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HEMP SEED OIL EFFECT ON THE RAT TISSULAR OXIDANT/ANTIOXIDANT BALANCE IN EXERCISE WITH PROGRESSIVE INTENSITY

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ABSTRACT. Introduction. Pro oxidant effect of intense physical effort, its consequences and the importance of combating nitrosative stress is a topical issue of interest, according to the literature. Combating this effect is achieved by administration of exogenous nutritional or non nutritional, natural or synthetic antioxidants. The influence of nutrition, the antioxidant and pro oxidant effect of moderate exercise is added to this aspect. Hemp seed oil is a natural source of antioxidants whose beneficial effects on the balance of serum oxidant / antioxidant in exercise rats was the subject of our previous research. **Objectives**. We aim to study the effect of the administration of hemp oil on indicators of tissue oxidant/antioxidant balance in the myocardium and brain in the exercise training with gradual intensity. **Methods.** The research was conducted on two groups of male, adult Wistar rats. Group I consisted of rats trained at progressive intensity exercise, group II consisted of rats trained with progressive intensity exercise and supplemented with hemp oil. Indicators of oxidative stress: malondialdehyde (MDA), protein carbonyls (PC) were determined. Antioxidant defense capacity indicators were hydrogen donor (DH), total sulfhydryl groups (SH), glutathione (GSH). For statistical analysis, data for normal distribution we used the t (Student) and one-way ANOVA analysis of variance. **Results.** The intensity progressive exercise and dietary supplementation with hemp oil produce significant changes in the myocardium and brain with increased indicators of oxidative stress and increased antioxidant defense versus unsupplemented group. **Conclusions.** Hemp oil supplementation and progressive intensity exercise affect the oxidant/antioxidant balance in the tissues, causes significant increases in oxidative stress and increases antioxidant defense in brain and myocardium.

Keywords: *oxidative stress, nutritional antioxidants, myocardium, brain*

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REZUMAT. ***Efectul uleiului din seminţe de cânepă asupra balanţei tisulare oxidanţi/antioxidanţi în efortul fizic cu intensitate progresivă la şobolani.*** **Introducere.** Efectul prooxidant al efortului fizic intens, consecinţele acestuia şi importanţa combaterii stresului oxinitrozativ, este o temă actualitate şi interes, conform datelor din literatură. Combaterea acestui efect se realizează prin administrare de antioxidanţi exogeni nutriţionali sau nenutriţionali, naturali sau sintetici. Acestui aspect i se adaugă şi influenţa nutriţiei, ca prooxidant şi antioxidant şi efectul antioxidant al efortului fizic moderat. Uleiul din seminţe de cânepă este o sursă naturală de antioxidanţi ale cărui efecte favorabile asupra balanţei serice oxidanţi/antioxidanţi în efortul fizic la şobolani a făcut obiectul cercetărilor noastre anterioare. **Obiective**. În acest studiu s-a urmărit efectul administrării uleiului de cânepă asupra indicatorilor tisulari ai balanţei oxidanţi/antioxidanţi la nivelul miocardului şi encefalului în antrenamentul la efort fizic cu intensitate progresivă. **Metode.** Cercetarea a fost efectuată pe două loturi de şobolani masculi, adulţi, rasa Wistar. Lotul I a cuprins şobolani antrenaţi la efort cu intensitate progresivă, lotul II a cuprins şobolani antrenaţi la efort cu intensitate progresivă şi suplimentaţi cu ulei de cânepă. Indicatorii stresului oxidativ determinaţi au fost: malondialdehida (MDA), proteinele carbonilate (PC). Indicatorii apărării antioxidante au fost capacitatea de donori de hidrogen (DH), grupările sulfhidril totale (SH), glutationul (GSH). Pentru analiza statistică a datelor, în cazul datelor cu distribuţie normală s-a utilizat testul t (Student) şi analiza variantei one-way ANOVA. **Rezultate.** În efortul fizic cu intensitate progresivă şi suplimentarea dietei cu ulei de cânepă determină la nivelul miocardului şi la nivelul encefalului se produc modificări semnificative cu creşterea indicatorilor stresului oxidativ şi creşterea apărării antioxidante, faţă de lotul nesuplimentat. **Concluzii.** Suplimentarea cu ulei de cânepă şi efortul fizic cu încărcare progresivă influenţează balanţa oxidanţi/antioxidanţi la nivel tisular, determină creşteri semnificative ale stresului oxidativ şi creşteri semnificative ale apărării antioxidante în miocard şi în creier.

