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The effect of BCAA supplementation on the oxidant/ antioxidant balance during physical exercise

Efectul suplimentării cu BCAA asupra balanţei oxidanţi/ antioxidanţi în efortul fizic

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Abstract

Background. Experimental studies have shown that branched-chain amino acids (BCAA) can downregulate the expression of some antioxidant (AO) genes and alter tissue redox homeostasis in the brain.

Aims. The increase of oxidative stress (OS) during intense exercise and the increase of AO defense during moderate exercise led us to study the effect of BCAA supplementation on aerobic exercise capacity (AEC) and biochemical redox profile.

Methods. The research was performed in 6 groups (n=10 animals/group): group I – controls, group II – controls + exercise (5% load), group III – controls + exercise (10% load), group IV – BCAA, group V – BCAA + exercise (5% load), group VI – BCAA + exercise (10% load). AEC was determined by the swimming test, the measurements for the O/AO balance were performed for malondialdehyde (MDA) and total sulfhydryl (SH) groups.

Results. Our research shows the energogenic effect of BCAA supplementation, with the increase of AEC after chronic administration for 28 days, and the systemic effect of AO in exercise trained animals. BCAA supplementation causes an alteration of redox homeostasis, with the increase of AO defense in the serum of exercise trained animals, an effect that is not reported in the literature, which might contribute to the increase of exercise capacity.

Conclusions. BCAA supplementation determines: an increase of exercise capacity in animals compared to unsupplemented controls and to initial values; a significant increase of AO defense in the serum of sedentary animals; a significant increase of AO defense in the serum of exercise trained animals.

Key words: BCAA, aerobic exercise capacity, oxidant/antioxidant balance, oxidative stress.

Rezumat

Premize. Unele studii experimentale au aratat că aminoacizi esenţiali cu lanţ ramificat (branched-chain amino acids) (BCAA) pot hiporegla expresia unor gene antioxidante (AO) şi altera homeostazia redox tisulară în creier.

Obiective. Creşterea stresului oxidativ (SO) în cursul efortului fizic intens şi creşterea apărării AO în efortul fizic moderat ne-a determinat să studiem efectul suplimentării cu BCAA asupra capacităţii aerobe de efort (CAE) şi profilului biochimic redox.

Metode. Cercetările au fost efectuate pe 6 loturi (n=10 animale/lot): lotul I - martori, lotul II - martori + efort (încărcare 5%), lotul III - martori + efort (încărcare 10%), lotul IV - BCAA, lotul V - BCAA + efort (încărcare 5%), lotul VI - BCAA + efort (încărcare 10%). CAE s-a determinat prin proba de înot, determinările pentru balanţa O/AO s-au realizat pentru malondaldehidă (MDA) şi pentru grupările sulfhidril totale (SH).

Rezultate. Cercetările noastre arată efectul energogen al suplimentului de BCAA, cu creşterea CAE după administrarea cronică timp de 28 zile şi efectul AO la nivel sistemic, efect prezent la animalele antrenate. Suplimentarea cu BCAA determină modificarea homeostaziei redox, cu creşterea apărării AO la nivel seric la animalele antrenate la efort, efect nesemnalat în literatură, ceea ce ar putea contribui la creşterea capacităţii de efort.

Concluzii. Suplimentarea cu BCAA determină: creşterea capacităţii de efort la animale faţă de martorii nesuplimentaţi şi faţă de valorile iniţiale; creşterea semnificativă a apărării AO la nivel seric la animalele sedentare; creşterea semnificativă a apărării AO la nivel seric la animalele antrenate la efort.

Cuvinte cheie: BCAA, capacitate aerobă de efort, balanţa oxidanţi/antioxidanţi, stres oxidativ.

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Introduction

The use of energogenetic means to increase physical performance is based on the quantitative use and qualitative requirements of macronutrients: carbohydrates, lipids and proteins, and micronutrients in various sports.

A supplement widely used by athletes is represented by essential and non-essential amino acids, basic components of proteins, involved in physical exercise.

AA play the following roles in physical exercise:

- an indirect energogenic role;
- a plastic trophotropic role on muscle mass and optimization of protein anabolism, with positive effects on nitrogen balance;
- an indirect functional role of transport and use of O_2 in active tissues (HIS), an antioxidant role in musculoskeletal tissue (His, Cys, Glu), muscle vasodilation (Arg), prevention and diminution of muscle fatigue (Asp), an immunostimulatory effect (Gly), a glucogenic and protective role of muscle glycogen (Glu, Asp), a cytoprotective effect on muscle enzymes during exercise - taurine (Dudgeon et al., 2015) and a decrease of adipose mass.

