Effects of Supplementation with Neptune Krill Oil (Euphausia Superba) on Selected Redox Parameters and Pro-Inflammatory Markers in Athletes during Exhaustive Exercise

by
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This study investigated the effects of supplementation with Krill oil on levels of pro-oxidant/antioxidant balance markers and levels of pro-inflammatory cytokines in professional rowers submitted to exhaustive exercise.

This double-blind study included 17 members of the Polish National Rowing Team. Subjects were randomly assigned a supplemented group (n=9) which received two capsules (500 mg) of Krill oil daily for 6 weeks, or a placebo group (n=8). At the beginning and at the end of preparatory camp, subjects performed a 2000 m maximum effort test on a rowing ergometer. Blood samples were taken from the antecubital vein prior to each exercise test, 1 minute after completing the test, and after 24-hours of rest. The following redox parameters were assessed in erythrocytes: superoxide dismutase (SOD), glutathione peroxidase (GPx), and thiobarbituric acid reactive substances (TBARS) concentration. Additionally, creatine kinase (CK) activity was measured in plasma samples, while tumor necrosis factor (TNF-α) concentrations were measured in the serum.

Exercise significantly increased values of SOD, TNF-α and TBARS in both groups, but recovery levels of TBARS were significantly lower in athletes receiving Krill oil compared with the control group.

Based on these results we conclude that supplementation with Krill oil (1 g per day) in trained rowers diminished post exercise oxidative damage to erythrocytes during recovery, but had no effect on antioxidant enzymes, TNF-α and serum lipid profiles.

Key words: oxidative stress, TNF-α, rowers, ergometer test

Introduction

Physical exercise, especially intense, is reflected with several adverse effects on the body, including the shift of the pro-oxidant/antioxidant balance towards oxidation, hyperthermia, metabolic acidosis, hypoglycemia or hemoconcentration. These processes are responsible for the decrease in the osmotic resistance of erythrocytes (Chatard et al. 1999; Reeder and Wilson 2001; Robinson et al. 2006). Yusof et al. (2007) suggest that hemolysis associated with prolonged exercise results mostly from injury to older erythrocytes which are less elastic and consequently more susceptible to injury. The same authors observed a significant inverse correlation (r= – 0.911, p < 0.05) between levels of spectrin and the degree of hemolysis. This finding confirmed the hypothesis explaining how free oxygen radical-mediated struc-
tural changes of cellular membranes increases the susceptibility of erythrocytes to lysis. Hemolysis resulting from prolonged physical exercise is reflected by elevated body levels of free iron, a catalyzer of free radical reactions and particularly of Fenton’s reaction (Córdova Martínez et al. 2006; Zunquin et al. 2006).

Studies carried out by numerous authors (Barros et al. 2001; Deutsch, 2007; Kidd, 2007; Park et al. 2010) revealed that Neptune Krill (Euphausia superba) oil contains more than 30% of eicosapentaenoic acid (EPA, C:20:5,n-3) and docosahexaenoic acid (DHA, C: 22:6, n-3) along with 200 ppm to 400 ppm of astaxanthin (Kolakowska, 1994), and may improve the stability of cellular membranes by effectively preventing free radical-mediated injuries.

EPA was also shown to control proper synthesis of prostanoids (eicosanoids) which participate in the regulation of inflammatory processes (Calder, 2007; James et al. 2000; Pischon et al. 2003). The content of EPA in Krill oil is markedly higher compared to fish oil (240 mg/g EPA in Krill oil vs.180 mg/g in standard fish oil, Logan, 2004). Astaxanthin, another component of Krill oil, not only proved to be efficient as an antioxidant but also significantly modulates the activities of inflammatory markers (Chew et al. 1999; Park et al. 2010).

However there is little published data on the effects of Krill oil in preventing exercise-induced peroxidative and inflammatory changes. Consequently, the purpose of this study was to analyze the effects of supplementation with Neptune Krill (Euphausia superba) oil – rich in polyunsaturated omega-3 fatty acids and anti-oxidants (astaxanthin), on exercise-induced oxidative stress and inflammation.

Materials and methods

Subjects

The research material consisted of 17 male members of the Polish National Rowing Team (15 heavy-weight and 2 light-weight rowers). The basic characteristics and sports classes of the athletes are shown in Table 1. The study was performed in March/May during a 6-week training camp between preparatory and competition periods. Data concerning the training profiles of the subjects includes the intensity, volume (in minutes), and type of exercise performed (specific – rowing: endurance, technical, speed, etc.; nonspecific: jogging, strength) and was recorded daily. All training data was analyzed for intensities below and above the lactate (LA) threshold of 4 mmol/L, as shown in Figure 1, and further classified according to workload which was extensive (below the lactate threshold) or intensive (above the lactate threshold).

