Effects of Dietary Supplementation with Krill Meal on Serum Pro-Inflammatory Markers after the Iditarod Sled Dog Race

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Running title: “Effects of Krill Meal on Inflammation in Sled Dogs”

Abstract

A seafood-based supplement from krill, rich in omega-3 phospholipids and proteins was tested on a group of dogs competing in the 2016 Iditarod dog sled race to investigate the effects of krill meal on exercise-induced inflammation and muscle damage in comparison to a control group.

A single team of 16 dogs received 8% krill meal for 5 weeks prior to the start of race, while another team of 16 dogs received no supplementation. Ten dogs of the treatment and 11 dogs of the control group finished the race and their blood was analyzed for omega-3 index, inflammation (CRP) and muscle damage (CK).

The omega-3 index of the krill meal-fed dogs was significantly higher at the beginning of the race (mean 6.2% in the supplemented vs 5.2% in the control group, $p < 0.001$). CRP concentrations increased from 7.05 ± 2.27 to 37.04 ± 9.16 µg/mL in the control and from 4.26 ± 0.69 to 16.56 ± 3.03 µg/mL in the treatment group, with a significant difference between the groups ($p < 0.001$). CK activity was increased from 90.75 ± 8.15 IU/L to 715.90 ± 218.9 IU/L in the control group and from 99.55 ± 12.15 to 515.69 ± 98.98 in the supplemented group, but there were no differences between groups ($p = 0.266$). The results showed that krill meal supplementation led to significantly higher omega-3 index, which correlated with lower inflammation and a tendency for reduced muscle damage after this long-distance sled dog competition. However, these results need to be confirmed by more controlled studies, since it was a field study and effects of race speed or other performance-related factors such as fitness and musher skill on the results cannot be excluded.

Keywords

Krill meal, feed ingredient, dog, omega-3 phospholipids, exercise, inflammation

Abbreviations and symbols

APR, acute phase response; ARA, arachidonic acid; CK, creatine kinase; CRP, C-reactive protein; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; n-3 PUFA, omega-3 polyunsaturated fatty acid; NADPH, Nicotinamide adenine dinucleotide phosphate; n-3 PL, omega-3 phospholipids; RBC, red blood cells;

Introduction

Iditarod is a 1600 km long dog sled race in Alaska, with the winning team normally completing the race in under nine days. This race is perhaps the most extreme endurance exercise performed by any domestic animal. Markers of oxidative stress, inflammation and skeletal muscle damage, and an exercise-induced acute-phase response (APR) are increased after long-distance sled dog races (1-3).

Sled dogs have a high metabolic energy expenditure during endurance exercise (4, 5), and it was shown that repetitive endurance exercise is associated with lipid peroxidation and reduced plasma antioxidant concentrations in sled dogs (6). Excessive free radial production induced by an extreme exercise challenge, such as the Iditarod race, can induce inflammation and generate muscle damage. The enzyme creatine kinase (CK) is localized in myocytes. Plasma CK activity increases, when the enzymes leak into the bloodstream due to cell membrane disruption or cell death, which has been measured in sled dogs as marker of muscle damage in previous studies (6). Beyond lipid peroxidation and muscle damage, rigorous exercise also induces the APR, a non-specific immune response to disturbances in homeostasis in humans. Serum C-reactive protein (CRP) is widely used as a marker of the APR (7). The reasons for the exercise induced CRP elevation are not yet understood (2). On this basis, researchers are searching for nutritional strategies to counteract the acute phase, inflammatory, oxidative stress and muscle damage response.

Long-chain omega-3 polyunsaturated fatty acids (n-3 PUFA), in particular eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been shown to have a positive influence on the immune system in both humans and dogs (8, 9). It occurs via various mechanisms (10), among others a shift from pro-inflammatory arachidonic acid (ARA)-derived mediators to less potent EPA/DHA-derived alternative inflammatory mediators (11, 12).

