLESTEROL CHOD-PAP), free fatty acids (FA 15) and triglycerides (TRIGS, GPO-PAP), and glycerol (GLY RX MONZA) were measured using commercially available test kits (RANDOX, UK). Glucose concentration was measured using a commercial kit (GLUC-PAP, RANDOX, UK). Total antioxidant status (TAS) was measured using commercially available test kits (TAS RANDOX, UK).

### *Statistical analysis*

All results are presented as the mean ± standard deviation. We analysed differences between pre- and post-interventions (placebo/n-3 PUFA) baseline and post-exercise variables. The results of the change in pre-intervention and post-interventions baseline NO level (ΔNO) and increase in maximal oxygen uptake ($\Delta\dot{V}O_{2max}$) were calculated. The data were analysed by two-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls test when appropriate. The statistical analysis includes a two-way ANOVA (placebo vs. n-3 PUFA and baseline pre-intervention vs. baseline post-intervention). Pearson correlation coefficients were analysed to determine the inter-variable relationships. All analyses were performed using the Statistica v. 9 statistical software package (StatSoft, Tulsa, OK, USA). Statistical significance was set at $P < 0.05$.

## Results

The effects of dietary supplementation with n-3 PUFA and placebo administration on total NO and ADMA concentrations, and vascular indexes, i.e. FMD and PWV in endurance-trained male cyclists were compared after 3 weeks of each treatment protocol.

ANOVA revealed a significant effect of n-3 PUFA supplementation on serum NO concentration ($F$ = 23.0; $P < 0.001$). Significant differences between pre-intervention and post-intervention baseline NO levels were observed after n-3 PUFA dietary protocol (13.9 ± 4.2 vs. 23.5 ± 3.6 μmol·l$^{-1}$; $P < 0.001$). Significant higher post-intervention baseline NO level was observed after n-3 PUFA diet compared with placebo (23.5 ± 3.6 vs. 15.3 ± 3.0 μmol·l$^{-1}$; $P <$ 0.01, respectively). The n-3 PUFA increased baseline NO concentration (ΔNO) by 6.7 ± 3.8 μmol·l$^{-1}$ and placebo by 1.6 ± 4.4 μmol·l$^{-1}$. Significant higher baseline ΔNO was observed in n-3 PUFA diet compared with placebo ($P < 0.01$). The n-3 PUFA diet and placebo increased NO levels after cessation of exercise test (26.7 ± 5.2 and 19.1 ± 5.0 μmol·l$^{-1}$; $P < 0.01$, respectively; Figure 1).

No significant effect of n-3 PUFA diet was observed regarding NO increase (ΔNO) at maximal exercise intensity compared with post-intervention baseline levels (e.g. placebo: 4.1 ± 4.8 vs. n-3 PUFA: 3.4 ± 4.8 μmol·l$^{-1}$; $P = 0.7$).

Significant positive correlation was observed between NO level and $\dot{V}O_{2max}$ both in response to supplementation ($r = 0.72$; $P < 0.01$) and placebo ($r = 0.78$; $P < 0.01$). However, importantly, the positive correlation was observed between ΔNO and $\Delta\dot{V}O_{2max}$ ($r = 0.54$; $P < 0.05$) only in response to omega-3 fatty acids supplementation. ANOVA did not reveal any significant effect of n-3 PUFA on ADMA levels ($F = 2.6$; $P > 0.05$). There was a trend for increased ADMA baseline levels after PUFA diet compared with pre-intervention levels (0.34 ± 0.09 vs. 0.43 ± 0.08 μmol·l$^{-1}$; $P = 0.052$). Significant increase in post-exercise ADMA levels was observed in investigated subjects after both treatment protocols compared with baseline post-interventions levels (Figure 2). After n-3 PUFA diet, significant negative correlation was seen between baseline ADMA level and NO ($r = -0.60$; $P < 0.05$).

The parameters of vascular endothelial function before and after a 3-week period of n-3 PUFA or placebo administration are presented in Table II. Resting HRs as well as SBP and DBP did not differ between both treatment protocols. The n-3 PUFA intervention significantly increases the values of the aortic pulse pressure (APP; $P < 0.01$) and decrease BAD ($P < 0.05$). The 3-week supplementation with n-3 PUFA did not significantly influence PWV although a tendency to lower levels was found in the supplemented group ($P < 0.07$). Omega-3 fatty acids supplementation resulted in a significant increase in FMD percentage ($P < 0.05$) compared with placebo. Endothelium independent vasodilatation (NMD) did not differ significantly between both dietary protocols ($P = 0.4$; Table II).