Cuvinte cheie*: stres oxidativ, antioxidanţi nutriţionali, miocard, creier*

Introduction

Exercise is a complex stress: neuromuscular, endocrine, systemic, biochemical and psycho-emotional. Depending on the intensity and duration, exercise can act paradoxically as a prooxidant or antioxidant (AO) factor, by modifying redox homeostasis: prooxidant, if high intensity and short duration and antioxidant if moderate and long duration (Fisher-Wellman & Bloomer, 2009). Increased oxygen consumption during exercise thereof causes the formation of oxigen catabolites and subsequently nitrogen catabolites, with oxinitrosative stress (ONS) triggering under conditions of intense effort (Tache, 2001).

Paradoxical effect of exercise is a topic of great interest and concern, according to the literature on the prooxidant effect of intense physical effort, its consequences and the importance of combating nitrosative stress by administration of exogenous nutritional or non nutritional, natural or synthetic antioxidants and antioxidant effect of moderate physical effort. To this effect is added the influence of nutrition, as prooxidant and antioxidant (Tache, 2006).

The myocardium has a high rate of oxidative metabolism and relatively low activity of main AO enzymes, which could increase susceptibility to oxidative damage after acute exercise. The exercise is considered an important stimulus for antioxidant systems as GSH and related AO enzymes that are important in protecting the myocardium, such as SOD and GSH-Px (Tache & Staicu, 2010).

Endurance training induces upregulation in some AO defense mechanisms protecting the heart muscle in potentially harmful situations that induce additional SO (Ascensão et al., 2003).

The brain uses 20% of the total oxygen consumed by the entire body at rest. Oxygen consumption increases by 10 to 15 times during the exercise. However, oxygen consumption of the brain is known to be constant during exercise. Thus, it is unlikely the exercise to induce oxidative stress (OS) in the brain. The brain may be sensitive to lipid peroxidation processes due to the high concentration of polyunsaturated fatty acids and low levels of antioxidant enzymes (SOD, CAT, GSH-Px) and GSH present in normal conditions (Radak et al., 2008).

Chronic physical effort increases the level of AO in the brain and helps protect brain oxidative lesions (Tache & Staicu, 2010).

Endurance training induces upregulation in some AO defense mechanisms protecting the heart muscle in potentially harmful situations that induce additional SO (Ascensão, 2003).

Physical training induces an adaptive biochemical response, which may require an increase of the intake and /or absorption of micronutrients. One hypothesis raised is whether acute or chronic exercise alter AO requirements. In this respect, the interaction of exercise with nutrition should be considered. SON cannot be avoided, but the imbalance between O and AO can be mitigated to reduce oxidative damage and consequences SON (Sies, 1997).

In the modern society there is excess of calories in the diet, what causes obesity and chronic metabolic stress. The calorie excess associated with elevated concentrations of plasma lipids and lipid accumulation in skeletal muscle, lead to decrease insulin sensitivity.

The exercise is commonly used to combat these consequences as it is considered an effective means of lipid oxidation (Kiens et al., 2001).

In high intensity exercise the O/AO balance is disturbed and nutritional AO supplements are administered to rebalance it. Doses of antioxidant supplements should be prescribed with respect to quantitative aspects relating to the effect of acute and chronic exercise on the O/AO balance and their impact on health.

Adopting certain nutritional strategies during exercise means administration of AO that must consider: the specific metabolic processes (aerobic, anaerobic or mixed) during various sports activities; intensity and duration of exercise during training or competitions. Intake of AO aims to increase intracellular concentrations of AO, delays muscle fatigue, improves performance, reduces the risk of injuries or illnesses related to the specific muscle effort (Tache, 2006).

There is growing evidence that a particular AO cannot prevent by itself the ONS production. As direct adverse effects of dietary supplements are synergistic adverse effects. Other effects are assumed to limit the effect of endogenous adaptive produced by chronic exercise. High doses of AO supplementation may reduce the favourable effects induced by the reactive species or generate prooxidant effects. The administration of nutritional supplements in athletes is needed only when the nutritional status is poor. Analysis of the risk/benefit evidence for an unknown risk of high doses of AO, namely an impairment of adaptive effects and long-term risk is still unknown (Margaritis & Rousseau, 2008).Caution should be applied in administering antioxidant supplements in excess. Atalay et al. (2006) recommends the following doses required to determine individual AO doses of each athlete performing a specific type of sport. Administration of several nutrients instead of mega-doses of any single nutrient form, appear to be a prudent choice and performance should not be the sole criterion for choosing supplementation doses AO. The general condition of the athlete, faster recovery and reduced oxidative damage could be affected by AO therapy (Arent et al., 2010).

