A 90 DAY SUPPLEMENTATION OF POLYUNSATURATED FATTY ACIDS (PUFA) HAS BENEFITS ON HEALTH MEASURES AND EXERCISE PERFORMANCE

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ABSTRACT

Background. Fish oil contains essential fatty acids that are linked to better cardiovascular health and even the prevention of sudden cardiac death in athletes. The purpose of this work was to examine the effects of 90 days of fish oil supplementation in elite-, leisure-, and non-athletes on body fat percent, body mass index (BMI), blood cholesterol and triglycerides, heart rate and blood pressure, and on exercise performance.

Method. Three groups of participants were tested with 12 equally distributed men and women in each: elite-athletes, leisure-athletes, and non-athletes. Participants received body weight-adjusted commercially available fish oil over 90 days. These nutritional supplements were taken in the morning, immediately following breakfast.

Results. The findings revealed that compared to the baseline, body fat percent decreased in all the three groups (p = .034), however, blood cholesterol and the cholesterol/high density lipoprotein (HDL) ratio increased (p < .05), with the elite-athletes showing the largest increase. Exercise performance, in terms of the time of running to voluntary exhaustion, increased (p = .05), but the largest benefits were observed in non-athletes (22%) in contrast to leisure-athletes (4%) and elite-athletes (1%), which could be linked to a ceiling effect. No statistically significant changes were observed in any other anthropometric, physiological, or biochemical measures.

Conclusion. These findings suggest that 90 days of fish oil supplementation may benefit body composition and increase exercise performance, especially in non-athletes, and increases cholesterol, as well as cholesterol/HDL ratio levels, primarily in elite-athletes. Based on these results, it appears that fish oil supplementation yields greater benefits in non-athletes than in athletes.

Keywords: anthropometric measures, biochemical measures, body composition, in-situ, real life setting.

INTRODUCTION

Long-chain omega (n)-3 polyunsaturated fatty acids (LC-PUFAs; e.g. eicosapentaeoic acid – EPA, or docosahexaenoic acid – DHA) have well-documented anti-inflammatory and anti-oxidant effects (Deckelbaum & Torrejon, 2012). PUFAs were found to be beneficial in the prevention and treatment of various cardiovascular diseases due to their triglyceride-lowering, anti-thrombotic, anti-inflammatory, and anti-hypertensive properties (Breslow, 2006; Deckelbaum & Torrejon, 2012; Kotwal, Jun, Sullivan, Perkovic, & Neal, 2012; Marik & Varon, 2009). In addition to cardiovascular effects, PUFAs promote early eye and brain development (Gódor-Kacsándi, Felszeghy, Ranky, Luiten, & Nyakas, 2013; Walker, Jebb, & Calder, 2013), regulate the immune function (Gorjão et al., 2006), and can improve inflammatory conditions such as arthritis (Calder, 2013; Serhan, Chiang, & Van Dyke, 2008). Based on these considerations, recent dietary guidelines recommend regular consumption of seafood to increase intake of PUFAs (Papanikolaou, Brooks, Reider, & Fulgoni, 2014).

Beyond health promotion and disease prevention, PUFAs also reduce exercise-related oxidative stress, immune and inflammatory responses to exercise, and soreness (Andrade, Ribeiro, Bozza, Costa Rosa, & do Carmo, 2007; Gray, Chappell, Jenkinson, Thies, & Gray, 2014; Jouris, McDaniel, & Weiss, 2011; Lembke, Capodice, Hebert, & Swenson, 2014). These positive effects can lead to the improvement of exercise performance. For example, in a recent study examining 30 male athletes, a 21-day administration of PUFAs (5 ml sea oil per day, containing, 375 mg EPA, 230 mg DPA, and 10 mg DHA) significantly improved peripheral neuromuscular function and aspects of fatigue compared to olive oil placebo. On the other hand, no beneficial effect on the central neuromuscular function was reported (Lewis, Radonic, Wolever, & Wells, 2015). In another placebo-controlled study, no improvement in the maximal aerobic power, anaerobic threshold and running performance was found in 28 well-trained male soccer players following a 10-week supplementation of PUFAs (1600 mg/day EPA, 1004 mg/day DHA) which led to the conclusion that the maximal aerobic performance of endurance athletes cannot be improved by PUFAs (Raastad, Høstmark, & Strømme, 1997). According to a recent review, available empirical data regarding the positive impact of PUFAs on exercise performance are inconclusive, and, therefore further studies are needed to assess the effectiveness of PUFAs supplementation on delayed onset muscle soreness, and subsequent exercise performance in elite-athletes and military personnel (Shei, Lindley, & Mickleborough, 2014).

