

**Research article**

## Exercise x BCAA supplementation in young trained rats: what are their effects on body growth?

Patricia Lopes de Campos-Ferraz<sup>1</sup>, Sandra Maria Lima Ribeiro<sup>2</sup>, Silmara dos Santos Luz<sup>1</sup>, Antonio Herbert Lancha Jr.<sup>3</sup> and Julio Tirapegui<sup>1</sup>✉,

<sup>1</sup> Faculty of Pharmaceutical Sciences, <sup>2</sup> School of Arts, Sciences and Humanities, <sup>3</sup> School of Physical Education and Sport, University of Sao Paulo, Brazil

### Abstract

The purpose of this study was to evaluate whether Branched-chain amino acids (BCAAs) supplementation had any beneficial effects on growth and metabolic parameters of young rats submitted to chronic aerobic exercise. Thirty-two young rats (age: 21-d) were randomly assigned to four experimental groups (n = 8): Supplemented Trained (Sup/Ex), Control Trained (Ctrl/Ex), Supplemented Sedentary (Sup/Sed) and Control Sedentary (Ctrl/Sed). The trained groups underwent a five-week swimming protocol and received supplemented (45 mg BCAA/body weight/day) or control ration. Trained animals presented a lower body length and a higher cartilage weight, regardless of supplementation. Physical activity was responsible for a substantial reduction in proteoglycan synthesis in cartilage tissue, and BCAA supplementation was able to attenuate this reduction and also to improve glycogen stores in the liver, although no major differences were found in body growth associated to this supplementation.

**Key words:** Branched-chain amino acids, exercise, growth, metabolism, leucine, rat.

### Introduction

Many people from all age groups are currently involved in regular physical activity. Among them, children and adolescents deserve special attention (Ribeiro et al., 2010). Although moderate physical exercise has beneficial effect on growth and development, excessive exercise training could lead to opposite results (Caine et al., 2001; Georgepoulos et al., 2010).

Body growth processes (including bone, cartilage and muscle), demand a high amount of energy and nutrients. As such, nutritional deficits are expected to jeopardize growth as a whole. For instance, Yahya and Millward (1994) showed that short term protein deficits were capable of disbalancing the well-coordinated bone and muscle growth.

It is important to note that epiphysis cartilage development and growth consists of proteoglycan synthesis as well as cell multiplication (chondrogenesis). As such, proteoglycan synthesis is a measurement of cartilage and long bone growth. Indeed, Ribeiro et al. (2010) considered proteoglycan synthesis a biological marker of nutritional status.

Thinking about situations where the body faces energy/nutrient deficits, we can mention physical exercise. Exercise, single bout or regular training, characterizes a

state of increased glucose uptake by skeletal muscle during (as an energy substrate) and after (for glycogen and protein synthesis) physical effort (Blomstrand and Saltin, 1999; Hargreaves, 1998; Tipton and Wolfe, 2001).

Considering the need for energy in protein synthesis, and consequently for body growth, there is a strict relationship between muscle protein synthesis and glycogen stores once glycogen is an available source of energy to the muscle (Newsholme and Leech, 1988). Muscle growth will occur adequately only if bone growth is guaranteed by an adequate nutrient supply (Yahya and Millward, 1994).

Branched-chain amino acids (BCAAs) are sources of nitrogen for the synthesis of non-essential amino acids such as glutamine and alanine (Tom and Nair, 2006). Leucine is a potent inhibitor of muscle protein degradation, enhances insulin secretion (Nicastro et al., 2011) and may also be an important precursor for gluconeogenesis in both liver and muscle (Araujo et al., 2006; Campos-Ferraz, data nonpublished).

To date, no studies have been conducted relating BCAAs supplementation (isoleucine, leucine and valine) on bone and cartilage development in growing trained animals. The few studies that used BCAA supplementation on cartilage development did not evaluate BCAA effects on training individuals. In one study with sedentary pigs, BCAA supplementation did not reduce severity of osteochondrosis lesions compared to the control group (Frantz et al., 2008).

With this in mind, we used an experimental model to reproduce the situation of young individuals submitted to a regular training in order to identify the effects of this practice in biomarkers of muscle and bone growth. Therefore, the aim of the present study was to evaluate if whether BCAA supplementation could feature any benefit regarding muscle and bone growth in growing rats submitted to aerobic exercise.