A preparation based on a triad of essential branched-chain amino acids: L-leucine, L-isoleucine and L-valine (BCAA) is frequently used/recommended by/for athletes. Of these, the most studied one, which can apparently provide the most benefits, is leucine.

BCAA are anabolizing supplements, which increase muscle resistance, playing an important role in muscle protein metabolism, being oxidized in muscle and representing the main source of calories. They produce glycogen having a gluconeogenetic role, balancing insulin secretion.

L-leucine is involved in hemoglobin formation, stabilization of glycemia, muscle anabolism, energogenesis. L-isoleucine plays a role in muscle anabolism and harmonious muscle distribution. L-valine is involved in muscle development, muscle anabolism and glucose formation (Jafari et al., 2016; Chen et al., 2016; Gil & Kim, 2015).

Although BCAA are abundant in the normal daily diet, they are the only AA that are not decomposed in the liver; the ingested BCAA amount is directly found in the plasma and peripheral tissues, particularly muscle and adipose tissue (Layman, 2003).

Other studies have shown that BCAA administration has beneficial effects in various diseases, in patients, for the improvement of protein synthesis, hepatic encephalopathy, insulin resistance, suppression of hepatocarcinoma, with the increase of the survival rate and the improvement of the quality of life (Jia et al., 2014; Sun & Wang, 2014).

Experimental studies have shown that BCAA can downregulate the expression of antioxidant genes and alter tissue redox homeostasis in the brain (Piscopo et al., 2011).

A number of studies have evidenced the favorable effects of the administration of BCAA supplements in endurance athletes (Gil & Kim 2015; Jafari et al., 2016; Dieter et al., 2016).

Favorable aspects have been attributed to the anti-fatigue and energogenic effects (Chen et al., 2016; Dudgeon et al., 2016); to the stimulation of protein synthesis and the role of signal molecules for cell growth and metabolism regulation (Sun & Wang, 2014); to the antiinflammatory role in skeletal muscle (Buonocore et al., 2015), and to the reduction of muscle lesions during exercise.

Hypothesis

The favorable effects of BCAA supplements on aerobic exercise capacity and the antioxidant effect of moderate physical exercise led us to study the influence of BCAA supplementation on redox homeostasis in animals subjected to exercise.

Material and methods

Research protocol

The research took place at the Ambulatory Sports Medicine Clinic and was approved by its manager, by the Ethics Board of the "Iuliu Hatieganu" University of Medicine and Pharmacy Cluj-Napoca, and the informed consent of the subjects was obtained.

a) Period and place of the research

The experimental study was performed on male Wistar rats from the Biobase of the "Iuliu Haţieganu" University of Medicine and Pharmacy Cluj-Napoca. The rats had a mean weight of 180-190 grams and were aged 16 weeks old. The study was approved by the Ethics Board, according to the Good Practice Guidelines. The requirements of the Helsinki Declaration, Amsterdam Protocol, Directive 86/609/EEC and the regulations of the Bioethics Commission of the "Iuliu Haţieganu" University of Medicine and Pharmacy Cluj-Napoca were met. The research was carried out in the Experimental Research Laboratory of the Department of Physiology of the "Iuliu Haţieganu" University of Medicine and Pharmacy Cluj-Napoca.

b) Subjects and groups

The measurements were performed in 6 groups of rats (n=10 animals/group):

Group I – controls
Group II – controls + exercise (5% load)
Group III – controls + exercise (10% load)
Group IV – BCAA
Group V – BCAA + exercise (5% load)
Group VI – BCAA + exercise (10% load)

BCAA (Natural Plus Preparation) were administered by oropharyngeal gavage, in a dose of 0.1 ml per rat, calculated in relation to the daily dose recommended for humans. The ratio between the amino acids (AA) in the preparation was 2:1:1 (1000 mg L-leucine, 500 mg L-isoleucine and 500 mg L-valine). The administered amount was 30 mg/animal/day for 28 days.

c) Tests applied

The aerobic exercise capacity (AEC) was determined by the swimming test, which was carried out in a plastic pool, with thermostatic water at 20°C. The pool had the following characteristics: length 100 cm, width 40 cm, height 60 cm, water level 30 cm (Nayanatara et al., 2005).

