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OMEGA-3 SUPPLEMENTATION ATTENUATES THE PRODUCTION OF C-REACTIVE PROTEIN IN MILITARY PERSONNEL DURING 5 DAYS OF INTENSE PHYSICAL STRESS AND NUTRITIONAL RESTRICTION

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ABSTRACT: The effects of omega-3 (n-3) polyunsaturated fatty acid (PUFA) supplementation on the serum concentration of C-reactive protein (CRP) and activity of creatine kinase (CK) were investigated in military personnel. The concentrations of CRP and CK were used as inflammatory and muscle damage markers, respectively. Twenty subjects were divided into two groups and were given capsules containing either n-3 PUFA (SUP) (n=10) or placebo (PLA) (n=10) for four weeks. During the fourth week of supplementation, the subjects participated in a military boot camp that restricted both their calorie intake and rest, and increased their physical stress. Blood samples were taken in four instances: 1) pre-supplementation; 2) pre-camp; 3) during camp; 4) after camp. During the three weeks of supplementation and prior to boot camp, a significant reduction was observed in the serum concentration of CRP (50%) only in group SUP (p=0.04). Significant increases in CK activity of 103.9% in SUP (p=0.0001) and 225.5% in PLA (p=0.004) after camp confirmed the strenuous nature of this procedure. Serum CRP increased during camp in both groups but the SUP group presented a significantly lower concentration of CRP at the end of boot camp in comparison to the PLA group (6.18 ± 2.6 $U \cdot L^{-1}$ and 8.6 ± 2.1 $U \cdot L^{-1}$ for SUP and PLA respectively, when p=0.04). These results suggest that supplementation with n-3 PUFA can exhibit a protective effect against the inflammatory process induced by a regimen of intense physical stress and food restriction.

KEY WORDS: eicosapentaenoic acid, docosahexaenoic acid, omega-3 fatty acids, C reactive protein, exercise

INTRODUCTION

Dietary fats, especially LDL cholesterol and trans fatty acids, are nutrients that contribute to the greatest risk for developing cardiovascular and metabolic diseases such as insulin resistance, diabetes, endothelial dysfunction, systemic inflammation, cancer, and obesity. In recent decades, an increase in the consumption of these types of fats was observed in the populations of both developed and developing countries alike [29].

However, in the 1970s, some types of fats began to be considered healthy due to the epidemiological observation that Greenland Eskimos had very low death rates from heart problems, even though their diets were rich in fats and the majority of them were highly overweight/obese. It was found that the lipids consumed by this population came from whale blubber, seals, and deep-water fish, which are the main sources of omega-3 (n-3) polyunsaturated fatty acids (PUFA) [11].

The molecular properties of n-3 PUFA, as well as the mechanisms by which they promote cardiovascular protection, still need to be better elucidated. However, we already know that these fatty acids have the ability to decrease production of inflammatory cytokines, among them the tumour necrosis factor (TNF-α), interleukin-1 (IL-1), and interleukin-6 (IL-6), and are substrates for the production of series 3 eicosanoids (prostaglandins, thromboxanes, and leukotrienes) whose inflammatory responses are less potent than those eicosanoids originating from arachidonic acid [5,6].

In recent decades, research has shown that the same inflammatory markers that are found in elevated levels in cardiovascular diseases are also present in athletes who are in a state of overtraining (with a mismatch between an elevated training load and insufficient periods of rest/nutrition) [26]. Intense physical exercise produces muscular micro lesions that trigger a natural inflammatory response at the site of injury. If the recovery period is insufficient, the previously local and acute inflammation can evolve to a chronic and systemic stage, mediated by the action of prostaglandins and pro-

inflammatory cytokines (IL-1, IL-6, TNF-α) [1]. IL-6 controls the release of cortisol, catecholamine, and gonadal hormones, and stimulates the liver to produce C-reactive protein; this last marker is an excellent indicator of overtraining [26]. So the overtraining process that athletes can be subjected to clearly involves an inflammatory activity, such that n-3 PUFA supplementation could attenuate the deleterious effects of overtraining in athletes. In fact, Andrade et al. [2] demonstrated that supplementation of n-3 PUFA resulted in lower elevations of inflammatory markers in elite swimmers at the national level during a training season.