### Methods

#### Animals

Male Wistar young rats (21 days old) were kept in individual cages on a 12 h dark/light cycle for 6 weeks. The animals were distributed into four experimental groups (n=8 each): supplemented trained (group Sup/Ex), control diet and trained (group Ctrl/Ex), supplemented diet and sedentary (group Sup/Sed) and control diet and sedentary (group Ctrl/Sed). The local Ethics Committee approved

all experimental procedures, and the guidelines for animal care and use were followed (National Research Council, 1996). Both food intake and animal weight were measured three times a week. Protein ingestion was calculated based on total food intake (g) and protein content of the diet.

### Diets

The control diet consisted of a standard ration designed for growing rodents by REEVES et al. (1993) and was prepared in our laboratory. It provided adequate levels of BCAA derived from casein, vitamins, minerals, fiber, carbohydrates and lipids. The protein content was assessed at 17% using the method provided by AOAC (1980). The supplemented diet was prepared using the same type of food as described above with the addition of BCAA (powdered, provided by Aji-No-Moto, Brazil) in order to reach 1.5 times the recommended level for each of the three amino acids (isoleucine, leucine and valine, 45 mg BCAA/kg body weight/day). The protein content of the supplemented diet was analyzed and resulted in 17.4% from the total energy content of the diet.

### Physical activity

The swimming training protocol was that described by LANCHETA Jr. et al. (1995), in which 10 individual PVC pools were connected to a boiler to allow the circulation of water and to keep the water temperature constant at 32°C. The first week of exercise consisted of an adaptation period during which the animals swam for 20 minutes. After this period, the animals started to swim longer (30 min - week 2, 40 min - week 3, 50 min - week 4 and 60 min - week 5, respectively) with a 5% body weight load attached to the tail, five times a week, always between 8 and 11 am. On the last week, if the rats were not able to stand the 5% body weight load, it would be reduced to 4%, to assure they completed 60 min of exercise.

### Euthanasia

Throughout the study, care was taken to avoid animals' unnecessary suffering. After the last training session, which was performed on the day before euthanasia, animals were fasted overnight. The animals were euthanized by decapitation. Blood was collected and handled in heparin-stabilized tubes, centrifuged at 2500g for 10 min at 4°C, and plasma was stored at -80°C. Soleus and gastrocnemius muscles were weighed, immersed in liquid nitrogen and stored at -80°C for further analysis.

### Biometric measurements

A few minutes before euthanasia, body weight and body length were measured using a digital scale and a graded wooden device, respectively (Hughes and Tanner, 1970). After euthanasia, muscle, liver, tibia bone, tibia cartilage and adipose tissue were collected and weighed.

### Biochemical analysis

**Glucose determination:** Plasma glucose was determined according to Trinder (1969) using colorimetric-enzymatic reactions. Basically, glucose is oxidized to gluconic acid and H<sub>2</sub>O<sub>2</sub> by glucose-oxidase. H<sub>2</sub>O<sub>2</sub>, in the presence of peroxidase, catalyzes phenol oxidation with 4-amino-

phenazon, giving a red color to the solution, whose peak absorbance is read at 505 nm.

**Plasma aminogram:** Plasma samples were deproteinized with 6% sulfosalicylic acid (1:5) and centrifuged at 9,000 g for 2 minutes, and the supernatant was diluted in NaS buffer, separated by ultra filtration and stored at -80°C. The concentrations of essential amino acids and ammonia were determined by reverse-phase HPLC (Busse and Carpenter, 1976).

**Muscle protein and total RNA:** Muscle protein content was determined according to Lowry et al. (1951). Protein was pretreated with copper (II) in a modified biuret reagent (alkaline copper solution stabilized with sodium potassium tartrate). The addition of Folin & Ciocalteu's phenol reagent forms chromogens that give increasing absorbance between 550 nm and 750 nm. Normally, absorbance at the peak (750 nm) or shoulder (660 nm) is used to quantify protein concentrations between 1-100 µg/ml. In our case, bovine albumin was used to build the standard curve and read the samples at 660nm.