The value of AEC was calculated by measuring the length of time, expressed in seconds, from the time of placement of the animals in the pool to their exhaustion (refusal to swim).

The intensity of exercise was changed by loading the

Table I

Comparative analysis of aerobic exercise capacity values (measured in sec) in the studied groups and statistical significance

Time	Group	Mean	SE	Median	SD	Min.	Max.	Statistical significance (p)		
T1	II	321	10.7703	310	34.0588	290	385	T1-T14-T28	II	< 0.001
	III	292	7.0427	292.5	22.2711	255	325		III	< 0.001
	V	336	6.3666	330	20.1329	306	375		V	< 0.001
	VI	328	6.3386	323	20.0444	306	368		VI	< 0.001
T14	II	428	11.0000	420	34.7851	385	482	II	T1-T14	< 0.01
	III	382	11.3490	385	35.8887	335	430		T1-T28	< 0.01
	V	427	4.9058	423.5	15.5134	406	456		T14-T28	< 0.001
	VI	423	4.5631	422	14.4299	405	450	III	T1-T14	< 0.001
T28	II	525	8.2476	524.5	26.0811	495	560		T1-T28	< 0.001
	III	503	4.8120	504	15.2169	469	520		T14-T28	< 0.001
	V	623	6.9730	619	22.0504	595	669	V	T1-T14	< 0.001
	VI	645	10.0786	644.5	31.8713	600	698		T1-T28	< 0.001
Statistical significance (p)		Time	II-III-V-VI	II-III	V-VI	II-V	III-VI		T14-T28	< 0.001
		T1	< 0.01	NS	NS	NS	< 0.01	VI	T1-T14	< 0.01
		T14	< 0.01	< 0.01	NS	MNS	< 0.01		T1-T28	< 0.001
		T28	< 0.001	< 0.05	NS	< 0.001	< 0.001		T14-T28	< 0.001

animals with different weights, 5% and 10% of the animal's weight, in the standard linear loading variant.

The duration of the experiment was 28 days. The studied days were day 1 (T1), day 14 (T14) and day 28 (T28).

Biochemical determinations were performed in the Laboratory for the Study of Oxidative Stress of the Department of Physiology of "Iuliu Haţieganu" UMPh Cluj-Napoca. For the measurement of the indicators of the O/AO balance in the blood, venous blood samples were taken from the retro-orbital sinus. The serum was separated from the collected blood by centrifugation, for the measurement of the indicators.

Malondialdehyde (MDA) was measured using the fluorescence method, according to Conti et al., (1991). Concentration values were expressed in mol/ml. Total sulfhydryl (SH) groups were measured using the method of Hu (1994). Concentration values were expressed in µmol/ml.

d) Statistical processing

Statistical processing was performed using the StatsDirect v.2.7.2 software, with the OpenEpi 3.03 application and the Excel application (Microsoft Office 2010). The results were graphically represented using the Excel application (Microsoft Office 2010).

Results

a) Aerobic exercise capacity (Table I, Fig. 1).

The exercise capacity was studied in groups II, III (control groups) and V, VI (groups supplemented with BCAA) at 3 time points (T1, T14 and T28).

The statistical analysis of aerobic exercise capacity values, considering all groups, showed the following:

- very statistically significant differences between at least two of the groups at times T1 and T14 ($p < 0.01$)
- highly statistically significant differences between at least two of the groups at time T28 ($p < 0.001$).

The statistical analysis of aerobic exercise capacity values, considering all time points, evidenced highly statistically significant differences between at least two of the studied time points in all four groups ($p < 0.001$).

The statistical analysis of the aerobic exercise capacity values for unpaired samples showed the following:

- at time T1: very statistically significant differences between groups III-VI ($p < 0.01$);
- at time T14: very statistically significant differences between groups II-III and III-VI ($p < 0.01$);
- at time T28: highly statistically significant differences between groups II-V and III-VI ($p < 0.001$), statistically significant differences between groups II-III ($p < 0.05$).

The statistical analysis of the aerobic exercise capacity values for paired samples indicated:

- in group II - very statistically significant differences between times T1-T14 and T1-T28 ($p < 0.01$); highly statistically significant differences between times T14-T28 ($p < 0.001$)
- in group III - highly statistically significant differences between times T1-T14, T1-T28 and T14-T28 ($p < 0.001$)
- in group V - highly statistically significant differences between times T1-T14, T1-T28 and T14-T28 ($p < 0.001$)
- in group VI - very statistically significant differences between times T1-T14 ($p < 0.01$); highly statistically significant differences between times T1-T28 and T14-T28 ($p < 0.001$).

