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ORIGINAL ARTICLE

Effects of supplementation with L-glutamine and L-alanine in the body composition of rats submitted to resistance exercise

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KEYWORDS
Glutamine;
Alanine;
Resistance exercise;
Body composition

Abstract We investigated the effects of glutamine and alanine supplementation on body composition of rats submitted to resistance exercise. Wistar rats were submitted to eight-week of resistance exercise, which consisted of climbing a ladder with progressive loads (25–100% of body weight). In the last 21 days of training, animals were supplemented with L-glutamine and L-alanine, as a dipeptide or in their free form (DIP, GLN + ALA and ALA groups), or water (SED and CTRL groups). RE attenuated body weight gain and lipid contents of CTRL group ($p < 0.05$ vs. SED) and DIP supplementation promoted an increase in tibialis muscle weight, as well as in protein content ($p < 0.05$ vs. CTRL). Taken together, our data indicated that resistance exercise improves body composition and dipeptide potentiated the muscle hypertrophic effect.

PALAVRAS-CHAVE
Glutamina;
Alanina;
Exercício resistido;
Composição corporal

Efeitos da suplementação com L-glutamina e L-alanina na composição corporal de ratos submetidos ao exercício resistido

Resumo Foram investigados os efeitos da suplementação com glutamina e alanina na composição corporal de ratos submetidos a exercício resistido. Ratos Wistar foram submetidos, durante oito semanas, ao exercício resistido, que consistia em subir uma escada com cargas progressivas (25 a 100% do peso corporal). Nos últimos 21 dias de treinamento, os animais foram suplementados com L-glutamina e L-alanina, como dipeptídeo ou em sua forma livre (DIP, GLN + ALA ALA e grupos) ou água (grupos SED e CTRL). Exercício resistido atenuou o ganho de peso corporal e conteúdo lipídico do CTRL ($p < 0,05$ vs. SED) e o DIP promoveu aumento no peso do músculo tibial, bem como no

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teor de proteína (p < 0,05 vs. CTRL). Os nossos dados indicam que o exercício resistido, melhora a composição corporal e dipeptídeo potencializa o efeito hipertrófico muscular.

PALABRAS CLAVE
Glutamina;
Alanina;
Ejercicio de resistencia;
Composición corporal

Efectos de la suplementación con L-glutamina y L-alanina en la composición corporal de ratones sometidos a ejercicio de resistencia

Resumen Se investigaron los efectos de la glutamina y la alanina en la composición corporal de ratones sometidos a ejercicio de resistencia. Algunos ratones Wistar fueron sometidos a 8 semanas de ejercicio de resistencia, que consistía en subir una escalera con cargas progresivas (del 25 al 100% de la masa corporal). En los últimos 21 días, los animales recibieron un suplemento de L-glutamina y L-alanina, en forma de dipéptido o en su forma libre (grupos DIP, GLN + ALA y ALA) o agua (grupos SED y CTRL). El ejercicio de resistencia redujo el aumento de masa corporal y la concentración de lípidos del CTRL (p <0,05 vs. SED). La suplementación con DIP promovió un aumento de peso del músculo tibial, así como en el contenido de proteína (p < 0,05 frente a CTRL). Nuestros resultados indican que el ejercicio de resistencia mejora la composición corporal y el DIP potencia el efecto hipertrófico.

Introduction

Body composition is influenced by genetic factors, physical activity, nutrition, diseases and others. Changes in body composition, such as raising in fat and reduction of muscle tissue, are associated with the development of pathologies (Miller et al., 2013). Fat accumulation, characteristic in overweight and obesity, increases the risk of chronic diseases, such as diabetes, hypertension and cardiopathies. Similarly, decreased levels of muscle mass interfere negatively in the resting metabolic rate, reduce physical capacity and life quality (Daly et al., 2014).