Total RNA concentration was determined in muscle samples as follows: protein in the homogenate was precipitated by the addition of an equal volume of 10% trichloroacetic acid (TCA) followed by two washings of the precipitate with 5% TCA to remove acid-soluble compounds. Lipids were removed by two washings with 95% ethanol: ether (3:1 v/v). The precipitate was suspended in 1.5 ml 0.5 N perchloric acid (PCA) and heated to 70°C for 20 minutes to hydrolyze DNA. The hydrolysis step was repeated with another 1.5 ml PCA. The samples were compared against a 2% perchloric acid solution as standard. For the calculation of RNA, each extinction degree at 260 nm was considered to be equal to a 32 µg RNA/ml solution (Munro and Fleck, 1966).

**Plasma free fatty acid (FFA):** FFA levels were determined using a modified method previously described by Regow et al. (1971). Plasma was treated with an organic solvent and CuNO<sub>2</sub>. Next, sodium diethyldithiocarbamate was used to produce a yellow color solution whose intensity was proportional to the FFA levels of the sample. The samples were read in a spectrophotometer at 435 nm.

**Glycogen:** Muscle and liver glycogen concentrations were determined according to Hassid and Abrahams (1957). Briefly, tissues were treated with 6 N KOH for 30 minutes in a 37°C water bath, and 70% ethanol was added to precipitate glycogen. Next, the solution was centrifuged for 20 minutes at 3,000 rpm and the pellet was diluted in hot deionized water. Anthrone diluted in sulfuric acid was then added to turn the solution green, and the reaction was analyzed with a spectrophotometer at 650 nm.

**Proteoglycan synthesis:** The proteoglycan synthesis was measured in the tibia epiphysis as the incorporation of <sup>35</sup>S-sodium sulfate into acid-precipitable proteoglycan. The animals were injected i.p. with the <sup>35</sup>SO<sub>4</sub> in 0.9% (w/v) NaCl (0.5 mL/100 g, 40µCi/100 g) and were euthanized 1 h after the injection. The epiphysis (including the growth plate) was cleaned of all visible traces of muscle and tendons, pulverized and homogenized in ice-cold 10% trichloroacetic acid (TCA), washed twice with 10% TCA and with perchloric acid to remove

**Table 1.** Parameters related to food ingestion and body weight variation of the experimental animals. Values expressed as mean ( $\pm$  standard deviation).

Groups	$\Delta$ body weight	Gastrocnemius weight (%) <sup>1</sup>	Muscle/Tibia (mg/mm) <sup>3,85</sup>	Food Intake (g)	Epydidimal Adipose Tissue weight (%) <sup>1</sup>
Sup/Ex	4.0 (.5)	.55 (.07)	1.96 (.50)	600 (54)	1.05 (.38)
Ctrl/Ex	3.9 (.6)	.62 (.02)	2.00 (.40)	615 (98)	1.26 (.20)
Sup/Sed	5.2 (.3) †	.59 (.04)	2.16 (.60)	689 (29) †	1.63 (.46) †
Ctrl/Sed	5.3 (.8) *	.59 (.04)	1.95 (.70)	740 (62) ‡	1.58 (.49) ‡

$\Delta$  body weight: final weight-initial weight/initial weight; <sup>1</sup> tissue weight in relation to total body weight; \* p < 0.05 when compared to Ctrl/Sed; † p < 0.05 when compared to Sup/Ex; ‡ p < 0.05 when compared to Ctrl/Ex.

any residual free <sup>35</sup>SO<sub>4</sub> and solubilized in 2 ml 90% (w/v) formic acid, after incubation at 90°C overnight. <sup>35</sup>S radioactivity was measured in a Beckman LS-150 liquid scintillation counter and expressed as d.p.m/mg tissue (Yahya et al., 1994). Proteoglycan synthesis was measured 23h after the last training session in order to analyze the differences among the different protocols of the study.

### Statistical analysis

The results are reported as mean  $\pm$  standard deviation and were analyzed with the software SPSS 15.0 for Windows. A two-way analysis of variance (ANOVA) was used among all experimental groups, to determine any effect of supplementation and exercise on experimental parameters. If the two-way ANOVA was significant (p  $\leq$  0.05), individual differences between trained and untrained and between supplementation type were tested with a *post-hoc* test (Scheffé).