Objectives

In this study we aimed:

1. the determination of tissue indicators of the O/AO balance in progressive intensity exercise training;

2. the effect of hemp oil administration on tissue indicators of the O/AO balance in the myocardium and brain in exercise training with progressive intensity.

Material and method

Lots

The research was conducted on two groups of adult male Wistar rats, weighing 200-300g average (n = 10 animals/group). Group I consisted of rats trained to progressive intensity exercise, group II consisted of rats trained with progressive intensity exercise and supplemented with hemp oil. Exercise intensity was progressively modified by loading the animals with weights attached, carried as follows: 7 days without charge, day 8 to14 with10% charge, day 15 to 21 with 15% charge, day 21 to 28 with 20% of the weight load. The time moment analyzed was on day 28.

Hemp oil administration was made by oral gavage in amount of 0.1 ml per rat, the dose was calculated in relation to the recommended daily dose for humans oil.

Training lasted 28 days, with the swimming test, which was performed in a plastic basin with water at 20 ° C, by measuring the time interval, in seconds, timed from the moment the animal were introduced in the pool, until exhausted (refusal to swim).

For the determination of the oxidant/antioxidant (A/AO) balance indicators in the myocardial tissue and brain, samples of myocardial and brain tissue were harvested following euthanasia of the animals.

Biochemical determinations were performed in the Laboratory for the Study of Oxidative Stress of the "Iuliu Haţieganu" University, in Cluj-Napoca.

We determined the following indicators of oxidative stress:

Malondialdehyde (MDA) was dosed using the fluorescence method after Conti et al. (1991). Protein carbonyls (PC) were determined using the method according to Reznik and Packer (1994). We determined the following indicators of antioxidant defense: Hydrogen donor ability (DH) was determined using the assay method after Janaszewska and Bartosz (2002). Total sulfhydryl groups (SH) were dosed according to the Hu (1994) method. Glutathione (GSH) concentration was assayed by the fluorescence method (Hu, 1994).

For statistical analysis of data, t test (Student) was used for data with normal distribution, one-way ANOVA analysis of variance followed by post hoc analysis for multiple comparison (Scheffe test/ Bonferroni/LSD test), the variations being tested with Levene test for variance. Kolmogorov-Smirnov test was used for normal distribution.

Results

Comparative statistical analysis between tissue indicators of the O/AO determined in samples of myocardium and brain is shown in Table 1.

Table 1. Statistical analysis of comparative indicators of tissue O/AO balance in myocardium and brain, the groups studied.

Myocardium homogenate - brain homogenate		MDA		PC		SH		DH		GSH	
Lot I	Average values	0,14	0,137	0,741	0,656	0,023	0,030	35,463	39,877	0,670	0,838
	p	0,865		0,482		**0,002**		**0,006**		**0,004**	
Lot II	Average values	0,268	0,153	1,122	4,051	0,016	0,043	51,479	30,413	0,52	3,941
	p	**0,001**		**1,38 x 10^{-5}**		**2,06 x 10^{-7}**		**1,92 x 10^{-7}**		**2,45 x 10^{-9}**	

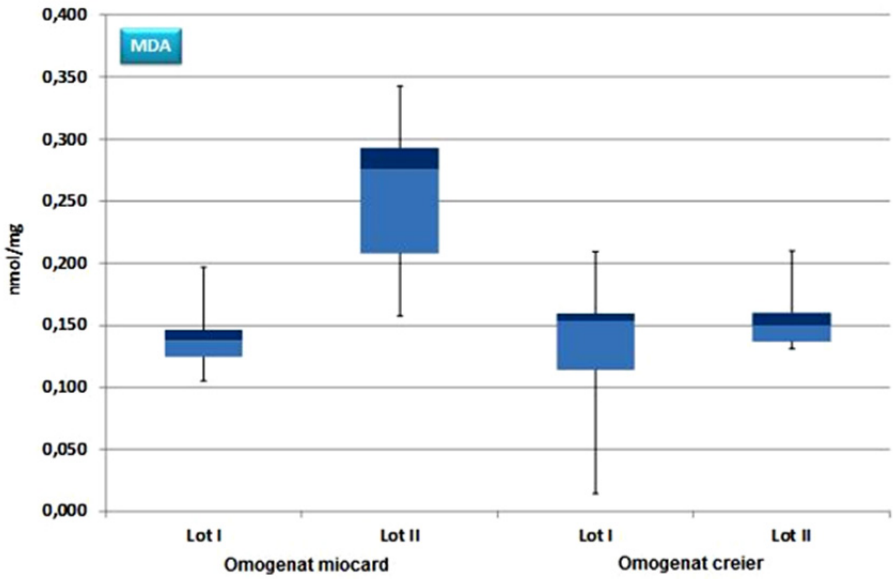

Fig. 1. MDA values for the studied lots (nmol/mg)