The sedentary lifestyle and high calorie food intake contribute to the increase in body fat, through positive energy balance (Ekelund et al., 2014) and disturbance in homeostasis of hormones involved in appetite and weight regulation (Stiegler and Cunliffe, 2006). Muscle mass gain is compromised in a sedentary lifestyle due reduced stimulation of muscle protein synthesis (Shahar et al., 2013). Similarly, low-calorie diets promote reduction in muscle mass, indicating the importance of balanced diets for weight reduction programs (Miller et al., 2013). Dietary programs and physical exercise are the main interventions used for prevention and treatment of metabolic disorders related to body composition disturbance (Kreider et al., 2010).

Studies demonstrate the effectiveness of aerobic exercise in reducing body weight and adiposity (Sillanpaa et al., 2009; Aadland et al., 2014; Miller et al., 2014). However, there are evidences that resistance exercise promotes similar effects (Arnarson et al., 2014; Villanueva et al., 2014; Young et al., 2014), and the main hypothesis is related to an increase in muscle mass, increase in resting metabolic rate and energy expenditure (Stiegler and Cunliffe, 2006), reduction in appetite and energy consumption (Rogero et al., 2005), as well increased lipid oxidation after an exercise session (De Feo, 2013).

Muscle hypertrophy, induced by resistance exercise, is promoted by myofibrils replication into the muscle fibers. This mechanism promotes growth of the fibers and increase in the production of muscle strength (Villanueva et al., 2014). The protein synthesis and muscle hypertrophy are enhanced by supplemental protein and essential amino acids. These nutrients provide substrates for anabolic reactions and repair after a physical exercise session. Hence, amino acids supplementation is a very popular alternative (Hartman et al., 2007).

Glutamine, the most abundant amino acid in the body, is a potentially useful supplement for athletes (Cruzat et al., 2010). Besides its important role for immune function, glutamine has been related to hypertrophy. The hypothesis that glutamine optimize protein synthesis and hypertrophy induced by exercise is based on the increase in cell volume (Kreider et al., 2010), as well as through optimizing the energy stores, preventing proteolysis (Fontana et al., 2003) and modulation of the immune system, reducing the release of cytokines, which can impair muscle mass gain (Cruzat et al., 2010). However, the effectiveness of the hypertrophic potential of glutamine is not well elucidated (Kreider et al., 2010).

Oral or enteral supplementation with glutamine has reduced influence on glutaminemia and tissue glutamine, due to the high metabolism of this nutrient in the intestine. In this sense, administration with dipeptide L-alanyl L-glutamine has been considered an alternative to improve glutamine absorption through to intestinal oligopeptide transporter (Pept-1), which is more efficient in transport dipeptide than free amino acids (Cruzat et al., 2014).

The aim of this study was to investigate changes in body composition of rats submitted to resistance training and chronic supplementation with glutamine and alanine in their free form or as dipeptide. We hypothesized that the synergy

between the interventions could improve the body composition, reducing lipid content, increasing fat-free mass as well as muscle protein content.

Materials and methods

Animals

Forty adult male Wistar rats were provided by the animal house of the University of São Paulo for use in this study. Animals were housed three per cage in a control environment at 22 ± 2 °C and relative air humidity of 55 ± 10%, under a 12-h light/12-h dark cycle (lights on 4 PM, lights off 4 AM) for a period of eight weeks. Animals were distributed into five groups: sedentary (SED) and trained control (CTRL); trained and supplemented with L-alanine (ALA), L-glutamine plus L-alanine in their free form (GLN + ALA), and the dipeptide L-alanyl-L-glutamine (DIP). Rats had free access to water and standard chow (NUVILAB CR1, Nuvital Nutrients, Curitiba, Brazil). Food intake and body weight were measured three times per week and water intake was registered daily. All procedures were approved by the Ethics Committee on Animal Use of the University of São Paulo (protocol: CEUA/FCF/428).

Resistance exercise protocol

The exercise protocol has been adapted from Hornberger and Farrar (2004) and Scheffer et al. (2012). Physical training was performed during eight weeks and consisted of climbing a vertical ladder (1.1 m × 0.18 m, 2 cm grid, 80° inclined) with weight affixed to the base of rat tail. Sets consisted of eight ladder climbs and rest of 2 min. This procedure was repeated once at the first two weeks (adaptation period), with load equal to 5% of body weight (BW). After the adaptation period, the exercise training started with load of 25% of BW, with three sets, during two weeks. Subsequent exercise sessions were increased to four sets (50% of BW) for one week, five sets (75% of BW) for one and a half week and, in the last sessions, animals performed six sets carrying 100% of BW. Each day of exercise was considered one session, conducted every 48 h (Fig. 1).