Compared to the placebo administration, n-3 PUFA diet significantly increased maximal oxygen uptake ($\dot{V}O_{2max}$) and individual $O_2$-uptake/HR ($\dot{V}O_{2max}/HR_{max}$), which was associated with lower HR at maximal exercise intensity ($P < 0.05$; Table II).

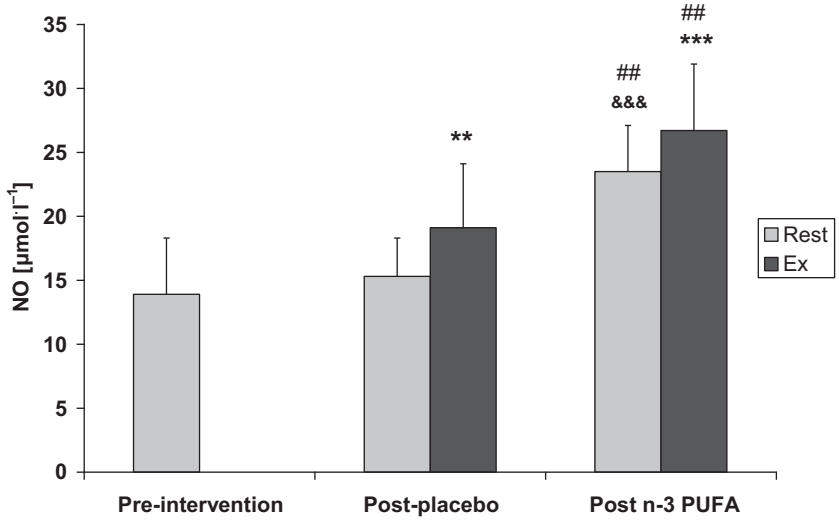


Figure 1. NO serum concentrations before diet (pre-intervention), after placebo, and n-3 PUFA diet (post-intervention) at rest and at maximal exercise intensity.
##$P < 0.01$ significant differences post-intervention baseline placebo vs. n-3 PUFA.
**$P < 0.01$; ***$P < 0.001$ significant differences baseline vs. Ex.
&&&$P < 0.001$ significant differences baseline pre- vs. post-interventions.

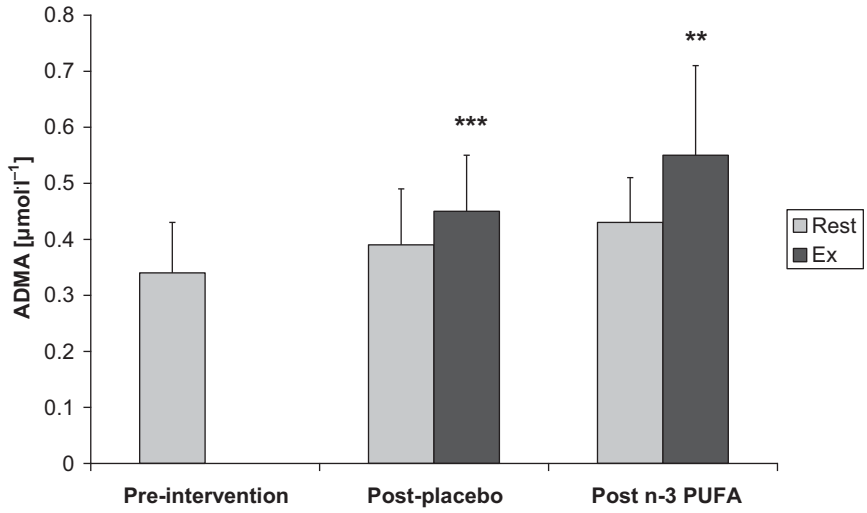


Figure 2. ADMA serum concentrations before diet (pre-intervention), after placebo, and n-3 PUFA diet (post-intervention) at rest and at maximal exercise intensity.
**$P < 0.01$; ***$P < 0.001$ significant differences post-intervention baseline vs. Ex.

Higher FMD percentage observed after n-3 PUFA supplementation correlated with $\dot{V}O_{2max}$ levels ($r = 0.68$; $P < 0.05$). The positive correlation was observed between increase in FMD percentage and $\Delta\dot{V}O_{2max}$ ($r = 0.52$; $P < 0.05$) in response to omega-3 fatty acids supplementation but not in placebo ($r = 61$; $P = 0.2$). Smaller BAD and negative correlation ($r = -0.57$; $P < 0.05$) between FMD percentage and BAD were observed in n-3 PUFA diet.