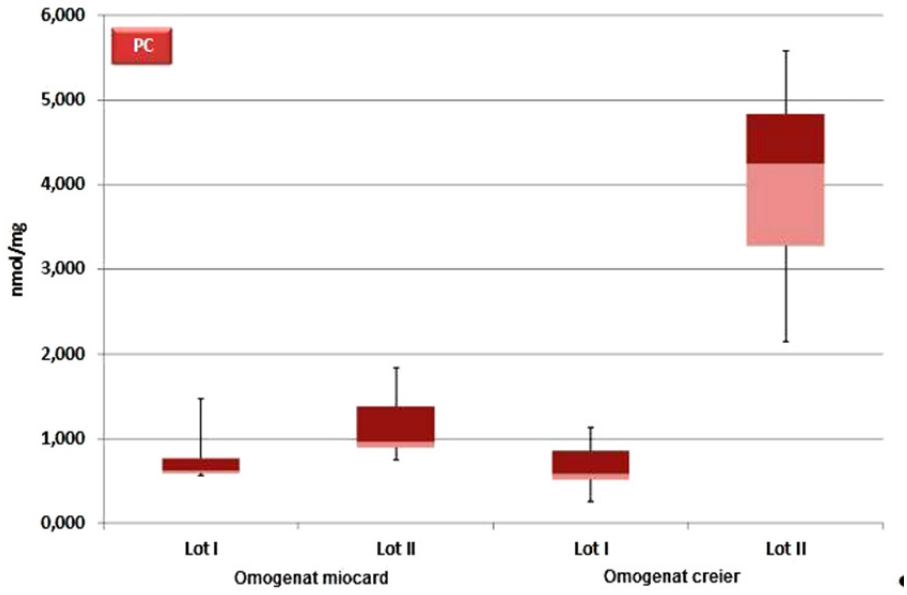

Fig. 2. PC values for the studied lots (nmol/mg)

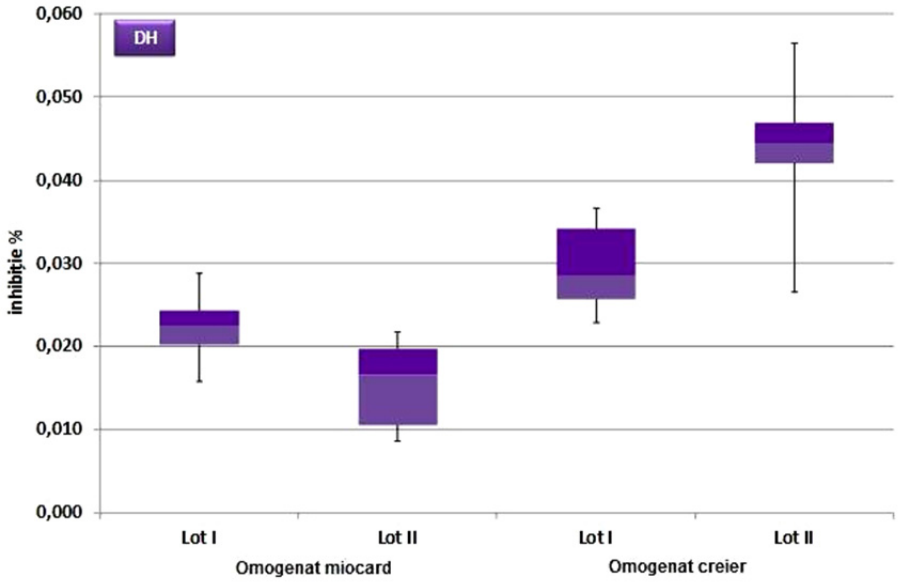

Fig. 3. DH values for the studied lots (% inhibition)