The effectiveness of PUFAs may also depend on the intensity of exercise and the training status of the participants. In a recent study, untrained males receiving a green-lipped mussel oil PUFA blend for 26 days showed a significant decrease of muscle damage and strength loss compared to the a placebo control group after a muscle damaging exercise (Mickleborough, Sinex, Platt, Chapman, & Hirt, 2015). In the current research, the effects of 90-day PUFA supplementation were assessed on measures of exercise performance, blood lipid profiles, and cardiovascular indices in three groups of participants with different training status (i.e. elite endurance athletes, leisure runners, and individuals with a sedentary lifestyle). Complementing past research, we report here the effects of PUFAs over a longer period (90 days) of administration, on anthropometric, physiological, biochemical, as well as exercise performance variables, and in function of training status. It was conjectured that this multi-level repeated measures factorial design will yield a clearer picture about the effects of PUFAs on health- and exercise-behaviour.

METHODS

Participants. Elite-athletes were competitive triathletes and elite endurance runners. They were recruited with the assistance of the national athletics and triathlon associations, who sent a call for participation e-mail to their athletes and also presented them with recruitment flyers in the upcoming competitions. Leisure-athletes were recruited before street running races in a large metropolitan area, while the control, non-athlete, participants were recruited from social media targeting groups interested in health and fitness. Thirty-six participants completed the study. Ethical permission for the study was obtained from the Ethics Research Board of the National Healthcare Scientific Committee. Elite-athletes trained between 24–36 hours per week. Their mean age was 22.35 ± 2.61 (men) and 23.63 ± 2.84 (women) years. The leisure-athletes (runners) trained an average of 9–12 hours per week and their mean age was 38.09 ± 8.37 (men) and 40.76 ± 7.46 (women) years. The non-athlete control participants did not report any scheduled exercise (0 hours per week). Their mean age was 31.97 ± 7.48 (men) and 27.55 ± 5.05 (women) years.

Materials. A known commercial brand of fish oil capsule was provided to all the participants. Based on the manufacturer's information, six (6) capsules contained 138.12 kJ (32.88 kcal) of energy, 0.786 g protein, 0.355 mg carbohydrates, 2.94 g fat, 741 mg saturated fatty acids, 703 mg monounsaturated fatty acids, 1293 mg polyunsaturated fatty acids of which 529 mg EPA, 353 mg DHA, and 6 mg vitamin E. Exercise was performed on a Spiro-ergometer (Schiller CS-200, ITAM) treadmill with heart rate and blood pressure reading functions (Schiller BP-200 Plus).

Procedure. Prior to the investigation, participants were informed verbally and in writing about the purpose of the research. They were interviewed about their health status and informed about any possible complications that may arise during the course of the study. If the participant agreed to take part in the study, she/he signed a written informed consent form. By signing the form, the participants declared that they will not make changes in their usual diet and that they will not consume any other unsaturated fatty acids during the course of the study, apart from the one supplied to them by the researchers. All tests (described below) were performed twice: on day zero (baseline) and on day 91.

Upon coming to the laboratory, participants were asked to relax in preparation for blood tests in which 15 ml of whole blood was collected by a qualified medical personnel. The cellular elements of the blood were destroyed after diagnosis as the norms of the relevant legislation require. Blood samples were stored in a refrigerator at 4ºC until they were transported to the laboratory in a cool box where the blood samples were analyzed for lipid profiles.