Over the entire study period, athletes resided in one of the Olympic Training Centers, and ate meals exclusively in the Centre. Their regular menu consisted of a mixed diet containing the recommended dietary allowance of carbohydrates, proteins, fats, and micronutrients (vitamins and minerals) following the Recommended Daily Allowances (RDA) of the Polish Nutrition Society (Ziemiański, 2001). The daily food and caloric intake, as well as the intake of fruits and vegetables, was sustained over the study period. The subjects informed scientific staff that they had not been taking any drugs, medications, or nutritional supplements for two weeks before and during the study.

Experimental procedure

Athletes enrolled in the study were randomly assigned to receive Krill oil (supplemented group, n=9) or placebo (placebo group, n=8). The rowers in the supplemented group were given Krill oil (produced by Enzymotec Ltd, India and supplied by Euro-Pharma Alliance Sp. z o.o., Wrocław, Poland) twice daily for 6 weeks. One gelatin capsule contained 500 mg of Krill oil. The capsule also contained the following inactive ingredients: glycerol (61 mg), and bovine gelatin (132 mg) with the average capsule weight around 693 mg (±5%). At the same time and with the same dosage regime, subjects in the placebo group received dyed gelatin capsules containing inactive ingredients used as standard filling.

All subjects were informed of the nature of the investigation, and gave their written informed consent. The Ethics Committee at the University School of Medical Sciences in Poznań approved the study protocol.

Athletes performed a 2000 m time trial on the first day of the training camp and at the end of it. Using a rowing ergometer (Concept II, USA), each subject had to cover the distance in as short a time as possible. The results of both tests were taken into consideration during the selection of athletes to the first national team, the athletes were well-motivated to perform both tests with maximal effort. Before each test, subjects performed a 5-minute individual warm-up.

Sample treatment
Blood samples were taken from the antecubital vein, using dipotassium ethylene diamine tetra-acetic acid (K2EDTA) as anticoagulant, before each 2000 m test (in the morning, after an overnight fast), 1 minute after completing the test, and following a 24-hour recovery period. Samples were centrifuged immediately to separate red blood cells from plasma. Packed erythrocytes were washed three times with saline and lysed with ice-cold, redistilled water. Plasma, serum, and lysed erythrocytes were frozen immediately and stored at -28°C until use (up to one week). Additionally, finger capillary blood samples were taken by finger prick before and after each exercise test to assess lactate levels (LA).

**Measurements**

Superoxide dismutase (SOD) activity was measured in washed erythrocytes after their lysis by means of a commercially available kit (Randox-Ransod, Cat No. SD 125, UK). SOD catalyzes dismutation of superoxide anion (O2●-) leading to the formation of oxygen and hydrogen peroxide. Determination of SOD activity was based on the production of O2 by the xanthine and xanthine oxidase system. The superoxide dismutase activity was expressed in U/g Hb.

Glutathione peroxidase (GPx) activity in the hemolysate samples was measured using a commercially available kit (Randox-Ransel, Cat No. RS 506, UK). GPx catalyzes the oxidation of reduced glutathione in the presence of cumene hydroperoxide. The rate of glutathione oxidation was measured by monitoring the depletion of NADPH+H+ in the reaction medium. Glutathione peroxidase activity was expressed in U/g Hb.

Concentrations of thiobarbituric acid reactive substances (TBARS) in the hemolysate samples were assessed as a measure of oxidative damage to red blood cells. TBARS concentrations were evaluated with the method described by Buege and Aust (1991), involving the acidic breakdown of lipid peroxides into malondialdehyde molecules. The concentrations of TBARS (malondialdehyde equivalents) were expressed in μmol/g Hb.

Plasma tumor necrosis factor (TNF-α) concentrations were determined by enzyme immunoassay methods using a commercially available kit (R&D Systems, USA).

Creatine kinase (CK) activity was determined from plasma samples with a commercially available kit (Dr Lange, Cat No. LCN 282, Germany). The results were expressed in U/L.

Serum concentrations of total and HDL cholesterol, along with triglyceride levels, were determined by enzymatic procedures using commercially available test kits (Cormay). LDL cholesterol was calculated using the Friedewald formula.

Concentrations of hemoglobin in the hemolysate were assessed using the cyanmethemoglobin method with Drabkin's reagent and maximal absorbance measured at 540 nm. The results were expressed in g/100 mL.

Lactate levels in capillary blood were determined immediately after collection of samples using a commercially available kit (Dr Lange, Cat No. LKM 140, Germany). The lactate concentration was expressed in mmol/L.