### Results

To assess nutritional status, body weight variation throughout the experiment was measured. Table 1 shows the variation of body weight of the animals. Sedentary rats, regardless of their diet intake, were heavier than trained animals. This table also shows total food intake. A significant difference was observed between trained and sedentary rats, as the latter showed a higher food intake regardless of the diet. Absolute gastrocnemius weight, relative gastrocnemius (% of body weight) muscle weight and muscle/tibia ratio (according to Tirapegui et al. 1994) are shown. Sedentary rats featured greater absolute gastrocnemius muscle weight compared to trained rats. Nevertheless, no significant differences were found in relative gastrocnemius weight and muscle/tibia ratio. Epydidimal adipose tissue relative weight was significantly higher in Ctrl/Sed animals, when compared to the Ctrl/Ex group.

Body growth was assessed by skull-tale length. Table 2 shows the skull-tale and tibia length; the first was significantly higher in sedentary rats; tibial length featured no significant differences among the experimental groups. Muscle weight was examined between the different

groups; however, absolute weight was higher in sedentary animals, irrespective of the diet. Epiphysis cartilage weight and sulfate incorporation are also present in this table. In trained rats, cartilage weight is increased compared to sedentary animals, however, proteoglycan synthesis is remarkably lower in trained compared to sedentary rats, albeit the Sup/Ex group featured increased sulfate incorporation compared to the Ctrl/Ex group.

Liver and muscle glycogen content are shown in Table 3. Liver glycogen levels were higher in supplemented rats, regardless of their physical activity (Sup/Ex and Sup/Sed). Muscle glycogen was significantly higher in the Ctrl/Ex group than in Ctrl/Sed.

**Table 3.** Tissue glycogen content. Values expressed as mean ( $\pm$ standard deviation).

Groups	Liver glycogen (mg%)	Muscle glycogen (mg%)
Sup/Ex	16.2 (3.4) ‡	3.5 (.3)
Ctrl/Ex	6.4 (1.6)	4.2 (.4) *
Sup/Sed	12.4 (4.8) *	3.0 (1.1)
Ctrl/Sed	5.7 (1.7)	2.7 (.8)

\* p < 0.05 when compared to Ctrl/Sed; ‡ p < 0.05 when compared to Ctrl/Ex.

Protein and RNA concentrations in gastrocnemius muscle are shown in Table 4. A significant difference in protein concentration was only observed between the groups Sup/Sed and Sup/Ex, with reduced protein concentration in the Sup/Ex compared to the Sup/Sed group. An increase in RNA levels was observed for the Sup/Ex group compared to both groups Ctrl/Ex and Sup/Sed.

Plasma glucose concentration is shown in Table 5. It was significantly higher in Ctrl/Sed compared to