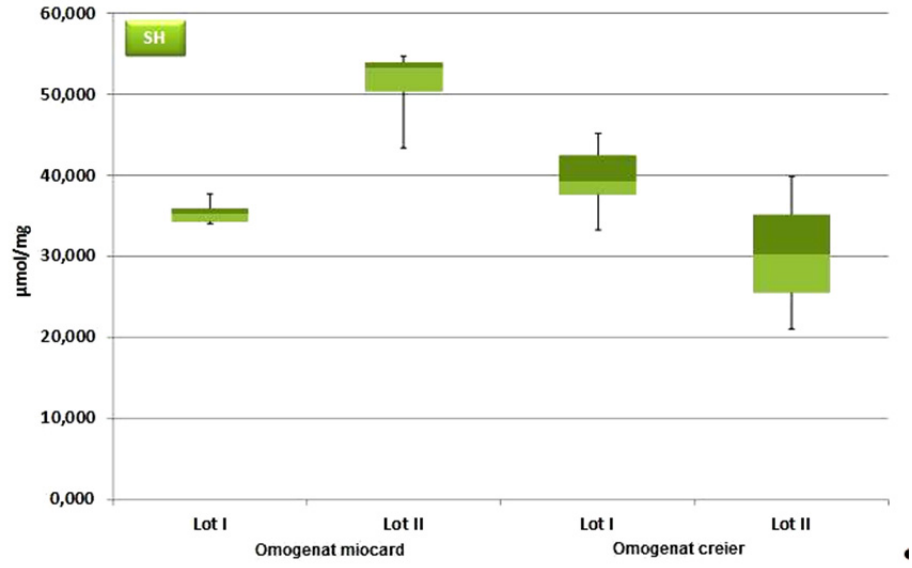

Fig. 4. SH values for the studied lots (µmol/mg)

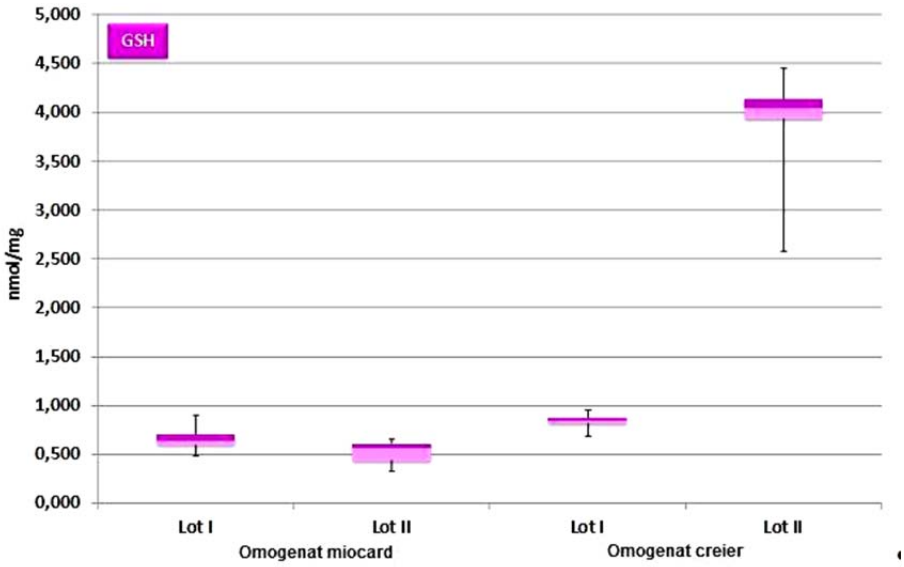

Fig. 5. GSH values for the studied lots (nmol/mg)

Discussion

The exercise with progressive intensity and hemp oil supplementation causes significant changes in the rat myocardium: increased ONS on account of MDA and decreased AO defense on behalf of SH groups and DH growth versus un-supplemented group.

In the brain, progressive intensity exercise and dietary supplementation with hemp oil, determine significant changes with increasing ONS due to PC and decreased AO defense due to the decrease in SH groups and GSH compared to un-supplemented group.

Conclusions

• Hemp oil supplementation and exercise with progressive loading influences O/AO balance at the tissue level.

• Hemp oil supplementation and exercise with progressive loading cause significant increases in ONS, on behalf of MDA in the myocardium and on behalf of PC in the brain.

• Hemp oil supplementation and exercise with progressive loading causes significant increases in AO defense on account of DH in the myocardium and on behalf of SH groups and GSH in the brain.

• Proper nutrition and strategies on intake of dietary nutritional agents, for supporting exercise capacity and post effort recovery, requires the development of recommendations by specialist physicians in the field of sports medicine in collaboration with nutritionists.

• To increase the performance should be considered use of antioxidants through proper nutrition and nutritional supplements in exercise, to increase aerobic capacity and antioxidant defense.

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