Supplementation

Supplements were administered in the last 21 days of experiment, diluted to 4% in drinking water and provided *ad libitum*. The supplement intake was daily assessed. Amino acids amount was calculated based in commercial dipeptide concentration (Dipeptiven® solution consists of 20 mg of L-alanyl-L-glutamine dissolved in 100 mL of water, which equals 8.2 g of L-alanine and 13.46 g of L-glutamine). Free L-glutamine and free L-alanine were manufactured and supplied by Labsynth (Synth, São Paulo, SP, Brazil) and L-alanyl-L-glutamine was manufactured by Fresenius Kabi S.A. (Bad Homburg, HE, Germany).

Tissue measurements

Rats were sacrificed by decapitation 1 h after the last session of resistance exercise. Epididymal adipose tissue and tibialis muscle were surgically excised after sacrifice, weighted and aliquots were stored at −80 °C for further analysis. Muscle samples (1 g) were homogenized in 2 mL of lysis buffer and protein extraction containing 20 mM Tris–HCl (pH 7.4), 150 mM NaCl, 2% Nonidet P-40, 1 mM EDTA (pH 8.00), 10% glycerol, 20 mM sodium fluoride, 30 mM sodium pyrophosphate, 0.2% SDS, 0.5% sodium deoxycholate and ultrapure water. Was added to the buffer 1 mM PMSF, manufactured by Sigma–Aldrich.

For homogenization was used electric type homogenizer Polytron (IKA T10 basic), keeping samples chilled to reduce the activity of proteolytic enzymes and phosphatases. After this process, the samples were centrifuged for 15 min at 14,000 rpm at −4 °C temperature. The supernatant was diluted ten times in demineralized water and homogenized by vortexing.

Protein content was quantified in tibialis skeletal muscle using BCA Protein Assay kit (Thermo Fisher Scientific, Massachusetts, USA, BCA Protein Assay Kit). Briefly, 10 μL of each standard curve point and 10 μL of diluted sample were pipetted in triplicate in a 96 wells plate, then 200 μL of working solution was added into each well. The plate was incubated at 37 °C for 30 min and absorbance was measured in 450 nm by Microplate Reader 340–750 nm UV/vis (Biorad Benchmark – California, EUA).

Body fat

According to analytical standards of the Adolfo Luts Institute (1985), after drying of carcasses, the lipid fraction was extracted with ethyl ether for 48 h in extractor of soxhlet (Infratec Multi TE188). After extraction, the material remained in the incubator at 105 °C for an hour and then

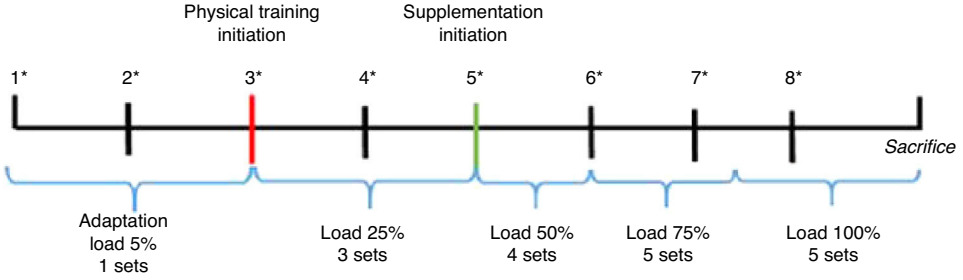

Figure 1 Physical training protocol and experimental design.

Table 1 Food intake, body weight gain, body fat and percentage of epididymal adipose tissue in rats submitted to resistance exercise and supplementation with L-glutamine and L-alanine.