**Table 4.** Muscle protein and RNA content. Values expressed as mean ( $\pm$ standard deviation).

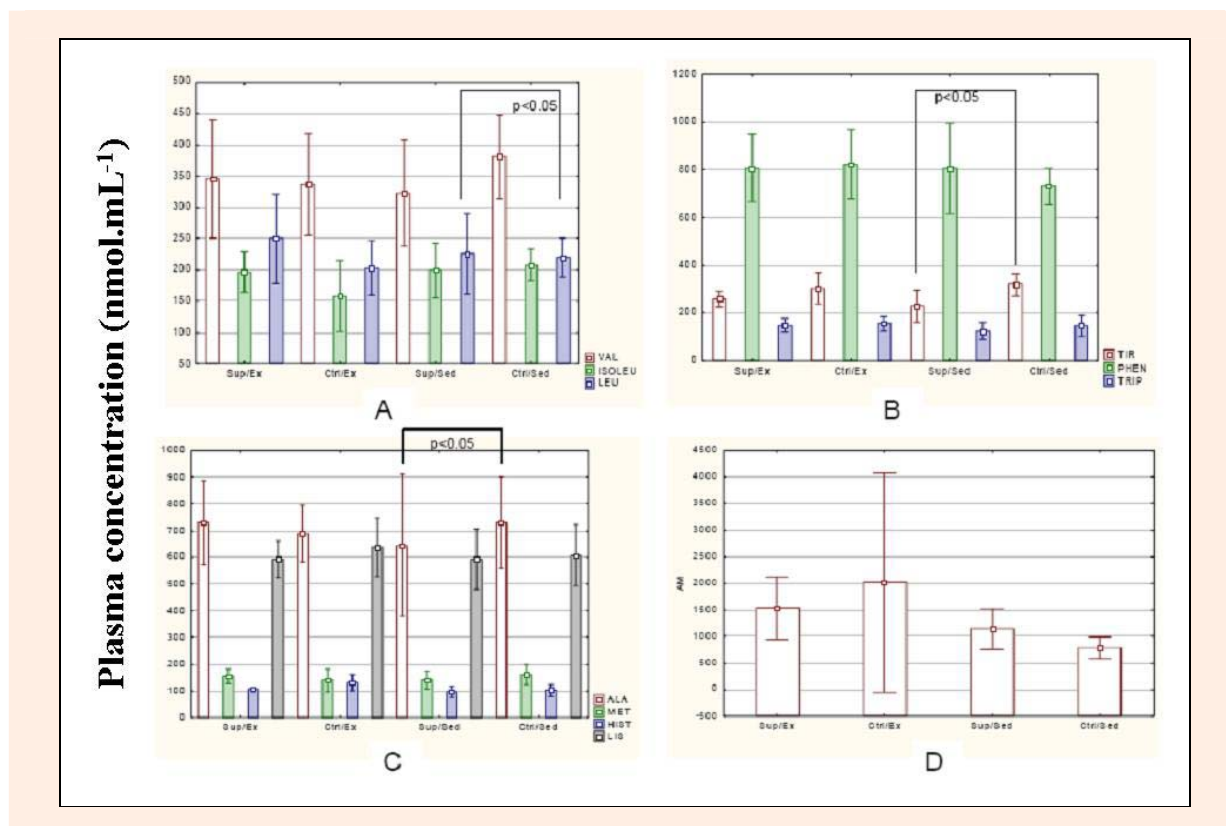
Groups	Protein (mg%)	RNA (ug/100 mg)
Sup/Ex	3.92 (1.22)	147.05 (19.98) ‡ §
Ctrl/Ex	5.52 (2.14)	124.00 (7.78)
Sup/Sed	6.37 (.47) †	118.66 (9.43)
Ctrl/Sed	5.08 (1.91)	128.86 (15.50)

† p < 0.05 when compared to Sup/Ex; ‡ p < 0.05 when compared to Ctrl/Ex; § p < 0.05 when compared to Sup/Sed.

**Table 2.** Parameters of bone growth. Values expressed as mean ( $\pm$ standard deviation).

Groups	Skull-tale length (cm)	Tibia length (mm)	Cartilage weight (% of body weight)	Proteoglycan synthesis <sup>1</sup> (cpm/100 mg tissue).
Sup/Ex	36.07 (1.20)	32.82 (1.68)	.039 (.001) §	301.95 (81.50) ‡
Ctrl/Ex	35.92 (2.33)	33.33 (2.39)	.041 (.003) †*	218.52 (80.40)
Sup/Sed	38.28 (.80) †	34.12 (2.19)	.034 (.003)	6395.95 (2066.20) †
Ctrl/Sed	37.07 (1.30) ‡	35.03 (2.67)	.034 (.003)	6284.27 (1877.30) ‡

<sup>1</sup> by <sup>35</sup>SO<sub>4</sub> incorporation on cartilage; \* p < 0.05 when compared to Ctrl/Sed; † p < 0.05 when compared to Sup/Ex; ‡ p < 0.05 when compared to Ctrl/Ex; § p < 0.05 when compared to Sup/Sed.



**Figure 1. Plasma amino acid and ammonia concentration in the experimental animals.** A = Branched Chain Amino Acids (valine, isoleucine, leucine); B = aromatic amino acids (tyrosine, phenylalanine, tryptophan); C = neutral and sulfur amino acids (alanine, methionine, histidine, lysine); D = ammonia. \* =

Ctrl/Ex and Sup/Sed. In this case, plasma glucose concentration was similar for Sup/Ex and Ctrl/Sed. Regarding FFA (Table 1), the exercised groups (Sup/Ex and Ctrl/Ex), irrespective of their diets, had lower plasma FFA levels than the sedentary groups (Sup/Sed and Ctrl/Sed).