Supplementation with n-3 PUFA did not have any effect on body mass with a slight impact on lipid profile of the study subjects (Table II). The n-3

Table II. Vascular indexes, physiologic characteristics, lipid profile, glucose concentrations and TAS of the subjects (mean ± SD)

| Variables | Pre-intervention | Diet | |
|---|---|---|---|
| | | Placebo | n-3 PUFA |
| HR (beats·min$^{-1}$) | 60.0 ± 12.0 | 59.0 ± 11.0 | 56.0 ± 11.0 |
| SBP at rest (mmHg) | 126.0 ± 13.0 | 128.0 ± 12.0 | 129.0 ± 16.0 |
| DBP at rest (mmHg) | 83.0 ± 11.0 | 82.0 ± 10.0 | 80.0 ± 14.0 |
| ASP (mmHg) | 110.9 ± 11.1 | 109.5 ± 14.1 | 109.5 ± 14.1 |
| APP (mmHg) | 25.3 ± 6.2 | 26.2 ± 7.1 | 30.2 ± 9.1[ab] |
| AP (mmHg) | 0.0 ± 2.9 | −0.3 ± 2.5 | −0.6 ± 4.6 |
| PWV (m·s$^{-1}$) | 7.0 ± 1.4 | 6.5 ± 1.8 | 6.2 ± 2.0 |
| BAD (mm) | 4.2 ± 0.2 | 4.2 ± 0.2 | 4.1 ± 0.1[bc] |
| FMD (mm) | 4.6 ± 0.2 | 4.5 ± 0.1 | 4.7 ± 0.3 |
| FMD (%) | 10.3 ± 5.3 | 10.0 ± 4.6 | 15.2 ± 7.6[bc] |
| NMD (%) | 17.5 ± 5.5 | 17.6 ± 5.0 | 19.0 ± 4.8 |
| $\dot{V}O_{2max}$ (ml·kg$^{-1}$·min$^{-1}$) | 69.8 ± 4.9 | 71.0 ± 4.1 | 74.8 ± 5.6[bc] |
| Peak power (watt) | 420.0 ± 42.0 | 420.0 ± 39.0 | 430.0 ± 39.0 |
| $HR_{max}$ (beats·min$^{-1}$) | 195.0 ± 6.0 | 196.0 ± 3.0 | 186.0 ± 5.0[bc] |
| $\dot{V}O_{2max}/HR_{max}$ | 0.36 ± 0.04 | 0.36 ± 0.01 | 0.40 ± 0.01[bc] |
| Triglycerides (mg·dl$^{-1}$) | 112.8 ± 66.8 | 113.1 ± 65.0 | 123.1 ± 84.0 |
| Total cholesterol (mg·dl$^{-1}$) | 204.1 ± 36.5 | 207.7 ± 40.4 | 197.1 ± 13.0 |
| LDL-cholesterol (mg·dl$^{-1}$) | 97.6 ± 24.6 | 102.0 ± 27.0 | 110.8 ± 27.3 |
| HDL-cholesterol (mg·dl$^{-1}$) | 58.5 ± 11.8 | 55.4 ± 14.0 | 65.2 ± 14.4 |
| Free fatty acids (mmol·l$^{-1}$) | 0.8 ± 0.4 | 0.9 ± 0.4 | 1.9 ± 1.0[bc] |
| Glycerol (µmol·l$^{-1}$) | 93.0 ± 44.3 | 87.1 ± 47.0 | 60.1 ± 26.4 |
| Glucose (mg·dl$^{-1}$) | 77.6 ± 15.6 | 81.5 ± 13.8 | 89.6 ± 10.1[b] |
| TAS | 1.7 ± 0.3 | 1.8 ± 0.2 | 1.81 ± 0.4 |

[a]$P < 0.01$ significant differences normal vs. n-3 PUFA.
[b]$P < 0.05$ significant differences placebo vs. n-3 PUFA.
[c]$P < 0.05$ significant differences normal vs. n-3 PUFA.
HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; ASP, aortic systolic pressure; APP, aortic pulse pressure; AP, augmentation pressure; PWV, pulse wave velocity; BAD, brachial artery diameter; FMD, flow-mediated dilatation; NMD, nitroglycerin-mediated dilatation; $\dot{V}O_{2max}$, maximal oxygen uptake; TAS, total antioxidant status.

PUFA intervention resulted in a significant increase in free fatty acids level ($P < 0.05$), non-significant LDL-cholesterol decrease and higher HDL concentrations. The post-placebo and post-supplementation levels of triglycerides, total serum cholesterol, glycerol concentrations did not differ significantly between the placebo and supplementation protocols. Significantly higher glucose levels were observed in response to n-3 PUFA diet compared with placebo ($P < 0.05$). TAS was similar for all subjects and no significant differences were observed in post-placebo and post-supplementation levels (Table II).