However, that study was developed with athletes who had theoretically adequate training, rest, and nutrition programmes for the entire season. Thus, there has been no consistent evidence yet regarding the relationship of n-3 PUFA in the prevention of overtraining and the health of persons who submit themselves to exercises of great physical stress, nutritional restriction, and inadequate rest. Military personnel in training are exposed to daily loads of physical exercises, with both aerobic and anaerobic characteristics. At determined periods in the graduation year of military training, boot camps are held, whose objective is to prepare military personnel for adverse situations. During these boot camps, which last approximately one week, caloric intake and rest periods are significantly restricted while physical and psychological stresses are greatly increased, leading these military personnel to a state similar to that of athletes in the process of overtraining. Therefore, we hypothesized that n-3 PUFA supplementation can attenuate the inflammatory process resulting from the exposure of military personnel to a boot camp regime. Thus, the objectives of this study were as follows: to confirm the occurrence of an inflammatory process induced by exercise performed by military personnel submitted to a boot camp characterized by intense physical stress, few hours of rest, and nutritional restriction for several consecutive days; and to assess the effects of n-3 PUFA supplementation on inflammatory and muscle damage markers.

MATERIALS AND METHODS

Subjects of the study. The research was conducted with subjects from the 16th Regiment Field Artillery, located in the city of Bayeux – PB. The study was performed with a squad composed of 20 subjects between the ages of 18 and 20 years old, students of the Preparation Nucleus for Reserve Officers (NPOR). All of the military personnel took part in the same physical exercise programme five days a week, as part of their military training, and had a normocaloric, normoglycidic diet. None of the military personnel had apparent chronic-degenerative diseases or used any medications. The 20 subjects were randomly divided into supplementation (SUP) and placebo (PLA) groups. The project was submitted to the Committee for Ethics in Research of the Paraíba Federal University Health Sciences Center, being approved under protocol no. 0051. After approval, a didactic presentation was made to the military organization, with all military personnel present, to explain the objectives of the study, clarify procedures, and to obtain consent by way of signing the Terms of Free and Clarified Consent (TCLE), in accordance with 196/96 of the National Health Council. Although they were part of a military organization, it was made emphatically clear that their participation in the study was completely voluntary and able to be withdrawn as well. During the study, three subjects expressed a desire to no longer participate in the research, and were promptly attended to. As a result, the study concluded with 17 subjects, 9 of whom were in the SUP group and 8 in the PLA group.

Design of the study

The participants' dietary intake of n-3 PUFA and their body composition were previously determined. They then received either n-3 PUFA or placebo supplementation for four weeks. At the start of the fourth week of supplementation, the military personnel underwent a five-day military survival boot camp, characterized by constant physical exertion throughout the day and night, and nutritional and sleep restrictions. Blood samples were taken before the start of supplementation and immediately before, during, and after camp. During the entire study period, the subjects' physical activities were supervised, following the annual macrocycle defined in that unit's military physical training schedule.

Assessment of food intake

Food intake was assessed by the 24-hour recall method, which consists of defining and quantifying all foods and beverages consumed in the period preceding the interview, which may be the preceding 24 hours, or more commonly, the previous day [14]. We opted for this method because it is considered the most frequently used instrument in assessing food and nutrient intake [14]. The questionnaire was applied three times for each subject; two days were representative of food consumption related to weekdays and one day was representative of consumption for a weekend day. To characterize the subjects' habitual diet and consumption of fatty acids, we took an average of the values from the three questionnaires. To analyse the recollections, we used NutWin software (version 1.5, 2002).

Assessment of body composition

The selected subjects underwent anthropometric measurements of weight, height, and body fat percentage before the supplementation period. For body fat percentage, we used the three folds protocol by Jackson and Pollock [16].