Anthropometric measurements were performed according to the International Biological Program recommendations (Weiner & Lourie, 1981). In this analysis, the two-component body composition estimation is based on Pariscova's recommendation and requires body weight and 5-skinfold (plica of biceps, triceps, scapula, hip, medial crural) measurements (Parízková & Bůzková, 1971), which were recorded on the basis of the International Society for the Advancement of Kinanthropometry (ISAK) method (Stewart & Marfell-Jones, 2011). Anthropometric variables included height, weight, abdominal circumference, skinfold measurements to determine body fat, body mass, relative bone-muscle ratio and fat mass determination based on the Drinkwatter and Ross procedure (Drinkwater & Ross, 1980).

Participants took fish oil capsules every morning after their breakfast for 90 days. Based on their body weight assessed on Day 0 (baseline), those under 55 kg received 4, those between 55 and 70 kg received 5 and those above 70 kg received 6 capsules for every day in the study. On days 0 and 91 we have performed anthropometric tests, collected venous blood for lipid analysis, measured resting heart rate and blood pressure, and tested participants on a two part ergometer test using a treadmill. After obtaining all measures at rest, the participants completed the "Vita Maxima" ergospirometric test (Fostikov, 1971), consisting of running at 8 km/hour steady speed starting with a 4.0% elevation (incline) that was increased by 1.5% every minute until exhaustion. After a half an hour of relaxation, the participants run again at the elevation corresponding to their 85% of maximal oxygen uptake (VO_2 max) until voluntary exhaustion.

Data were analysed with the SPSS 20.0 statistical software package. Since none of the variables violated the assumption of normal distribution – as based on preliminary Kolmogorov–Smirnov statistical tests – the data were examined with parametric statistics. Mixed (between (groups) and within (change with time) repeated measures analyses of variances were used, which were followed up with Bonferroni-corrected post hoc tests. For tests involving repeated measures, the Greenhouse-Geisser correction of the degrees of freedom was adopted.

RESULTS

The descriptive statistics of the dependent measures are presented in Table 1. A repeated measures analysis of variance (RMANOVA) of body fat percent yielded a time main effect ($F(1, 33) = 4.918$, $p = .034$), as well as a group main effect ($F(2, 33) = 4.624$, $p = .017$), but no group by time interaction ($F(2, 33) = 1.081$, $p = .351$). Post-hoc tests showed that the overall body fat percent has decreased 4.8% ($t_0 = 20.15 \pm 6.15$, $t_1 = 19.18 \pm 5.39$, $p = .034$). The between-group main effect was due to the fact that elite-athletes already differed on Day 0 from non-athletes ($p = .023$) and also – but only marginally – from the leisure-athletes ($p = .071$), while the latter two did not differ from each other (Table).

The analysis of the body mass index (BMI) only yielded a statistically significant group main effect ($F(2, 33) = 3.452$, $p = .044$), which substantiated that the BMI differed between elite-athletes and non-athletes ($p = .044$) already on Day 0. Blood cholesterol levels showed a statistically significant time main effect ($F(1, 33) = 5.325$, $p = .027$), but no group main effect and no group by time interaction. Post hoc tests revealed that overall blood cholesterol levels have increased 4.1% after 90 days of fish oil consumption ($t_0 = 4.95 \pm 0.82$, $t_1 = 5.16 \pm 0.84$, $p = .027$). The analysis of the ratio of total blood cholesterol to high-density lipoprotein (HDL) yielded a statistically significant time main effect (F(1,33) = 16. 914, p < 0.001), a group main effect (F(1,33) = 3. 44, p = 0.044), but no group by time interaction. Post hoc tests showed that the