A source of omega-3 phospholipids (n-3 PLs) is krill, a small crustacean that is harvested in the Southern Ocean. Whole dried krill can be used as a feed additive in pet diet formulations, mainly in the form of a high-protein krill meal. Typically, 25% of krill meal is krill oil, which is shown to increase post-exercise immune function (PBMC IL-2 production and NK cell cytotoxicity) after a single bout of endurance exercise in humans (11). Krill meal can be used as an alternative to fish meal in feed formulations, which is characterized by around 11% n-3 PLs and 110-140 ppm astaxanthin (13). The antioxidant astaxanthin is known to have anti-inflammatory properties as free radical scavenger, thereby counteracting oxidative stress (14-17).

The aim of this study was to determine whether supplementation with krill meal is associated with decreased exercise-induced muscle damage and markers of the inflammatory response.

Material and methods

Animals and blood sampling

Two Norwegian competing teams took part in this study of the 2016 Iditarod sled dog race from Anchorage to Nome, Alaska over a distance of around 1600 km. The dogs of the two teams were Alaskan Huskies with ages ranging from two to six years, including both males and females. One team of 16 Alaskan Huskies received 8% of a proprietary krill dietary supplement provided by Aker BioMarine Antarctic AS (QRILL™ Pet, Oslo, Norway) for five weeks prior to the start and also during the race. The second team of 16 dogs received no krill meal supplementation. The standard feed for all dogs was based on Eukanuba working and endurance dry food (Sulzbach, Germany), into which the 8% krill meal was added, before it was distributed between the dogs of the krill meal-supplemented group. Both groups were occasionally given beef, chicken and fish snacks in between, but the majority of calories came from the dry food. During the five-week treatment before the race, the dogs were fed and slept in individual doghouses. During the race, they were given straw to sleep on and fed from individual feeding bowls.

Both teams underwent a physical examination and blood sample collection as part of the required screening prior to the start of the race by Iditarod veterinarians. During this examination 6-8 ml of venous blood was collected from the cephalic vein into a vacutainer containing EDTA for plasma collection. Both teams competed with a finish in Nome, Alaska after between 9-10 days. Within one hour of finishing the race, all dogs of both teams underwent a second blood collection of 6-8 ml. Red blood cells (RBC) and plasma were separated by centrifugation at 3000 rpm for 15 min at room temperature and kept on dry ice until stored at -80°C. Plasma was used for analysis of muscle damage and inflammatory markers, and RBC were used for fatty acid analysis.

The study was approved by the Institutional Animal Care and Use Committee at the University of Alaska Anchorage and the Animal Welfare and Ethics Committee of the University of Glasgow.

Red blood cell and plasma fatty acid analysis

Fatty acid methyl esters were generated from RBC (18) and plasma (19) as described previously at OmegaQuant Analytics, LLC (Sioux Falls, South Dakota, USA) using capillary column gas chromatography. Fatty acid composition was expressed as a percent of total identified fatty acids and the omega-3 index as the sum of EPA and DHA in RBC membranes. The omega-3 index is considered a strong biomarker in humans as erythrocytes have a less variable fatty acid composition than plasma (20).

Plasma analysis

Canine CRP was measured was measured using an Olympus AU640 chemistry analyser and an immunoturbimetric assay kit (CRP Bioanalyser, Avacta animal health, UK). The lower limit of detection of this assay was 0.78 ng/ml and the higher limit of detection 16mg/ml. Performance characteristics were assessed periodically with assay range 0.78 – 25 ng/mL, and were less than or equal to intra-assay variation of 2.3% (range 0.7-3.5%) and inter-assay variation of 11.3% (range 8.7-13.9%). CK was measured using a biochemical analyser (Olympus AU640 chemistry analyser) to detect production of NADPH following reaction of CK standards and samples with creatine phosphate to produce creatine and adenosine triphosphate (Creatine Kinase (NAC) reagent; Olympus). The rate of increase of absorbance at 340/660 nm is due to the formation of

NADPH and directly proportional to the activity of CK in the sample. Intra- and inter-assay variations were less than 5 and 10%, respectively.