## Discussion

The present study was undertaken to investigate whether n-3 omega fatty acids supplementation might exert a beneficial effect on serum NO and ADMA concentrations, ultrasound vascular indexes of brachial artery dilatation and muscle oxidative capacity in cyclists. Our results have demonstrated that a 3-week omega-3 fatty acids supplementation caused elevation of baseline serum NO compared with pre-supplementation levels. An increase in baseline and post-exercise serum NO levels were also observed in contrast to the placebo administration. Moreover, the increased NO production seems to have significant effect on FMD and resting artery diameters, a tendency was observed to PWV reduction, and significantly higher APP values. The major findings of our study are that incremental exercise is more effective in stimulating NO expression and that this effect is more pronounced in athletes after n-3 PUFA supplementation. Three weeks of n-3 PUFA supplementation had a beneficial effect on endothelial function. Endothelial function improvement, might, in turn, have significantly increased individual peak $\dot{V}O_2$ and peak $\dot{V}O_2/HR_{max}$.

Data concerning positive impacts of omega-3 fatty acids consumption on vascular function in intensely trained athletes are still sparse (Angerer & von Schacky, 2000; Khan et al., 2003). Most studies support the clinical benefits of dietary supplementation with fish oil, rich in n-3 PUFA, in patients diagnosed with cardiovascular disease (Anderson et al., 2010, Lovegrove & Griffin, 2013) and diabetes (Rizza et al., 2009). Several mechanisms have been reported that may be responsible for the cardioprotective effect of n-3 PUFA (Wang et al., 2012). The proposed mechanisms include a role of chronic supplementation with n-3 PUFA in stimulating fat oxidation in liver and skeletal muscle (Huffman, Michaelson, & Thomas, 2004) and maintenance of vasodilatation through the stimulation of NO production (Nestel, 2000). A significant effect of both exercise training and n-3 PUFA supplementation on suppression of enhanced cardiac sympathetic activity (Nishimura et al., 2000), increased sensitivity to insulin and antioxidant status has also been suggested (Anderson et al., 2010; Simopoulos, 2007). In our study, we concluded that NO production after n-3 PUFA diet has a significant effect on FMD – a non-invasive indicator of endothelial function. FMD is routinely expressed as percentage change in arterial diameter from resting baseline to post-ischemic peak. However, it was recently suggested that FMD percentage did not scale accurately for interindividual differences in baseline artery diameter. Therefore, in order to analyse differences in FMD between various populations, and/or to predict of further cardiovascular diseases, it may be useful to estimate an allometric expression ($D_{baseline}/D_{peak}^{0.89}$), rather than a simple ratio (Atkinson, Batterham, Thijssen, & Green, 2013). Smaller BAD and negative correlation between FMD percentage and BAD were observed in our study in n-3 PUFA diet. In athletes, the evaluation of the endothelial function based on brachial artery size was only carried out to confirm the effectiveness of the diet; thus, we did not reanalyse the FMD percentage using allometric scaling of diameter change. However, in our further research into the endothelial response to physical effort in athletes, we will apply the method recommended by the above literature data.

The potential mechanisms for arterial remodelling in elite athletes may be complex and influenced by endocrine and neural signals (Green et al., 2012). Moreover, all of these mechanisms may be differently affected in response to training duration and intensity (Jensen et al., 2004). In the present study, the decrease in BAD observed after endurance training and n-3 PUFA diet might have been a result of greater activation of the sympathetic nervous system and the lack of inhibition of endothelium-dependent vasoconstriction, which may occur in the post-training period. In this study, omega-3 fatty acid supplementation resulted in serum NO elevation compared with the pre-supplementation level and also with placebo group levels. Both resting NO level and post-exercise NO concentrations were significantly increased. It was also observed that omega-3 intake improved the cardiovascular adaptation to exercise. HRs of supplemented cyclists were lower at the same maximal power output compared with pre-supplementation HRs. This was accompanied by higher individual maximal oxygen uptake and $\dot{V}O_2/HR$ at maximal exercise intensity (Table II). Thus, our results suggest, but do not prove, that serum NO increase in response to 3-week omega-3 fatty acids supplementation may act as a potent causative factor leading to endothelial function improvement. The endothelium plays an important role in the maintenance of vascular function and structure in response to physical exercise. By combining the effects of vasoconstriction, repeated exposure to shear stress and an increase in peripheral

resistance, exercise exerts higher pressure load on the vasculature (Delp, 1995). Increased shear stress and/or up-regulation of eNOS gene expression enhance vascular NO production and stimulate the regulatory mechanisms of the cardiovascular system (Bailey et al., 2012; Kojda & Hambrecht, 2005; Trinity et al., 2012).