Supplementation protocol

The entire study was developed according to the double-blind model. The supplementation consisted of three gelatin-coated daily capsules containing 1000 mg of n-3 PUFA, 180 mg of eicosapentaenoic acid (EPA), 120 mg of docosahexaenoic acid (DHA) and 700 mg of vehicle gel without caloric value (Vitamin Life, Matão, Brazil) totalling 540 $mg \cdot day^{-1}$ of EPA and 360 $mg \cdot day^{-1}$ of DHA. The equivalent ingested in placebo form consisted of capsules similar to the n-3 PUFA

ones, containing 80 mg of maltodextrin, produced in a preparation pharmacy (Farmafórmula, João Pessoa, Brazil). During the first three weeks, the researcher responsible for the study administered the capsules directly to each subject in the study and instructed the subject to ingest it in his presence. In the fourth week, during the boot camp, the capsules were administered by a lieutenant responsible for the group, who was participating in the study as a collaborator.

Military regimen

When the supplementation protocol started, the subjects were in the 23rd week of their military regimen. Up to that moment, the physical training of the subjects had been prescribed by a non-military physical education professional. It was based on the training planning theory of Matvéiev [20], following the scientific precepts of sports training. We found in the programme rippling aspects of training loads and the application of cycle concepts (mesocycles and microcycles). The microcycles were designed to contain shock phases (weeks of intense training), recuperation phases (weeks of light, regenerative training), and ordinary training phases (weeks of training according to the actual capacity of the subject). Hence, the military personnel were exposed to a rational and balanced training programme which included practising sports, regular running, and weight training.

The boot camp regimen consisted of five days of controlled caloric intake below nutritional requirements, both in terms of calories and number of meals, combined with few hours of sleep and intense physical stress. On the first day of boot camp, each subject received an Operational Combat Ration kit. The purpose of this kit is to feed one person for 24 hours during combat situations. The total caloric value of the kit varies between 3,000 and 3,600 kcal, being nutritionally balanced with an average value of 12 to 15% protein, 20 to 35% lipids, and 50 to 70% carbohydrates, of the total caloric value. The foods in the kit are thermo processed, ready for consumption, and packed in flexible, sterilized packaging, when it comes to main meals (lunch and dinner), and in lesser quantities for lyophilized and dehydrated foods employed during breakfast. Each complete unit consists of four meals: breakfast, lunch, dinner, and supper. However, the subjects only consumed the portions established in their activity plan. The caloric intake obtained during the boot camp regimen is presented in the table below (Table 1).

On the fourth day of the camp there began military survival training, in which the subject was not allowed to use the Operational Combat Ration kit as a meal. Instead, the subject had lunch of chicken and goat meat, both raw, if found in the environment of the camp. At the end of camp, the military held a luncheon in the same regiment in barracks without caloric restriction.

Sleep restriction was very strict. Only on the third day of camp were subjects granted a scheduled rest of exactly two hours (7:00 am - 09:00 am).

TABLE 1. MEALS EATEN BY SUBJECTS DURING BOOT CAMP WEEK

Meal	Instance	Caloric Value
Breakfast	Day 1	~ 1000 kcal
Lunch	Days 1 and 3	~ 700 kcal
Dinner	Days 2 and 3	~ 700 kcal
Raw animal	Days 4 and 5	Indeterminate

Note: Meals for days 4 and 5 depended on the animals subjects found in camp. The animals were eaten raw.

Blood samples

The subjects had their blood samples taken on four occasions: before the start of supplementation, preceded by three days of no physical exercise; the night before the start of boot camp; the afternoon of the third day of boot camp; and immediately two hours after boot camp. The entire blood collection process was performed by an experienced nursing professional. The blood samples were all taken in single use BD Vacutainer® vacuum tubes. 5 ml of venous blood was taken from the antecubital vein and the blood was immediately transported to the Lauro Wanderley University Hospital clinical analysis laboratory. The plasma was obtained by centrifugation at 3000 rpm for 10 minutes at 4ºC and refrigerated at 4°C until the analyses took place, usually on the same day or the day after the collection.

Determination of creatine kinase

For the analysis of CK, a commercial kit was used (Labtest, Minas Gerais, Brazil). A volume of 20 ul of serum was added to 1 ml of working reagent according to the kit's instructions, and the results were read on a spectrophotometer, model SP 22, at a wavelength of 340 nm.