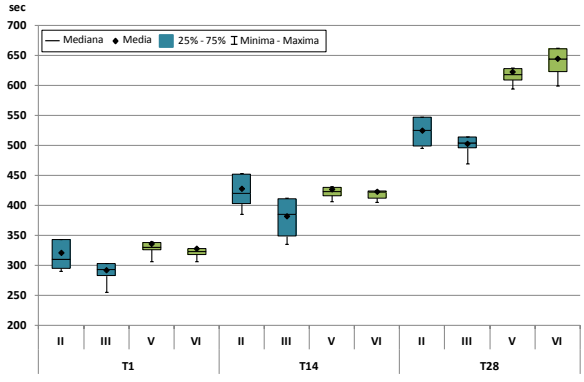

Fig. 1 – Aerobic exercise capacity in the 4 studied groups

b) The O/AO balance in the serum (Table II, Fig. 2 & Table III, Fig. 3).

The statistical analysis of malondialdehyde (MDA) values, considering all control groups, showed very

Table II

Comparative analysis of malondialdehyde values (measured in nmol/ml) in the studied groups and statistical significance

Group	Mean	SE	Median	SD	Min.	Max.	Statistical significance (p)			
I	1.467	0.0875	1.476	0.2768	1.002	1.944	I-II-III	< 0.01	IV-VI	NS
II	1.717	0.1675	1.545	0.5298	0.979	2.697	IV-V-VI	< 0.01	V-VI	< 0.001
III	2.023	0.0676	2.130	0.2138	1.712	2.238	I-II	NS	I-IV	< 0.05
IV	1.905	0.1661	1.916	0.5253	1.278	3.109	I-III	< 0.001	II-V	NS
V	1.687	0.0917	1.650	0.2898	1.221	2.314	II-III	NS	III-VI	NS
VI	2.240	0.1525	1.997	0.4822	1.920	3.448	IV-V	NS	nmol/ml	

Table III

Comparative analysis of SH values (measured in µmol/ml) in the studied groups and statistical significance.

Group	Mean	SE	Median	SD	Min.	Max.	Statistical significance (p)			
I	0.128	0.0123	0.124	0.0388	0.083	0.197	I-II-III	< 0.05	IV-VI	NS
II	0.150	0.0076	0.154	0.0239	0.111	0.179	IV-V-VI	NS	V-VI	NS
III	0.166	0.0044	0.163	0.0140	0.143	0.192	I-II	NS	I-IV	< 0.01
IV	0.182	0.0075	0.188	0.0238	0.143	0.214	I-III	< 0.05	II-V	< 0.01
V	0.190	0.0094	0.183	0.0297	0.158	0.255	II-III	NS	III-VI	< 0.05
VI	0.190	0.0073	0.185	0.0229	0.164	0.225	IV-V	NS	µmol/ml	

Table IV

Statistical analysis of correlation between the values of the studied serum indicators

Indicators		Group I		Group II		Group III		Group IV		Group V		Group VI	
MDA -	SH	0.3423	**	0.2253	*	0.2121	*	0.3082	**	-0.1026	*	-0.0545	*

Correlations: **** very good, *** good, ** acceptable, * weak.

statistically significant differences between at least two of the groups ($p < 0.01$).

The statistical analysis of MDA values, considering all groups with BCAA supplementation, evidenced very statistically significant differences between at least two of the groups ($p < 0.01$).

The statistical analysis of MDA values for unpaired samples showed:

- highly statistically significant differences between groups I-III, V-VI ($p < 0.001$)
- statistically significant differences between groups I-IV ($p < 0.05$).

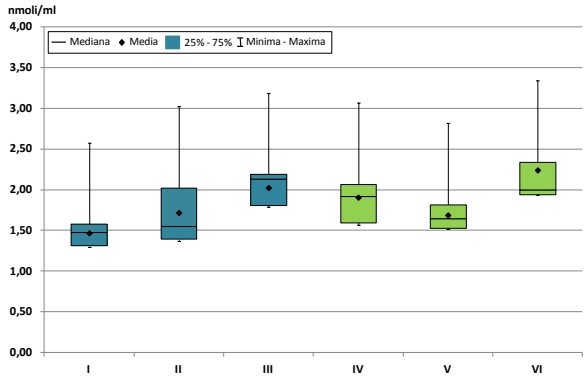

Fig. 2 – Serum MDA in the 6 studied groups.