Table. **Changes in the dependent measures over 90 days in three groups**

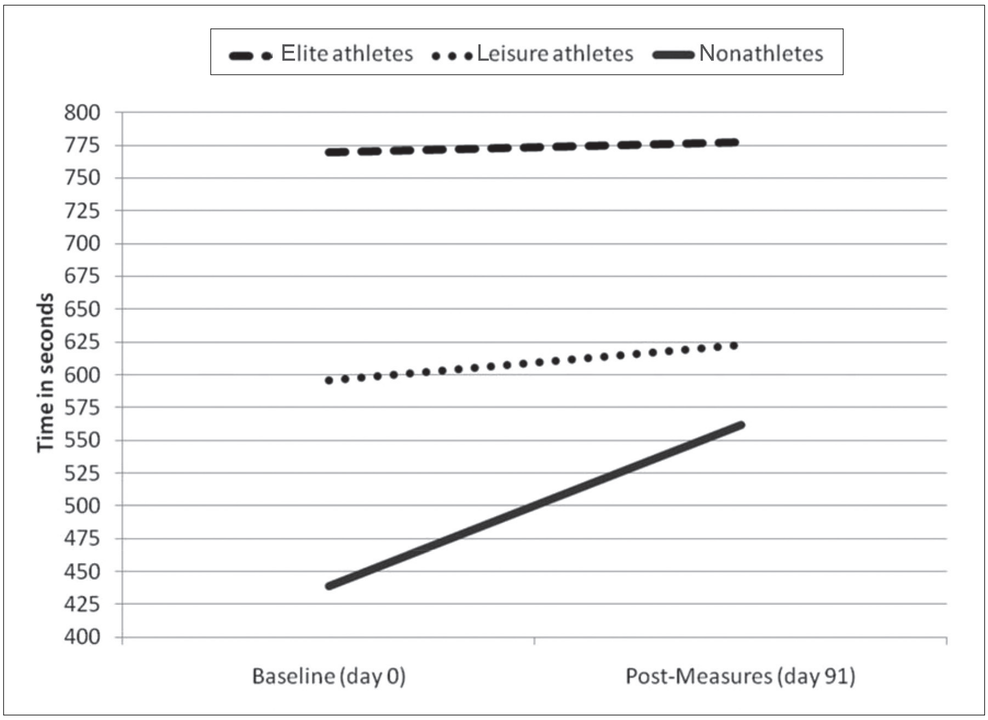
Time	Dependent Measure	Elite-athletes	Leisure- athletes	Non-athletes
Pre	Body fat (t0) (%)	16.06 ± 5.86	21.70 ± 4.68	22.69 ± 6.03
Post	Body fat (t1) (%)	16.00 ± 5.23	20.31 ± 5.47	21.24 ± 4.27
Pre	BMI (t0) (*kg/m*^{2})	20.49 ± 1.51	23.05 ± 3.35	24.33 ± 5.27
Post	BMI (t1) (*kg/m*^{2})	20.60 ± 1.33	23.17 ± 3.25	24.43 ± 5.17
Pre	Blood cholesterol (t0) (*mmol/l*)	4.54 ± 0.74	5.32 ± 0.96	5.00 ± 0.60
Post	Blood cholesterol (t1) (*mmol/l*)	4.92 ± 0.87	5.47 ± 0.87	5.08 ± 0.76
Pre	Total cholesterol/HDL (t0) (*mmol/l*)	2.39 ± 0.41	2.83 ± 0.59	3.12 ± 0.77
Post	Total cholesterol/HDL (t1) (*mmol/l*)	2.86 ± 0.59	3.31 ± 0.73	3.43 ± 0.94
Pre	Triglyceride (t0) (*mmol/l*)	0.99 ± 0.35	0.97 ± 0.37	1.20 ± 0.77
Post	Triglyceride (t1) (*mmol/l*)	0.86 ± 0.42	0.93 ± 0.34	1.26 ± 0.70
Pre	Resting heart rate (t0) (*bpm*)	57.08 ± 13.00	66.00 ± 9.22	71.92 ± 13.65
Post	Resting heart rate (t1) (*bpm*)	57.08 ± 10.20	63.50 ± 12.48	66.58 ± 11.99
Pre	Systolic blood pressure (t0) (*mm Hg*)	127.67 ± 12.82	126.92 ± 16.16	125.33 ± 11.11
Post	Systolic blood pressure (t1) (*mm Hg*)	125.75 ± 10.51	125.92 ± 11.76	120.42 ± 8.03
Pre	Diastolic blood pressure (t0) (*Hgmm*)	65.83 ± 9.25	71.17 ± 9.71	69.08 ± 5.81
Post	Diastolic blood pressure (t1) (*Hgmm*)	63.92 ± 7.57	70.92 ± 6.69	68.33 ± 8.44
Pre	Time to exhaustion (t0) (*s*)	770.00 ± 159.60	595.83 ± 151.75	439.17 ± 94.34
Post	Time to exhaustion (t1) (*s*)	777.50 ± 174.15	622.50 ± 150.10	561.67 ± 179.54
Pre	Exercise performance (t0) (*W*)	370.75 ± 178.26	287.25 ± 66.22	256.67 ± 69.56
Post	Exercise performance (t1) (*W*)	312.83 ± 69.65	285.75 ± 62.25	267.25 ± 70.44