Determination of ultra-sensitive C-reactive protein

CRP-us was analysed using the ultra-sensitive method, through the use of a Biosystems (Barcelona, Spain) commercial kit with an A25 (Barcelona, Spain) automatic photometer. After preparing the working reagent and samples, the working reagent and the photometer's door buckets were heated at 37ºC. A volume of 1.5 ml of working reagent was mixed with 20 ul of the sample, blank or default. A chronometer was used and readings were taken at 540 nm at 10 seconds and at five minutes. Calculation of the serum CRP concentration was made against a calibration curve.

Determination of total cholesterol

Analysis was performed following the recommendations of the Labtest (Lagoa Santa, Minas Gerais, Brazil) brand commercial kit. A volume of 10 μl of sample was added to 1 ml of working reagent in Eppendorf tubes. Next, the tubes were placed in a bain-marie at 37°C for 10 minutes. Finally, readings were taken on a spectrophotometer at a wavelength of 500 nm, which was previously zeroed against a blank containing only the working reagent.

Statistical analysis

Data are presented as mean and standard deviation from the mean. The Smirnov-Kolmogorov and Barlet tests were used to test the nor-

mality of the data from each group and the differences between the standard deviations of the data sets that were being compared. Based on these results, Student's t-test for independent samples, t-test for paired samples, and ANOVA test were adopted. When the data did not allow for the use of parametric tests, they were substituted by the Mann-Whitney, Wilcoxon, and Kruskal-Wallis tests respectively. A confidence level of 5% was adopted for all tests. The statistical procedures were performed in GraphPad InStat version 3.03 software (GraphPad, San Diego, CA, USA).

RESULTS

Characteristics of subjects. Groups SUP and PLA proved to be statistically similar in terms of height, weight, and body fat percentage. Likewise, the pre-supplementation levels of total cholesterol, CK, and CRP-us were similar between the two groups (Table 2).

TABLE 2. CHARACTERISTICS OF RESEARCH SUBJECTS

	SUP	PLA
Age	18.6 ± 0.5	18.6 ± 0.5
Weight (kg)	69.7 ± 6.5	70.3 ± 7.3
Height (cm)	174.3 ± 4.8	175.6 ± 6.8
BMI	23.01 ± 1.8	22.45 ± 1.8
Body fat percentage	7.2 ± 5.2	7.2 ± 4.1

Note: Mean and standard deviation data. No differences were found between the two groups for any variable.

TABLE 3. PATTERN OF DIETARY INTAKE OF FATS AND DERIVATIVES OF POLYUNSATURATED FATTY ACIDS

	SUP	PLA
Polyunsaturated fatty acid (g)	20.7 ± 12.7	13.3 ± 10.0
α-linolenic acid (g)	18.7 ± 11.5	11.8 ± 9.1
Linoleic acid (g)	1.7 ± 0.9	1.3 ± 09
Docosahexaenoic acid (g)	0.064 ± 0.053	0.033 ± 0.05
Arachidonic acid (g)	0.23 ± 0.16	0.15 ± 0.14

Note: Mean and standard deviation data. No differences were found between the two groups for any variable.

TABLE 4. CHOLESTEROL LEVELS OF SUBJECTS BEFORE THE SUPPLEMENTATION PROCEDURE (PRE-SUPPLEMENTATION), ON THE NIGHT PRIOR TO BOOT CAMP REGIMEN (PRE-CAMP), ON THE FOURTH DAY OF BOOT CAMP (CAMP), AND TWO HOURS AFTER THE COMPLETION OF BOOT CAMP (POST-CAMP)

	SUP	PLA
Pre-supplementation ($mg \cdot dl^{-1}$)	160 ± 28	134 ± 43
Pre-camp ($mg \cdot dl^{-1}$)	165 ± 3	137 ± 27
Camp ($mg \cdot dl^{-1}$)	165 ± 25	134 ± 30
Post-camp ($mg \cdot dl^{-1}$)	144 ± 26 †	111 ± 22 †

Note: Mean and standard deviation data. † indicates the statistical difference between post-camp values in comparison with the other occasions.