The statistical analysis of sulfhydryl (SH) group values, considering all control groups, evidenced statistically significant differences between at least two of the groups ($p < 0.05$).

The statistical analysis of SH values, considering all groups with BCAA supplementation, showed no statistically significant differences between the groups ($p > 0.05$).

The statistical analysis of SH values for unpaired samples showed: very statistically significant differences between groups I-IV, II-V ($p < 0.01$); statistically significant differences between groups I-III, III-VI ($p < 0.05$).

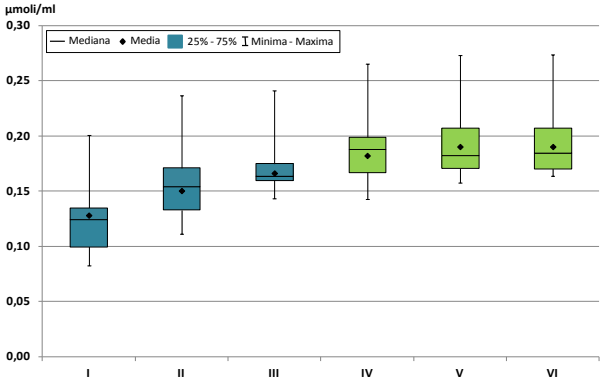

Fig. 3 – Serum SH groups in the 6 studied groups.

c) The O/AO balance (Table IV).

The statistical analysis of correlation between the values of the studied indicators showed:

- for group I, an acceptable positive correlation between MDA-SH;
- for group IV, an acceptable positive correlation between MDA-SH.

Table V

Statistical analysis of correlation between the values of the serum indicators of the O/AO balance and aerobic exercise capacity in the studied groups.

Indicators		Group II		Group III		Group V		Group VI	
AEC -	MDA	0.0697	*	0.3110	**	-0.2168	*	0.2606	**
	SH	-0.3028	**	-0.1896	*	-0.4723	**	-0.4942	**

Correlations: **** very good, *** good, ** acceptable, * weak.

d) The statistical analysis of correlation between the values of AEC and the indicators of the O/AO balance (Table V) showed for:

- group II, an acceptable negative correlation with SH
- group III, an acceptable positive correlation with MDA
- group V, an acceptable negative correlation with SH
- group VI, an acceptable negative correlation with SH and an acceptable positive correlation with MDA.

Discussions

BCAA supplementation in sedentary animals was performed in group IV compared to group I. Significant increases of serum MDA and SH were found.

BCAA supplementation in exercise trained animals shows: for group V compared to group II, significant increases of SH groups in the serum; for group VI compared to group III, significant increases of SH groups in the serum.

The comparative effect of BCAA supplementation in exercise trained animals, studied in group VI compared to group V, evidences significant increases in serum MDA.

Our results show the energogenic effect of BCAA supplementation, with the increase of AEC after chronic administration for 28 days, and the systemic AO effect, present in trained animals.

Our data are in agreement with the data of other authors, who show the favorable energogenic effect of BCAA preparations in endurance athletes (Dudgeon et al., 2015; Gacek et al., 2016; Jafari et al., 2016) and in animals (Chen et al., 2016), through provision of muscle energy and prolongation of resistance exercise. A study carried out by Gualano et al. (2011) demonstrated that BCAA supplementation increases resistance to fatigue and promotes lipid oxidation in subjects undergoing an exercise protocol for exhaustion of glycogen resources, and subsequently subjected to an exercise test until exhaustion.

BCAA supplementation causes an alteration of redox homeostasis, with the increase of serum AO defense in exercise trained animals, an effect unreported in the literature, which might contribute to the increase of AEC.

BCAA can be considered a beneficial supplement for high performance athletes, contributing to muscle recovery by promoting protein synthesis in muscle, by limiting muscle lesions during exercise, and to the regulation of the immune system (Negro et al., 2008; Shimomura et al., 2004; Howatson et al., 2012).

Conclusions

1. BCAA supplementation causes an increase of exercise capacity in animals compared to unsupplemented controls and to initial values.

2. BCAA supplementation induces a significant increase of AO defense in the serum of sedentary animals and exercise trained animals.

3. The oxidant/antioxidant balance does not show significant serum changes in animals supplemented with BCAA and subjected to exercise at different intensities.

Conflicts of interest

Nothing to declare.

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