Data Analysis

Linear regression analysis was employed to explore the influence of krill supplementation on the inflammatory parameters before and after the Iditarod race. Data for CRP and CK were found to follow a positive skewed distribution and were log transformed before fitting a mixed effects regression model to these data and other outcome variables (CRP, CK, EPA, omega-3, DHA and ARA). Main effects were treatment (control, krill meal) and time (pre- and post-race) and random effect was individual dog. Data analysis was performed using Stata software (StataCorp; Texas US), statistical significance was accepted at values of $p < 0.05$ (two-tailed), and data are presented as mean ± SEM, unless indicated otherwise.

Results

32 Alaskan Huskies were included in the study and samples were compared from the 21 dogs that completed the race. The dogs that could not complete the race were flown back to the start, where they were taken care of until they were reunited with the rest of the team. Reasons for dogs to be left behind at designated rest stops, where veterinarians perform health checks, included fatigue, injury, or musher strategy.

Ten out of the sixteen dogs of the krill meal supplemented team finished the race (4 males and 6 females, mean age 4.4 years) and eleven dogs of the control team (5 males and 6 females, mean age 3.7 years), which were used for the analysis. All of these dogs were without signs of injury or disease and the supplementation was well tolerated, as appetite was maintained during the race by all dogs. Both teams arrived after 9 days with the control group arriving 7.5 hours earlier. Dogs in the treated (krill supplemented) group had significantly increased pre-race RBC values for the percentage of EPA (mean 4.0% in the supplemented vs 3.3% in the control group, $p < 0.001$), DHA (mean 2.2% in the supplemented vs 1.8% in the control group, $p < 0.001$) and the omega-3 index (mean 6.2% in the supplemented vs 5.2% in the control group, $p < 0.001$).

In both groups were the post-race RBC samples higher for EPA ($p < 0.001$) and lower for DHA ($p < 0.001$), while the omega-3 index remained the same ($p = 0.424$) (Fig 1).

Serum CRP concentrations were increased in the post-race samples for both treated and control groups, but this effect was lower in the treated animals, as indicated by significant main effects of both race ($p < 0.03$) and treatment ($p < 0.001$) (Fig 2). Activity of CK was significantly higher in the post-race samples in both treated and control groups ($p < 0.001$), but there was no significant effect of treatment on CK activity ($p = 0.266$) (Fig 2).

Discussion

The search for nutritional strategies to counteract the detrimental effects of the APR, oxidative stress and muscle damage response in dogs performing strenuous exercise is ongoing. This is, to our knowledge, the first study showing a significant reduction in the exercise-induced CRP increase, with no significant change in other markers measured, after supplementation with krill in long-distance racing dogs.

EPA, DHA and omega-3 index were significantly elevated in the supplemented group. This is in agreement with previous studies in humans showing that the omega-3 index is directly associated with oral intake of EPA and DHA (21, 22). In accordance with this, supplementation with krill meal for five weeks before the race resulted in higher amounts of n-3 PUFA consumed and therefore a higher omega-3 index at start of race. The omega-3 index remained unchanged after the race, but it was interesting to note that EPA concentration increased, while DHA concentration decreased at the end of study. A possible explanation for the increase in EPA may be that the strenuous activity enhanced the breakdown of DHA to EPA in peroxisomes, which are responsible for β-oxidation of the very long-chain fatty acids.

Baseline CRP plasma concentrations were within the reference range reported for dogs in both groups. After the race, CRP concentrations were increased in both groups, but significantly less in the supplemented group. An exercise-induced increase has been shown previously, with a similar increase in CRP concentration in one study (2), but markedly higher increase in another study (7). Variability in the concentration of post-race CRP might relate to differences in the timing of the post-race blood sample, and future studies should collect multiple timed samples to define the kinetics of CRP release after racing, and the optimum time for sample collection.

The plasma CK activity increased in both groups after the race, which is in accordance with a previous study (6). Previous reports suggest that plasma CK activity in racing sled dogs increases over the first 320 km, then decreases again at 800 km (23). The reason for the decrease after initial peak is still unclear, but might be related to a decreased production or increased clearance of CK (24). In the current study, the post-race measurements were performed one hour after finishing the 1600 km race, when peak CK activity was likely to be already decreasing. Studies in humans have shown that CK increase after exercise was higher in untrained individuals compared to highly trained ones (25). Possible effects of training may play a role in CK activity changes. Further studies of the effects of krill supplementation on CK activity in blood samples collected during racing are required to establish, if supplementation affects CK production (26).