Previous studies demonstrated that endothelial NO production increases during exercise and correlates positively with oxygen consumption (Maroun, Mehta, Turcotte, Cosio, & Hussain, 1995). Our results revealed higher resting NO levels in a specifically trained supplementation group compared with the placebo. It should be emphasized that this finding is in line with the hypothesis that short-term training increases NO bioactivity, which acts to homeostatically regulate the shear stress associated with exercise (Prior et al., 2004; Vassilakopoulos et al., 2003) but may dissipate within weeks of extensive training (Iemitsu et al., 2000). Regardless of the fact that long-term exercise training might diminish NO concentrations, we conclude that a diet rich in n-3 PUFA improves cardiovascular function in athletes. Other studies have shown that dietary fish oil supplementation also has a beneficial effect on the function of the respiratory endothelium by suppressing exercise-induced bronchoconstriction in elite athletes (Mickleborough, 2013).

Still, a question arises whether diets rich in n-3 fatty acids could induce functional and structural remodelling of the vascular system. The effect of n-3 PUFA supplementation was analysed using ultrasound indices of vascular function, i.e. FMD, NMD and PWV. The relationships between serum markers of the endothelial function and the above-mentioned ultrasound indices were also examined. We demonstrated that NO concentration increase in response to a diet rich in n-3 PUFA was associated with significant differences in peak $\dot{V}O_{2max}$ level and higher muscle oxygen uptake positively correlated with FMD improvement. The effect of marine omega-3 fatty acids consumption on FMD was investigated in both healthy individuals and patients with cardiovascular disease (Anderson et al., 2010; Fahs et al., 2010). In the study of Goodfellow, Bellamy, Ramsey, Jones, and Lewis (2000), long-term treatment with n-3 PUFA resulted in a significant improvement in FMD and a significant reduction in triglycerides in subjects with hypercholesterolemia. In a study of Shah et al. (2007), a 2-week intake of fish oils significantly increased endothelium-dependent brachial artery flow-mediated vasodilatation. Shechter et al. (2009) reported that larger artery diameter related to remodelling due to atherosclerosis characterized individuals with lower cardiovascular risk; however, the authors did not presented any association between baseline brachial artery size and prediction of cardiovascular events (Shechter et al., 2009). An association between brachial FMD and LVMI has also been noted (Yeboah et al., 2011).

Our results do not seem to unambiguously confirm those obtained by other researches, who demonstrated a significant improvement in FMD, PWV and lipid profile after omega-3 supplementation (Goodfellow et al., 2000). This might be a result of differences between study populations; our subjects were healthy athletes characterized by high cardiovascular tolerance. Also, dietary supplementation with a dose of 1.3 g/day recommended for patients with increased cardiovascular risk might not have been sufficient to significantly improve PWV and metabolic function (as evidenced by LDL-cholesterol concentrations and beneficial changes in TAS) in elite athletes. However, the influence of n-3 PUFA diet on baseline glucose concentration was observed. The possible mechanisms responsible with a detailed characteristic of glucose metabolism in response to n-3 PUFA diet were not a major issue of the paper. We could only speculate that supplementation with omega-3 fatty acids increased fat oxidation in liver and probably skeletal muscle, resulting in lower glucose disposal and largest energy reserves during exercise.

There are several limitations but also some favourable aspects to our study. Although the sample size was limited, the study group and protocol characteristics allowed an objective analysis and important conclusions. Since endothelial function and brachial artery dilatation values can be influenced by different factors, we tried to adopt appropriate exclusion criteria. Also, to enhance reliability, all measurements were taken by the same examiner. As there are different methods of NO serum level/bioavailability analysis, some inconsistencies in results obtained by different researchers might partially be explained by different methodologies used. The technique used in our study which was different to many studies in the literature and that this may have limitations when comparing between studies.

We do not have software for automated edge detection, thus arterial diameters (5–7 measurements) were taken from the anterior to the posterior "M" line at the end diastole. In order to optimize the time of measurements, all images were acquired with an ECG gating with the end of the diastole corresponding to the onset of the R wave. We did not measure the shear rate area under the curve (as a measure of the stimulus for FMD) and one time point for determining peak. These parameters might bring new values to our further observations.

In summary, our results show that a 3-week omega-3 fatty acids supplementation had a beneficial effect on exercise performance and cardiovascular

adaptation to exercise. The improvement of endothelial function observed in our study population might have been induced by an increase in endogenous production of NO associated with a PUFA-rich diet.

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