Nutrition survey

Saturated and unsaturated fatty acids consumed in the diets of the subjects are presented in Table 3. Additionally, we evaluated the intake of nutrients that were variables in this study. The intake of polyunsaturated fatty acids and linoleic acids, α-linolenic acid, docosahexaenoic acid, and arachidonic acid, was statistically similar between the two groups involved in the study.

Effect of n-3 PUFA supplementation on total cholesterol

The three weeks of omega-3 supplementation that preceded the boot camp regimen were unable to significantly alter the serum levels of total cholesterol. However, the analysis performed at the end of camp revealed that in merely five days, the regimen adopted in this period promoted a significant reduction in total cholesterol levels as compared to the values found on the night before the start of camp. This phenomenon was observed both in the SUP group (-10.1%) and in the PLA group (-17.1%) (Table 4).

Changes in serum CK activity

Table 5 shows that CK values were significantly reduced during the three weeks prior to the start of camp, as compared to base values, both in the SUP group (-50.32%) and in the PLA group (-45.6%).

TABLE 5. CREATINE KINASE LEVELS OF THE SUBJECTS BEFORE THE SUPPLE-MENTATION PROCEDURE (PRE-SUPPLEMENTATION), ON THE NIGHT PRIOR TO BOOT CAMP REGIMEN (PRE-CAMP), ON THE THIRD DAY OF BOOT CAMP (CAMP), AND TWO HOURS AFTER THE COMPLETION OF BOOT CAMP (POST-CAMP)

	SUP	PLA
Pre-supplementation ($U \cdot l^{-1}$)	336 ± 148	282.3 ± 206
Pre-camp ($U \cdot l^{-1}$)	166.9 ± 39 ‡	153.5 ± 109 ‡
Camp ($U \cdot l^{-1}$)	333.7 ± 76 †	523.6 ±521 †
Post-camp ($U \cdot l^{-1}$)	340.4 ± 87 †	499.6 ± 435 †

Note: Mean and standard deviation data. ‡ indicates the statistical difference between pre-supplementation and pre-camp values; † indicates the statistical difference between pre-camp values. Pre-supplementation blood samples were taken after three days of no physical exercise. Pre-camp blood samples were taken the night before the start of boot camp. Blood samples during camp were taken in the afternoon of the third day of this regimen. Blood samples after camp were taken in the afternoon of the 5th day of boot camp, two hours after the conclusion of camp.

TABLE 6. CRP-US LEVELS OF THE SUBJECTS BEFORE THE SUPPLEMENTATION PROCEDURE (PRE-SUPPLEMENTATION), ON THE NIGHT PRIOR TO BOOT CAMP REGIMEN (PRE-CAMP), ON THE THIRD DAY OF BOOT CAMP (CAMP), AND TWO HOURS AFTER THE COMPLETION OF BOOT CAMP (POST-CAMP).

	SUP	PLA
Pre-supplementation ($mg \cdot l^{-1}$)	1.30 ± 0.4	1.41 ± 0.8
Pre-camp ($mg \cdot l^{-1}$)	0.86 ± 0.5 ‡	1.49 ± 1.4
Camp ($mg \cdot l^{-1}$)	2.04 ± 1.5 †	2.65 ± 1.6†
Post-camp ($mg \cdot l^{-1}$)	6.18 ± 2.6 †*	8.6 ± 2.1†

Note: Mean and standard deviation data. ‡ indicates the statistical difference between pre-supplementation and pre-camp values; † indicates the statistical difference between camp and post-camp values as compared to the pre-camp instance. * indicates differences between the supplementation and placebo groups

The boot camp regimen promoted a significant increase of CK in both groups—an increase of 99.9% on the 3rd day of the camp and 103.9% after camp in the SUP group, and 241.1% during and 225.5% after camp in the PLA group. However, Table 5 shows great individual variability for this marker, resulting in elevated standard deviation values. As a consequence, no differences between the SUP and PLA groups were observed for either of the instances evaluated.