**Table 5. Blood glucose and FFA. Values expressed as mean ( $\pm$ standard deviation).**

Groups	Glucose (mg.dL <sup>-1</sup> )	FFA (mEq.L <sup>-1</sup> )
Sup/Ex	113 (14) §	.21 (.06)
Ctrl/Ex	105 (8)	.37 (.09)
Sup/Sed	101 (19)	.93 (.10) †
Ctrl/Sed	117 (13) ‡ §	.94 (.20) ‡

† p < 0.05 when compared to Sup/Ex; ‡ p < 0.05 when compared to Ctrl/Ex; § p < 0.05 when compared to Sup/Sed.

Plasma amino acid concentrations are shown in Figure 1 and were also measured 24 h after the last workout session. Tyrosine, alanine and leucine concentrations were increased in the Ctrl/Sed group, compared to the Sup/Sed group.

## Discussion

This study investigated whether BCAA supplementation had any beneficial effects on body growth, long bone and muscle growth in long-term exercise trained young male rats.

The main findings were as follows: physical exercise showed to be responsible for an impressive reduction of proteoglycan synthesis in cartilage tissue, and BCAA supplementation was capable of slightly attenuating this

reduction. In addition, BCAA supplementation seemed to enhance glycogen stores in the liver, but not in the muscle. Exercise and BCAA supplementation together resulted in higher muscle total RNA and reduced muscle protein content compared to sedentary supplemented rats.

Food intake was lower in trained groups than in sedentary groups, especially among supplemented animals. This fact supports what has been observed in some studies, which have stated that exercise has an anorexic effect (Eckel and Moore, 2004). Also, authors such as Flier (2006) have demonstrated that BCAA, especially leucine, can act on central receptors and signal anorexigen peptide synthesis, probably via mTOR. This could help explaining our results.

Nutrition and physical activity are known environmental factors that can influence body growth. Dietary protein has to be highlighted, as rats fed a 20% protein diet for 7 days had increased body growth, muscle growth, epiphyseal cartilage width and epiphysis length compared to a 5% protein diet group. Indeed, it is known that body growth and long bone growth are correlated (Yahya et al., 1994). According to these authors, in an unfavorable metabolic situation such as a low protein diet, the body tends to preserve long bone growth, although muscle and cartilage growth are compromised first.

Our study showed that body growth has been compromised in response to physical training despite the fact that protein intake and long bone growth were preserved. These findings were in accordance with our previous study (Ribeiro et al., 2010)

The groups submitted to physical training showed

higher cartilage weight. Nevertheless, to our surprise, sulphate incorporation, an indirect marker of proteoglycan synthesis (Yahya et al., 1994) was extremely reduced in those trained groups.

The importance of measuring proteoglycan synthesis relies on the fact that these molecules, together with a variety of others, are components of the extracellular matrix. The ECM turnover is influenced by physical activity. Both transcriptional and posttranslational modifications, as well as local and systemic release of growth factors, are enhanced following exercise (Kjaer, 2004).

On the other hand skeletal muscle is extremely sensitive to body growth, as it is the body's greatest compartment of fat-free mass including protein. Thus, bone and muscle growth are intimately coupled. Endocrine and environmental stimuli allow bone growth, which stimulates muscle stretching in a passive way. Consequently, protein synthesis increases and growth occurs (Ribeiro and Tirapegui, 1996).

Cartilage weight, however, augmented with physical activity. As a nutritional marker, in our experimental conditions, we expected that it would be reduced in trained groups such as protein deficient rats in other studies (Yahya et al., 1994). This can mean that other mechanical factors (load) might be taking place, as further indicated.

In our study, proteoglycan synthesis was measured 24h after the last training session in order to analyze the chronic effects of regular physical training and BCAA supplementation. One of the limitations of this determination is that no analyzes were performed at the beginning of training (before the first week).

It is known that acute exercise performed by adult rats, especially exercise characterized by eccentric contraction, increases collagen synthesis, which is a component of the extracellular matrix, both in tendons and muscle (Heinemeier et al., 2007). However, in very young animals, the effect may be different. Van Weeren et al. (2008) working with foals aged 10 days to 18 months, observed that they featured a reduction in glucosaminoglycan and augmentation collagen synthesis in the proximal articular surface of the proximal phalanx of the right hind limb when submitted to a 30% workload increase, while at the same time, posttranslational modifications of collagen were augmented. This author concluded that increased exercise in these very young animals contributed to advanced maturation of the cartilage extracellular matrix. This hypothesis could explain why in our case trained rats featured heavier cartilage with reduced proteoglycan synthesis.