Notes. Day 0 (t_0) = baseline (Pre); time 2 (t_2) = after 90 days (Post); data are presented as means ± standard deviations.

total cholesterol to HDL ratio has increased by 13% over time (t_0 = 2.78 ± 0.67, t_1 = 3.20 ± 0.78) and that elite-athletes differed from the non-athletes only (p = .045), while the latter did not differ from the leisure-athletes. No statistically significant effects have emerged in triglycerides, resting heart rate, diastolic- and systolic blood pressure.

The analysis of the duration of exercise to exhaustion yielded a statistically significant time main effect (F(1, 33) = 8.972, p = .005), a group main effect (F(2, 33) = 10.815, p < .001), and a group by time interaction (F(2, 33) = 4.163, p = .024). The time main effect reflected an increased endurance performance across the 90 days of dietary fish oil supplementation (t_0 = 601.67 ± 23.054, t_1 = 653.89 ± 28.069, p = .005), and the performance of the elite-athletes was significantly better than that of the participants in the other two groups, which did not differ from each other. The elite-athletes' performance did not change after the treatment, probably due to a ceiling effect since their performance was much better already initially than that of the two other groups. On the contrary, the other two groups had increased their performance, the leisure-athlete group with a statistically significant increase of 21.8% (see Figure and Table) from baseline to the second assessment (t(11) = –2.73, p = .02).

Figure. **Diagram the group by time interaction obtained for the performance time to exhaustion in three groups**



DISCUSSION

The current findings show that PUFA supplementation may reduce body fat percent. In spite of the lack of emergence of a group by time interaction, from Table 1 it emerges that leisure-athletes and non-athletes profited the most since the overall 4.8% decrease over time was due to over 6% decrease in these two groups, while elite-athletes showed virtually no change in this measure. These findings agree with past research showing that PUFA supplementation reduces body fat (Couet, Delarue, Ritz, Antoine, & Lamisse, 1997; Hill, Buckley, Murphy, & Howe, 2007).

In spite of reports from the literature claiming that PUFA reduces triglyceride levels in the blood (Gidding et al., 2014; Oelrich, Dewell, & Gardner, 2013; Shearer, Savinova, & Harris, 2012), in the current work triglyceride profiles were unaffected by PUFA supplementation. The most likely explanation may be linked to the small dose (under 1g/day EPA and DHA) of PUFA administered in the current work. Earlier, it was suggested that a moderate dose of 4g/day is needed to lower triglyceride levels in the blood (Miller et al., 2011). According to the recommendations of the Federal Drug Administration, the intake of consumers should not exceed 3 g/day of EPA plus DHA with no more than 2 g/day from dietary supplementation (Bradberry & Hilleman, 2013). Therefore, the dose of PUFA used in the current inquiry was probably too small to yield changes in plasma triglycerides.

Fish oil supplementation resulted in a slightly increased blood cholesterol level (4.1%), as well as increased total cholesterol/HDL ratio (13%), independent of the participants' training status.

We have no clear explanation for this novel observation. There are three possible mechanisms, however: 1) Total blood fat content has increased as a result of the PUFA consumption, which triggered an increased cholesterol release from the cell membranes; 2) The liver could not process the increased fat intake (which is unlikely given the low dose used in the current study); 3) Some components of the PUFA containing fish oil capsules may be metabolised in the same way as the cholesterol, which may slow down cholesterol metabolism.