	SED	CTRL	ALA	GLN + ALA	DIP
Food intake (g/day)	62.08 ± 4.39	54.13 ± 1.63[a]	54.00 ± 1.56	53.96 ± 2.29	54.13 ± 0.91
Body weight gain (%)	43.48 ± 2.47	35.89 ± 1.14[a]	35.91 ± 1.88	35.94 ± 1.70	36.98 ± 0.85
Body fat (%)	24.83 ± 2.28	14.53 ± 2.41[a]	19.21 ± 2.48	18.86 ± 2.70	16.41 ± 2.93
Epididymal adipose tissue (%)	1.34 ± 0.19	0.94 ± 0.20[a]	0.69 ± 0.18	1.00 ± 0.08	0.97 ± 0.13

SED, sedentary group received water; CTRL, control group received water; ALA, supplemented with L-alanine; GLN + ALA, supplemented with L-alanine plus L-glutamine; DIP, supplemented with L-alanyl-L-glutamine. Supplements were diluted in drinking water in a 4% solution and offered *ad libitum* in the last 21 days of the experiment. Eight-week exercise protocol consisted of climbing a ladder with progressive load increase. The weight of the epididymal adipose tissue was determined by the percentage of total body weight. Data are presented as mean ± SDM ($n = 8$ per group).

[a] $p < 0.05$ vs. SED (*t* test).

was cooled in the desiccator prior to final weighing. The result was obtained by calculating:

$$\text{Calculation}: \left(\frac{B-A}{C}\right) \times 100 = \%\,\text{Lipids}$$

A is the weight of the empty balloon; *B* is the weight of the balloon with sample; *C* is the Carcass weight.

Statistical analysis

To test normality was used Kolmogorov–Smirnov test. To parametric data was used Student's *T*-test for comparison between SED and CTRL groups and One-way ANOVA, with post-test Tukey HSD (Honestly Significant Differences), was used to compare CTRL and supplemented groups. Mann–Whitney test and One-way ANOVA followed by Kruskal–Wallis were performed to analyze nonparametric data. Differences with p values <0.05 were considered statistically significant. Analyses were performed using Graph Pad Prism 5.0 and data were expressed as mean ± standard deviation of the mean (SDM).

Results

Food intake and body weight

Food intake was statistically higher in sedentary rats (62.08 ± 4.39 g/day) compared to animals submitted to resistance training (54.13 ± 1.63 g/day). Resistance exercise promoted reduced body weight in CTRL group (35.89 ± 1.14%) compared to sedentary (43.48 ± 2.47%) ($p < 0.05$ vs. CTRL). The differences between SED and CTRL groups, concerning food intake and body weight gain, consist of 15% and 21%, respectively. These data support the efficacy of progressive resistance training in reducing food intake and body weight gain. However, supplements did not influence these parameters (Table 1).

Body fat

As showed in Table 1, resistance exercise was able to reduce the lipid content of trained animals. Both body fat and weight of epididymal adipose tissue were higher in sedentary animals (24.83 ± 2.28% and 1.34 ± 0.19%, respectively), compared to trained group (14.53 ± 2.41% and 0.94 ± 0.20%) ($p < 0.05$ vs. SED). These values represent difference of 71% and 43% among sedentary and trained groups, concerning body fat and weight of epididymal adipose tissue, respectively. No difference was found among groups supplemented with amino acids.

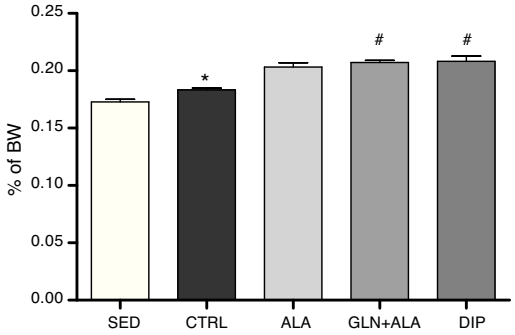

Figure 2 Percentage of tibialis muscle weight relative to the total final Wistar rats weight submitted to resistance training, except SED group. Animals supplemented for 21 days with 4% solution, containing dipeptide (DIP), L-glutamine and L-alanine in theirs free forms (GLN + ALA) or L-alanine (ALA). SED and CTRL received filtered water. Data are presented as mean ± SDM ($n = 8$ per group). * $p < 0.05$ vs. SED (*t* test); # $p < 0.05$ vs. CTRL (ANOVA, Tukey's HSD).