**Statistical analysis**

Statistical analyses were performed using the STATISTICA v. 9.0 software package. Redox parameters, TNF-α levels, and CK activity were analyzed for variance (ANOVA) in both groups at three points in time. The distribution of data was analyzed using the Shapiro-Wilk test. In cases where significant changes were observed in ANOVA tests, the Fisher's post-hoc test was applied to locate the source of significant differences. The unpaired Student’s t-test was used to compare the anthropometrical characteristics and hematological indices of the study groups. Results of the 2000 m tests performed before and after the training camp, along with parameters of lipid metabolism, were analyzed with the paired t-test for comparison within groups, and with the unpaired t-test for comparisons between the groups. All values were reported as the mean ± SD. Statistical significance was set at P<0.05.

**Results**

Subjects in the supplemented group were similar to subjects belonging to the placebo group with respect to mean age, height, body mass, and years of training experience (Table 1).

No significant differences in hematological indices were found between the supplemented and placebo groups (Table 2).

Changes in serum lipid profiles and the ratio of LDL to HDL are shown in Table 3. Plasma concentrations of HDL-cholesterol, LDL-cholesterol, triglycerides and the ratio of LDL to HDL did not change over the studied period.
The study groups did not differ in mean power output and total run time for the 2000 m test protocol performed at the beginning of the training camp. The post-exercise blood lactate levels in both groups were found to be higher after the training camp than during initial assessment (Table 4), what suggests greater work capacity, through higher intensity of training.

Training volumes (expressed in minutes per day) during the week preceding the first term of assessment (at the beginning of the training camp) and during the week preceding the second term of assessment (at the end of the camp), specified for extensive rowing, intensive rowing, and extensive non-specific training are shown in Figure 1. Before the first assessment, the training volume amounted to 990 min·per week, of which about 64% was extensive rowing, 27% was non-specific training such as power training, and the rest was intensive rowing. Total training volume before the second assessment was 1019 min-per week of which 59% was extensive rowing, 16% was intensive rowing, and 25% was land training.

Table 5 shows the comparative analysis of endogenous antioxidant potential parameters. ANOVA showed the significant interaction effects of exercise on SOD activity in both groups (P<0.001). In comparison with pre-exercise values, SOD activity directly after the exercise test was significantly higher in both groups (before and after the training camp), and following 24-hours of rest (only before the training camp).

In contrast, GPx activity was not significantly affected by the supplementation and did not change significantly over the exercise period (Table 5).

Table 6 presents changes in measured oxidative stress markers. The analysis of variance indicates that physical exercise significantly influenced the values of these parameters. Ergometry performed during the study yielded similar changes in levels of
Levels of thiobarbituric acid reactive substances (Table 6) were significantly affected by supplementation (main effect P<0.001) and the exercise test (P<0.001). Exercise caused a significant increase in oxidative stress parameters immediately after exercise, measured before (by 67% in the supplemented group, and by 70% in the placebo group) and after the training camp (57% and 39%, respectively). After the training camp, the recovery levels of TBARS were higher in the control group compared with the supplemented group.

ANOVA showed no significant interaction effects between supplementation and exercise on plasma creatine kinase activity (Table 6).

Discussion

Our study on parameters of pro-oxidative/antioxidative balance undoubtedly revealed that maximal physical exercise was reflected by an increase in oxidative processes. This finding was shown by the significant post-exercise increase in thiobarbituric acid reactive substances (TBARS) in all groups analyzed at each time point studied. These observations are consistent with the results of other authors. According to Kypraros et al. (2009), the 2000 m rowing test induces oxidative stress which manifests itself by an increase in lipid peroxidation products and carbonyl groups by 45% and 70%, respectively.
The authors observed disturbances in the pro-oxidative/antioxidative balance despite the high level of physical fitness of the tested subjects.

In our study, physical training in the week preceding each ergometric test was proven to be an additional factor modifying the analyzed variables (Fig. 1). An increase in the rower’s training volume and intensity at the end of the training camp was reflected by a post-exercise increase in TBARS in both the supplemented and control group by 51% and 56%, respectively. During the recovery period, however, further increase in TBARS was observed only in the control group. At the same phase of the training cycle, significantly lower values of lipid peroxidation were found in turn in erythrocytes of subjects being supplemented with Krill oil at 1 g per day. This finding suggests that the supplement reduced the extent of free radical mediated injuries. Fatty acids present in the Krill oil, particularly DHA and EPA, are incorporated into erythrocyte membranes, efficiently protecting them against free radical injury as shown by Maki et al. (2009).

Krill oil is a valuable source of astaxanthin (Kolakowska et al. 1991). Astaxanthin belongs to a group of carotenoids with the highest biological activity, but in contrast to other representatives of this group does not exhibit pro-oxidative properties (Beutner et al. 2001). Astaxanthin inhibits lipid peroxidation at a cellular level, protecting cell membranes and mitochondrial membranes against oxidation (Goto et al. 2001; Liang et al. 2009). Aoi et al. (2003) tested the effects of 3-week astaxanthin supplementation in rats who were subjected to intense exercise. Supplementation resulted in the decrease in 4-hydroxy-2-nonanal-modified protein and 8-hydroxy-2-deoxyguanosine concentrations in the gastrocnemius and heart of rats subjected to exercise. Moreover, the extent of plasma creatinine kinase and myeloperoxidase increase in the gastrocnemius and heart of supplemented rats was lower compared to non-supplemented controls.