Limitations of the study are small numbers of dogs and lack of randomization of the groups, since two different teams with two different mushers (dog sled drivers) were analyzed. The race setting of this study meant that it was not possible to standardize speed between teams, and the control group finished 7.5h before the treated group. For this reason, we cannot exclude an effect of race speed or other performance-related factors such as fitness and musher skill on the results. Indeed, higher average speed during the race would likely be related to higher level of exercise and could have biased the results towards higher CRP and CK in the control group. In a previous study conducted in a similar race setting, dogs that did not complete the race had higher CRP concentrations at the time of withdrawal from the race than dogs that finished the race (27). The reasons for withdrawal were not noted for each case. A review evaluating CRP as a marker of inflammation after surgical procedures showed that the risk for bias in studies about CRP in dogs is elevated due to its lack of specificity (28). We have encountered similar bias in our study, making it difficult to draw conclusions from a field study.

Further, the small sample size and lack of quantitative information on training and performance meant that we were unable to investigate potential effects of co-variables such as age, sex, ending body weight, body condition, training and racing factors like time on trail and trainer on the associations that we report. Furthermore, it was not possible to control for food intake during the race, as the race setting meant that the dogs could not be fed individually. Further blood sampling at different time points during the race may have been useful to determine peak concentrations of the measured parameters.

Conclusions

Five-week krill meal supplementation of sled dogs before a long-distance race led to higher amounts of n-3 PUFA consumed and therefore a higher omega-3 index at start of race, which was associated with a significant reduction in the exercise-associated rise in serum CRP concentration, and a tendency to minimize the increase in CK activity. The results of this study are encouraging for krill meal supplementation for dogs performing long-distance races, but further dose-ranging studies are needed, as are investigations into the mechanisms of the protective effects of krill n-3 PUFA. Biomarker measurements at multiple time points and larger sample sizes are recommended for further studies.

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Conflict of interest

LB is an employee of Aker Biomarine Antarctic AS, Norway that has provided the krill meal and has sponsored the study. The authors declare that they have no other competing interests.

Figure legends

Fig 1. Effect of krill meal supplementation on n-3 PUFA in RBC membranes before and after the Iditarod race. The percent of EPA was significantly increased in the treated animals, and was also increased in the post-race samples (A). DHA percent was decreased in the post-race samples for both treated and control groups, but concentrations were higher in the pre- and post-race samples in the treated animals (B). The overall omega-3 index was significantly increased in the supplemented animals, without a difference between the pre- and post-race samples (C).

Fig 2. Post-race blood samples showed significantly increased CRP (A) and CK (B) concentrations in both treated and control dogs compared to pre-race samples and supplementation with krill meal attenuated these increases in the treated animals. There was a significant main effect of treatment for CRP ($p = 0.001$), but not for CK ($p = 0.266$), when adjusted for age and sex.