Changes in serum CRP concentration

Table 6 shows the pre-supplementation values of CRP-us. It appears that the results are within appropriate benchmarks for both groups. N-3 supplementation in the three weeks prior to the boot camp regimen promoted a significant reduction in serum concentration of CRP-us, while the PLA group showed no changes in this variable over the same period. In the analysis performed during camp, both groups showed a significant increase in CRP-us concentration, in relation to pre-camp values, but both groups were able to maintain their values within normal limits. On this occasion, there were no significant differences between the values of both groups. At the end of boot camp, both groups showed significantly higher CRP-us values than those found pre-camp and during camp. However, the SUP group ended camp with a significantly lower serum CRP level than the PLA group.

DISCUSSION

The results indicate that supplementation of n-3 PUFAs: 1) resulted in a decrease of CRP-us in three weeks of supplementation without changing total cholesterol values; 2) attenuated inflammatory activity induced by a regimen of five days of military boot camp with great physical exertion and sleep and food deprivation; 3) was not able to mitigate the occurrence of muscle damage during the boot camp regimen. Additionally, a mere five days of military boot camp was able to promote a significant reduction in serum levels of total cholesterol.

The reduction of total cholesterol concentrations in only 5 days of the boot camp regimen was a fact that drew attention. Previous results have shown that cholesterol suffers little influence from training, with a small reduction in low-density lipoprotein (LDL) cholesterol and increased high-density lipoprotein (HDL) levels after several weeks of physical exercise [25]. This prior information leads us to believe that an acute protocol of food deprivation coupled with intense exercise promoted this important reduction in total cholesterol. As the measurement of total cholesterol was only to control the variable intake of n-3 PUFA, one cannot determine the fractional participation of HDL, LDL, and very-low-density lipoprotein (VLDL) in this reduction of total cholesterol. The n-3 PUFA supplementation was shown to improve the plasma lipid profile, despite differing in the amounts of n-3 PUFA administered [9, 30], but this phenomenon occurred only after a long period.

The influential role of n-3 PUFAs in the inflammatory response induced by obesity and several other cardiovascular and metabolic diseases has been widely investigated [19,30], due to the fact that n-3 PUFAs are substrates for the production of inflammatory mediators (eicosanoids and cytokines) less potent than those formed from arachidonic acid [6]. Supplementation of n-3 PUFA results in increase of EPA and DHA in plasma lipids, platelets, erythrocytes, leukocytes, heart tissue, and other cells and tissues [7]. Once embedded in the membrane of the phospholipids, the n-3 PUFAs are capable of inhibiting the production of eicosanoids and the classic pro-inflammatory cytokines, TNF-α, IL-1β, and IL-6 [6].

Recently, this anti-inflammatory function of n-3 PUFAs has been expanded to the scope of sports [2,3,26]. The basis for this line of research is that intense physical training promotes an inflammatory process in the muscles trained, mediated by the immune system, which can develop into a systemic inflammatory process, with production of cytokines, changes in the modulation of the hypothalamic-pituitary-gonads and hypothalamic-pituitary-adrenals with production of inflammatory enzymes and acute phase proteins [1]. So the relation between n-3 PUFA and the syndrome of overtraining is the fact that the imbalance between physical stress, diet, and recovery periods to which athletes and non-athletes are submitted is mediated by the same pro-inflammatory factors that are inhibited by the n-3 PUFAs.

The CRP levels found in this study, preceding the boot camp regimen, confirm the anti-inflammatory function of n-3 PUFAs. These data corroborate several previous studies that also showed the protective effect of n-3 PUFAs, by reducing the activity of either cytokines [2, 3], CRP [3, 8] or both [24]. However, other studies do not confirm the influence of n-3 PUFA in the activity of CRP [15,17,18,22], even when doses as high as 5.2 g daily in a period as large as 12 weeks were used [18].

Although there was a significant increase in CRP for the SUP and PLA groups during the boot camp regimen, n-3 PUFA supplementation was able to significantly attenuate the increase of this inflammatory marker. Some authors investigated the influence of n-3 PUFA supplementation during sports training [2,4,21,23,27]. Overall, these studies point to positive changes related to health, in the intake of n-3 PUFAs, such as marked reduction in the severity of bronchoconstriction in athletes [21], more efficient reduction of postprandial lipaemia when combined with physical exercise [27], lower availability of arachidonic acid and consequently lower production of prostaglandins [2], improved cardiovascular function, and reduced cardiac risk factors in athletes, but no improvement in running time on a treadmill until exhaustion nor in the recovery time of supplemented athletes [4].