From the comparison between trained groups (Sup/Ex and Ctrl/Ex), we can observe higher values of sulfate incorporation in Sup/Ex animals. The existence of proteoglycan leucine-rich molecules is known, and they have been studied not only for their role on bone and cartilage growth, but also for their role in many metabolic functions (Schaefer and Iozzo, 2008). From these observations, we can previously hypothesize that BCAA supplementation could be important to induce, at least in part, a higher production of proteoglycans in cartilage of young trained animals; thus neutralizing the effects of early cartilage maturation that seems to occur with exercise

training at very young ages.

One of the limitations of this study is that collagen synthesis and other cartilage functional determinations were not measured, since this work aimed on more general growth parameters. This can also be a direction for future investigations.

Regarding glycogen synthesis, BCAA supplementation was much more effective in increasing glycogen reserves in the liver than in the muscle, considering that the animals were in 24h fasting and in resting state, and this is especially interesting if one considers that the BCAA dose in this study was not very high (45 mg/body weight/day). Our data support what others have observed with histidine (Tipton and Wolfe, 1998), glutamine, alanine and proline supplementation (Aoyama et al., 1993) and BCAA supplementation (Araujo et al., 2006). In the liver, several important pathways play a role in glucose homeostasis by maintaining a balance between glucose uptake and storage that is determined by gluconeogenesis and glycolysis, as well as glucose release that is regulated by glycogenolysis and gluconeogenesis (Baquet et al., 1990). Some key enzyme sites regulate the interaction between glycolytic and gluconeogenic pathways, especially during fasting (e.g., glucose 1,6 diphosphate/fructose 1,6 bisphosphatase). Morifuji et al. (2005) trained and supplemented adult rats with whey proteins, which has plenty of BCAAs, and observed a higher activation of fructose 1,6 bisphosphatase and a lower activation of glucose-6-phosphatase in the liver, resulting in a significant increase in liver glycogen storage probably due to the glucose-alanine cycle.

Regarding muscle protein concentration and total RNA, trained supplemented rats were found to have lower protein levels than sedentary supplemented animals, while the opposite was observed for RNA concentrations. This effect on muscle RNA levels was not observed in trained rats that received a 21% protein diet (Ribeiro et al., 2010) and hence is especially due to BCAA supplementation.

Our results indicate that despite having a higher capacity for synthesizing protein in skeletal muscle, BCAA supplementation did not result in higher muscle protein concentration in trained rats. This is in accordance with another study, in which oral administration of 270 mg leucine to rats enhanced phosphorylation of the eukaryotic initiation factor (eIF) (4F-BP1), but no differences in protein synthesis were found in the liver, which led them to conclude that the translation of mRNA is disjointed from the rates of total protein synthesis but is related to the degree of SK61 phosphorylation (Anthony et al., 2001). Tipton & Sharp (2005) demonstrated that the addition of insulin or BCAA to perfused muscle increased polypeptide initiation and the protein synthesis ratio, concluding that the acute responses of protein synthesis to various types of low activity exercise in slow fiber muscles seemed to be under translational control, while pretranslational control was more evident in chronic alterations caused by muscle activity.

In our experimental protocol of moderate intensity physical activity, all metabolic parameters were measured in order to characterize the chronic effects of exercise, which might highlight the final mechanisms of protein synthesis. In addition to the effects of training *per se*,

some authors have suggested that there is either an enhancing effect on the synthesis or an inhibitory effect on the proteolysis caused by ingestion of either BCAA or leucine alone (Zanchi et al., 2008; Norton and Layman, 2006).

According to our results, BCAA supplementation increased the availability of plasma glucose when accompanied by a systematic training protocol. As it is known, both glycolysis and oxidative phosphorylation provide the energy bone and cartilage cells need; and recent evidence suggests that other amines (creatine) also play an energetic role in these tissues (Gualano et al., 2010). Thus we can consider the possibility that BCAA in this case may have contributed to an increased energy generation in this site due to their capacity of ATPs generation from their carbon skeletons.