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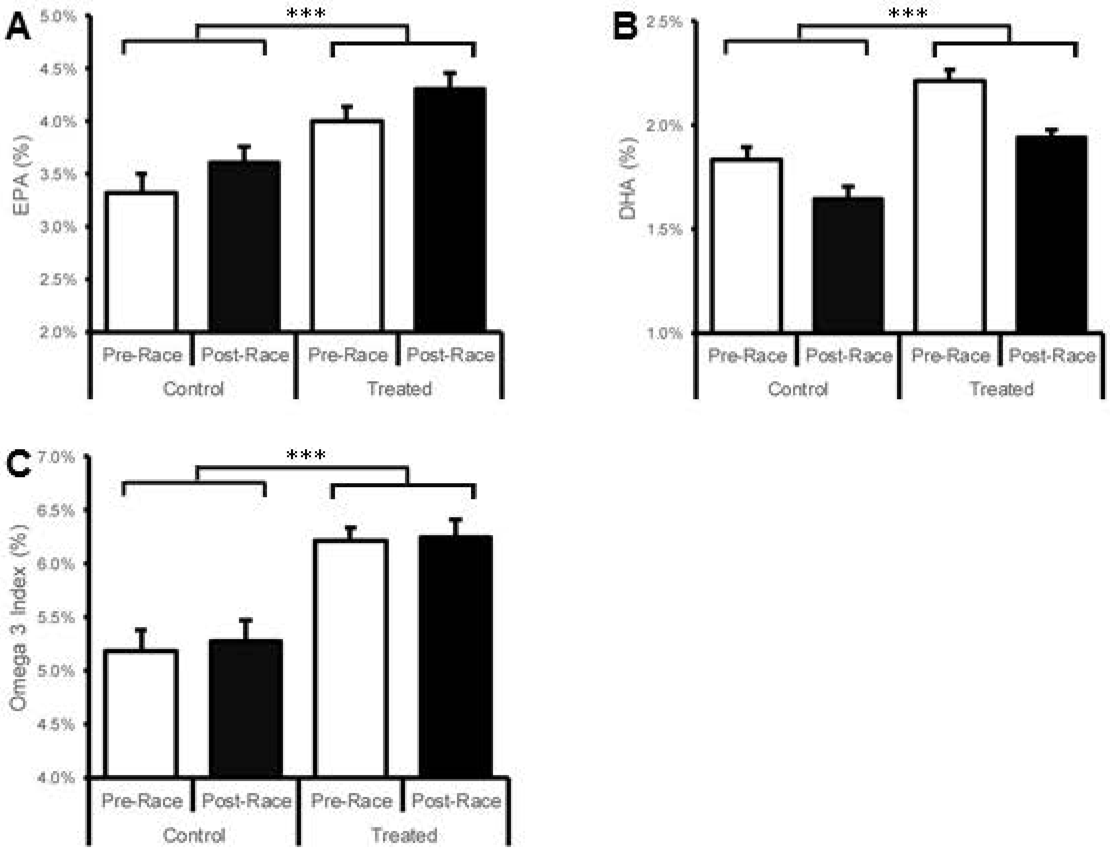
Fig. 1.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Pre-Race
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Omega 3 Index (%)
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Control
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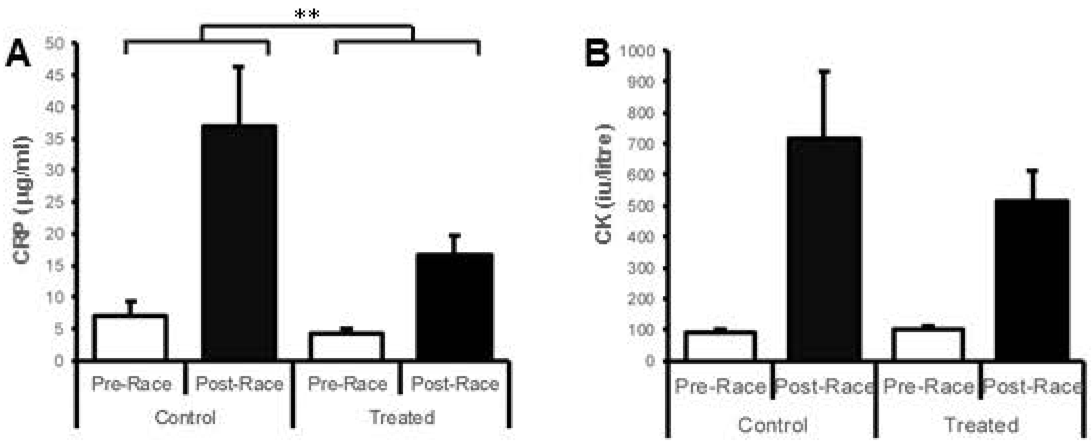
Fig. 2.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CRP (µg/ml)
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Post-Race
Pre-Race
Post-Race
Control
Treated
B
CK (iu/litre)
1000
900
800
700
600
500
400
300
200
100
0
Pre-Race
Post-Race
Pre-Race
Post-Race
Control
Treated