In the studies by Andrade et al. [2], the effects of n-3 PUFA supplementation (950 mg EPA and 500 mg DHA) in the immune response of athletes from the Brazilian swimming team were investigated. In that study, supplementation was able to attenuate the increase of E2 prostaglandin induced by exercise. However, it is important to note that the subjects were swimmers who had good physiological monitoring and a very controlled training load, such that these athletes were not highly exposed to the deleterious effects

of overtraining or lack of nutrition/rest. In the study by Bloomer [3], decreased inflammatory activity was found, with no change in oxidative activity after 6 weeks of supplementation with doses of EPA/DHA similar to those used in our study. However, after a session of aerobic exercise, there were no differences in the inflammatory markers, oxidants, or muscle damage in the supplemented subjects. These data indicate that EPA/DHA supplementation would not have a protective effect against overtraining, but in none of these studies were subjects exposed to high levels of physical stress, nor were food or sleep restriction investigated; therefore this question cannot be answered by the previous data.

In this respect, our study was able to investigate a situation where this question can best be answered from an overtraining standpoint and from the standpoint of a real situation commonly experienced by military personnel. In this sense, the smallest increase in CRP in the supplemented military personnel indicates a protective effect of the supplementation of EPA/DHA against situations of physical stress/inadequate nutrition and rest, which increases the possibility of protection by n-3 PUFAs in the scope of physical and sports training. However, these data should be weighted by the fact that we only used one inflammatory process marker. Other tools, such as the classic pro-inflammatory cytokines (TNF-α, IL-1, and IL-6), would bring more consistent results to confirm the protective effects of supplementation. Other researchers have also made use of oxidative stress markers (SOD, TBARS, MDA) [3,10,12], which may also increase the level of certainty of the ergogenic effect of EPA/DHA in athletes, military personnel, or other subjects exposed to work of great physical and nutritional stress.

The significant increase in CK serum activity confirmed the strenuous nature of the regimen that was adopted during boot camp. The increase in CK activity was as large as the one found by Takashima et al. [28] after a 50 km cross-country skiing competition, taking into account that skiing competitions are considered among the toughest events in sports. In these two studies, there were significant increases in CRP in conjunction with the observed increase in CK activity. According to Eston et al. [13], CK activity increases according to the intensity of physical exercise, and can be attenuated with the development of physical conditioning. Even considering that the military personnel had already gone through 26 weeks of systemized physical training, this pre-conditioning was not enough to prevent muscle damage as intense as that from the toughest athletic competitions. At the same time that the stressful nature of the boot camp regimen was confirmed, the CK data indicated that n-3 PUFA supplementation was not able to minimize the muscular stress induced by this regimen, even considering that the same supplementation was able to attenuate CK activity in the three weeks prior to camp.

Although the concentration of CK increased much more in the placebo group than in the experimental group, the great individual variability of this variable resulted in large standard deviations from the average, with a consequent lack of statistical differences. Our study presents the limitation of not including other markers, such as lactate dehydrogenase enzyme (LDH) and troponin I, which could better address the issue of supplemental protection against muscle damage.

CONCLUSIONS

Considered as a whole, the data from this study reinforce the prospect of a new line of research in the n-3 PUFA area, with data that extend the possible functions of this nutrient, from cardiovascular and metabolic protection to the protection of individuals exposed to regimens of intense physical activities and/or nutritional inadequacy— situations that can be found in athletes, military personnel in training, and various other categories of workers. Thus, not only athletes or military personnel would benefit from the consumption of n-3 PUFAs, but all people who have daily routines resembling those of the investigated group. The use of more biochemical variables in future studies, such as procytokines and anti-inflammatories, cholesterol sub-fractions, and other markers of muscle damage and oxidative stress, could confirm the results obtained in this research.

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