Muscle mass gain and intramuscular protein

Muscle protein was improved with both interventions. Weight of tibialis muscle (Fig. 2) and intramuscular protein (Fig. 3) were increased about 6% and 41%, respectively, in trained groups, compared to sedentary ($p < 0.05$ vs. CTRL), demonstrating hypertrophic effects of physical training on skeletal muscle. Weight of tibialis was elevated in GLN + ALA and DIP groups compared to CTRL (Fig. 2), and intramuscular protein was improved with dipeptide supplementation (Fig. 3). Therefore, dipeptide supplementation was able to improve protein synthesis and muscle mass gain, which suggests an improvement of the anabolic effect induced by progressive resistance exercise.

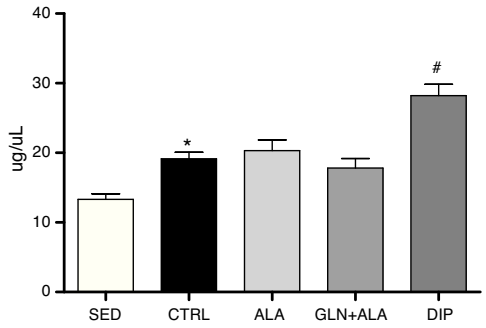

Figure 3 Intramuscular protein in tibialis muscle of Wistar rats submitted to resistance training, except SED group. Animals were supplemented for 21 days with 4% solution, containing dipeptide (DIP), L-glutamine and L-alanine in theirs free forms (GLN + ALA) or L-alanine (ALA). SED and CTRL received filtered water. Data are presented as mean ± SDM ($n = 8$ per group). *$p < 0.05$ vs. SED (t test); $^{\#}$ $p < 0.05$ vs. CTRL, ALA and GLN + ALA (ANOVA, Tukey's HSD).

Discussion

Progressive resistance exercise reduced food consumption, body weight gain and lipid content in trained rats, confirming the effectiveness to change the body composition. Interestingly, L-glutamine administration in the free form along with L-alanine or as dipeptide improved the RE effects enhancing tibialis muscle weight in trained animals. Similar effect was observed in intramuscular protein content, which was enhanced by progressive resistance exercise, however improved only by dipeptide administration.

Intense physical exercises are known to reduce appetite and food intake (Kreher and Schwartz, 2012) by increasing cytokines release and activation of hypothalamic nuclei (Rogero et al., 2005). The decrease in energy consumption and increase in energy expenditure promoted by physical exercise originate the negative energy balance state (Ekelund et al., 2014; Stiegler and Cunliffe, 2006). The main contributing factor of total energy expenditure is the exercise, corresponding to 30–40%. Muscle mass increase induces high energy demands and contributes to the energy balance and increased metabolic rate, consequently reducing fat mass (Stiegler and Cunliffe, 2006).

The lipid contents can be modified by the resistance exercise through various mechanisms. Subsequent to an exercise session, lipid oxidation is optimized as substrate to provide energy (De Feo, 2013). The optimization of metabolic rate, energy expenditure and reduced food consumption are also responsible for increased use of lipids as energy substrate (Stiegler and Cunliffe, 2006; Kreher and Schwartz, 2012). Furthermore, studies indicate that resistance exercise promotes changes in the profile of plasmatic lipoproteins and these findings were consistent with the reduction of adipose tissue and improvement of body composition (Arnarson et al., 2014).

Resistance exercises can promote muscle hypertrophy, which is understood as increasing in the thickness of the fibres, both due to the myofibrils accumulation, by the contractile proteins (actin and myosin), as for accumulation of non-contractile structures, such as water and glycogen (Bucci et al., 2005). The muscle hypertrophy caused by exercise occurs when there are successive periods of positive muscle protein balance, which indicates protein synthesis higher than degradation (Hartman et al., 2007). Myofibrils replication within the muscle fibres, resulting from the hypertrophy, promotes growth of fibres and increase in production of muscle strength (Villanueva et al., 2014).