On the other hand, a lower plasma FFA level was observed in trained rats (Sup/Ex and Ctrl/Ex) compared to sedentary animals (Sup/Sed and Ctrl/Sed). This parameter results from both the release of FFA from adipose tissue and liver as well as FFA uptake by muscle, indicating that exercise has enhanced these metabolic reactions, as expected.

Plasma tyrosine, alanine and leucine levels presented significant changes after intervention. Plasma tyrosine concentration was significantly lower in Sup/Sed animals than in the Ctrl/Sed group. Plasma alanine levels were higher in Sup/Ex than in Sup/Sed, while leucine levels were higher in the latter group (Sup/Sed) compared to Ctrl/Sed. Some interesting trends can be observed. First, in the Sup/Sed group, BCAA supplementation reduced the plasma concentration of tyrosine, an essential aromatic amino acid, which is used to indicate protein catabolism, since it is not generated or utilized by any other oxidation pathway (Libby and Goldberg, 1978).

Another relevant aspect, regarding the plasma amino acid profile, is the reduction of alanine in the Sup/Sed group compared to Sup/Ex and Ctrl/Sed groups. The role of alanine in the glucose-alanine cycle as an energy source for skeletal muscle has been well established (Pascoe and Gladden, 1996). In the present study, alanine concentrations were reduced in the Sup/Sed group compared to the other groups, indicating that alanine is less available for glucose synthesis. In the same group, plasma glucose was reduced compared to Ctrl/Sed. Considering the trained rats regardless of their diet, plasma alanine levels were the same, although a reduction in this parameter would be expected in the supplemented groups (Sup/Sed and Sup/Ex). This result suggests that BCAA supplementation during exercise may optimize alanine use to maintain plasma glucose levels and reduce proteolysis caused by an unfavorable metabolic situation for optimal growth.

## Conclusion

From our results, we can conclude that BCAA supplementation did not have major effects on improving growth on trained rats, but features mild results in enhancing liver glycogen deposition, reducing proteolysis and attenuating proteoglycan synthesis reduction associated to physical training.

More studies are necessary to identify the mechanisms underlying cartilage maturation, body growth and BCAA supplementation associated to exercise.

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### Key points

- Cartilage proteoglycan synthesis was dramatically reduced in trained animals as a whole.
- BCAA supplementation augmented liver glycogen stores and reduced proteolysis in our experimental conditions
- Trained animals receiving BCAA supplementation featured increased proteoglycan synthesis compared to sedentary ones, probably because BCAA may have attenuated the negative effects of exercise on cartilage development.
- BCAA supplementation was not capable of neutralizing directly the negative effects of long-term physical training and lower food intake in young male rats on body growth

### AUTHORS BIOGRAPHY

#### Patricia Lopes de CAMPOS-FERRAZ

##### Employment

Postdoctoral Fellow at University of Sao Paulo

##### Degree

PhD

##### Research interests

Protein metabolism, obesity and exercise.

**E-mail:** patfcamp@yahoo.com.br

#### Sandra Maria Lima RIBEIRO

##### Employment

Professor at University of Sao Paulo, School of Arts, Science and Humanities.

##### Degree

PhD

##### Research interests

Nutrition, aging and exercise.

**E-mail:** smlribeiro@usp.br

#### Silmara dos Santos LUZ

##### Employment

Applied Human Nutrition at the University of Sao Paulo.

##### Degree

PhD

##### Research interests

Nutrition, exercise and eating disorders, protein metabolism and growth.

**E-mail:** silmaral@yahoo.com

#### Antonio Herbert LANCH A Jr.

##### Employment

Professor at School of Physical Education and Sport of the University of Sao Paulo.

##### Degree

PhD

##### Research interests

Protein metabolism, amino acid supplementation and exercise.

**E-mail:** lanchajr@usp.br

#### Julio TIRAPEGUI

##### Employment

Professor at Faculty of Pharmaceutical Sciences of the University of São Paulo.

##### Degree

PhD

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**Research interests**

Protein metabolism and exercise.

**E-mail:** [tirapegu@usp.br](mailto:tirapegu@usp.br)

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**✉ Dr. Julio Tirapegui**

Faculdade de Ciências Farmacêuticas – USP., R. Prof. Lineu Prestes, 580, Bloco 14. Cid. Universitaria. São Paulo, SP, 05508-900 São Paulo - Brazil.