A 90 day of fish oil supplementation did not have a statistically significant effect on resting heart rate in the current work. To some extent these findings appear to be in contrast to a large scale population-based study that revealed a decrease in heart rate (Dallongeville et al., 2003). Indeed, it was shown that regular fish consumption may be associated with a heart rate reduction of approximately 3.2 beats per minute (bpm) (Mozaffarian, Prineas, Stein, & Siscovick, 2006). These authors also found that an estimate of 1 g/day PUFA intake may be associated with a heart rate reduction of 2.3

bpm. The current findings are still consistent with Mozaffarian et al.'s report in that resting heart rate decreased an average of 2.5 bpm in leisure-athletes (runners) and by more than 5 bpm in non-athletes, in spite of the fact that a statistically significant group by time interaction could not be disclosed* which was probably due to a high variance, as well as relatively low sample size, in the studied sample. The failure to see lower resting heart rate in elite-athletes (exhibiting nearly the same heart rate at pre- and post-assessment), may be ascribed to a floor effect.

The effect of PUFA supplementation in lowering blood pressure may be the most prominent in hypertensive patients; the reduction is usually relatively small and also dose dependent (Morris, Sacks, & Rosner, 1993). Several studies did not yield an effect on healthy subjects (Morris, Sacks, & Rosner, 1993), but at higher doses (i.e. > 3g/day) PUFAs were effective in reducing both systolic blood pressure (SBP) and diastolic blood pressure (DBP) (Appel, Miller, Seidler, & Whelton, 1993; Cabo, Alonso, & Mata, 2012). It was recommended that the hypotensive effects of lower doses of fish oil supplementation (< 0.5 g/day) should be further evaluated (Geleijnse, Giltay, Grobbee, Donders, & Kok, 2002). Using a lower dose in the current study, no hypotensive effects of the PUFAs were observed. These findings are in accord with the conclusion of a recent meticulous meta-analysis of clinical trials showing that administration of ≥ 2 g/day PUFA may reduce both SBP and DBP, with the largest benefits seen among hypertensive patients who are not on medication. Further, a lower dose (between 1 and 2 g/day – that is higher than the dose used in the current work - may reduce SBP, but not DBP (P. Miller, Van Elswyk, & Alexander, 2014).

In partial accord with a recent report (Lewis et al., 2015), fish oil supplementation resulted in increased exercise performance, however, the noticed increase was statistically significant only in the non-athletes, while no changes were seen in athletes. These findings are consistent with a recent review (Macaluso et al., 2013) showing that the majority of studies could not reveal a benefit of fish oil supplementation on exercise performance in athletes. Volpe also came to the same conclusion and recommended more systematic studies (Volpe, 2012). In the current work, two levels of athletic involvement were studied by testing a group of elite triathlon and endurance runner competitors and a group of regular, but leisure-athletes. None of them showed improvement in running to voluntary exhaustion after 90 days of fish oil consumption, thus adding further data to the bulk of the studies that failed to disclose a positive link between PUFAs and exercise performance. The increase in exercise performance in non-athletes could be the manifestation of a metabolic effect induced in some way, perhaps by active energy intake represented by long term fish oil consumption, or a placebo effect associated with the PUFA supplementation.

CONCLUSIONS

Regardless of athletic or exercise status, a relatively modest dose of fish oil supplementation lowers body fat and increases cholesterol, as well as cholesterol/HDL ratio; It also tends to reduce resting heart rate in leisure-athletes and non-athletes, but not in the elite-athletes. A low dose of PUFA supplement could be used to increase exercise performance, decrease resting heart rate, and alter body composition especially in non-athletes and leisure-athletes.

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Conflict of interest. The authors have no conflict of interest, of any kind, to declare.

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* A group by time RMANOVA excluding the elite athletes, yielded a statistically significant time main effect, showing a decrease in heart rate in the leisure athletes and nonathletes only ($F(1, 22) = 4.7, p = .041$).

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