In this study, there was an increase of protein synthesis, evidenced by higher levels of intramuscular protein in animals subjected to progressive resistance exercise and supplemented with dipeptide. This result, combined with the increase of the weight of muscle tissue, also higher in the exercising and supplemented group (GLN + ALA and DIP), corresponds to the aforementioned hypertrophy concept.

Arnarson et al. (2014) subjected individuals to resistance exercise and found increased body weight, due to the increased muscle mass, as well decrease of approximately 1% of body fat (Arnarson et al., 2014). The results of body weight differ from the present study, possibly due to intensity of the applied exercise, since exhaustive exercise reduces appetite, food intake and, therefore, body weight (Rogero et al., 2005).

Aadland et al. (2014) subjected individuals to predominantly aerobic exercises. There was reduction of body weight and body fat of 10% and 16%, respectively. However, the authors also observed reduction in muscle mass (Aadland et al., 2014). Contrary to aforementioned studies, Young et al. (2014) submitted rowers to sessions of resistance and aerobic exercise. There was reduction of body weight and body fat, as well as an increase in muscle mass (Young et al., 2014).

As a result of the benefits promoted by aerobic and anaerobic exercises, physical training covering both types can provide better results on body composition parameters, regarding the reduction in body fat through aerobic exercise, and potent elevation of the muscle mass, due to resistance exercise (Young et al., 2014). However, the exercise protocol applied in this study was effective in promoting improvement in body composition, especially due to its intensity, which allowed both body fat reduction and muscle mass increase. Muscle hypertrophy caused by exercise is enhanced by supplemental protein and amino acids, since it provides the substrates needed for anabolic reactions (Hartman et al., 2007). In addition, protein intake contributes to the positive protein balance and inhibits protein degradation (Villanueva et al., 2014).

Glutamine supplementation in exercise is based on its role in the immune and inflammatory systems, and related to repair of muscle injuries (Cruzat and Tirapegui, 2009). This amino acid is linked to optimization of protein synthesis and muscle hypertrophy by different mechanisms. The use of glutamine in the process of gluconeogenesis and maintenance of glycogen levels prevents protein degradation for energy purposes. The release of cytokines and acid–base imbalance after exercise contribute to muscle proteolysis, however, they are attenuated through supplementation with glutamine, whereas this amino acid is associated with the homeostasis of the immune system and carrying of ammonia (Fontana et al., 2003). Finally, the cellular transport of glutamine is dependent upon sodium (Na+), which results in water absorption and increased cellular volume. The increased hydration state promotes increased resistance of

the cell and is an anabolic signal for hypertrophy (Cruzat et al., 2010).

Candow et al. (2001) subjected young adults to glutamine supplementation and resistance exercise, however, different from the results presented in this study, supplementation did not attenuate muscle protein degradation, and there was no significant difference in *performance* and body composition (Candow et al., 2001). These findings can be explained by the supplementation protocol used, since the free glutamine administration has reduced effects on glutaminemia and tissue glutamine.

Supplementation with glutamine through dipeptide L-alanyl L-glutamine or a solution containing these amino acids in their free form, as applied in the present study, have been prioritized, since its absorption is more efficient than absorption of independent amino acids in free form (Cruzat et al., 2014). Previous studies corroborate this finding (Cruzat and Tirapegui, 2009, 2010; Petry et al., 2014). In this study, we observed a higher influence of supplementation with dipeptide L-alanyl L-glutamine on muscle protein, compared to the groups supplemented with a solution containing L-glutamine and L-alanine in their free forms.

Conclusion

We conclude that eight weeks of progressive resistance training resulted in improvements on body composition evidenced by reduction of body weight and body fat, as well as enhanced intramuscular protein content and increased muscle mass. Supplementation with the dipeptide L-alanyl-L-glutamine was able to optimize the hypertrophic effect promoted by exercise.

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Conflicts of interest

The authors declare no conflicts of interest.

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