Ikeuchi et al. (2006) observed that supplementation with astaxanthin (1.2, 6 or 30 g per kg of body mass) significantly improved the endurance of rats when compared to non-supplemented controls. With the highest astaxanthin doses, significant improvement of endurance was observed following only one week of supplementation. The endurance of animals given astaxanthin at the lowest doses (1.2 mg/kg b.w.) was significantly higher compared to controls no earlier than after 5 weeks of the study.

Although Krill oil is considered a valuable source of astaxanthin, this supplement was found not to have any effect on the mean power output and total run time observed in our study (Table 4). Elevated post-exercise blood concentrations of lactate found in both groups at the end of the training camp resulted from an increased level of training intensity in the period of time directly preceding the determination of that parameter (Fig. 1), along with improved adaptation to anaerobic exercise that takes place during a 2000 m ergometric test.

The observed positive adaptive changes in body acidity of rowers subjected to our study were not reflected with a decrease in the post-exercise synthesis of the pro-inflammatory cytokine TNF-α. More than a two fold increase in this parameter was observed one minute after exercise in all periods analyzed, returning to baseline values following 24 hours of recovery (Table 6). According to Starkie et al. (2001), injuries of muscle fibers rather than circulating monocytes are responsible for the post-exercise increase in TNF-α levels. Plomgaard et al. (2005) observed that expression of TNF-α is characteristic for fast-twitch fibers. However, in our study no significant changes were noted in creatinine kinase activity, a marker of muscle fiber injury. Tendencies towards a decrease in the serum activity of this intracellular enzyme are worth noticing following the period of supplementation (Table 6). According to Starkie et al. (2001), the decrease in TNF-α concentrations observed during a period of restitution post intense exercise is associated with activation of the hypothalamic-pituitary axis, resulting in significant elevation of blood adrenaline and cortisol levels. These latter changes result from the stress reaction to intense physical exercise. The intensity of the exercise test our subjects were exposed to (2000 m run) was proven by an average post-exercise concentration of lactate which exceeded 16 mmol/L (Table 4).

In our study, supplementation with Krill oil did not affect the activities of anti-oxidative enzymes, SOD and GPx (Table 5). In contrast, a decrease in the activities of the enzymes was observed in young women subjected to a study by Sadowska-Krepa and Poprzęcki (2005), who analyzed the effects of supplementation with polyunsaturated fatty acids in fish oil. The aforementioned authors associated this phenomenon with decreased levels of lipid auto-oxidation reflected by lower activity levels of the enzymes studied. Similarly, in other studies Poprzęcki...
et al. (2009) demonstrated a decrease in SOD activity after ω-3 fatty acid supplementation.

Previous studies on the effects of supplementation with Krill oil referred mostly to the lipid profile of blood. Bunea et al. (2004) revealed that 12 weeks of supplementation with Krill oil (1, 1.5, 2 or 3 g per day) positively modulated the composition of the lipid profile. The aforementioned authors observed a significant decrease in total cholesterol and triglyceride concentrations along with a reduction in low-density lipoprotein (LDL) and an elevation in high-density lipoprotein (HDL) levels. These positive changes were noted irrespective of the dose of Krill oil supplemented, but were not found in groups given fish oil (3 g per day) or placebo. It seems that krill oil was more effective at improving blood lipids and lipoproteins than fish oil.

Improvements in the lipid parameters of individuals supplemented with Krill oil were also proven by the results of other studies (Jia-Jin et al. 2008). The lack of significant changes in the lipid parameters of our subjects (supplemented with Krill oil) is plausibly explained by the fact that their baseline values were already within normal reference limits (Table 3). Moreover, we did not observe any significant effects of supplementation on TNF-α concentrations.

In conclusion, 6 weeks of supplementation with 1g of Krill oil per day did not inhibit markedly pro-oxidative changes found in rowers during the restituation phase of the training cycle. It is likely that the weak effects of supplementation resulted from a dose of Krill oil that was too low or from the extremely high training loads the rowers were subjected to during the initial period of direct pre-competition preparation.

The unique molecular composition of krill oil, which is rich in phospholipids, omega-3 fatty acids, and astaxanthin, surpasses the profile of fish oils. Thus, there is a valid reason to recommend krill oil supplements to athletes participating in acute high-intensity exercise. Additional studies are required to determine daily doses and timing of supplementation.

References


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