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ORIGINAL ARTICLE

Glutamine supplementation influences the secretory apparatus in the right atrial cardiomyocytes of resistance trained aged rats



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KEYWORDS

Atrial muscle cells;
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Abstract We investigated the effects of glutamine supplementation on the secretory apparatus of natriuretic peptides in atrial cardiomyocytes of aged rats undergoing resistance training. Two groups of resistance-trained rats were studied: resistance trained, and resistance trained and supplemented with glutamine group. Both groups of rats were trained to climb a 1.1 m vertical ladder with weights tied to their tail. The cardiomyocytes from resistance trained and supplemented rats showed increased density and sectional area of natriuretic peptides granules, higher relative volumes of the mitochondria, endoplasmic reticulum, Golgi complex and nuclear euchromatin, and nuclear pore density compared with resistance trained rats. In conclusion, glutamine supplementation caused hypertrophy of the secretory apparatus in the cardiomyocytes of aged rats undergoing resistance training.

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PALAVRAS-CHAVE

Células musculares do
átrio;
Suplementação de
aminoácidos;
Treinamento
anaeróbio;
Peptídeo natriurético

A suplementação de glutamina influencia o aparelho secretor dos cardiomiócitos do átrio direito de ratos idosos submetidos a treinamento de resistência

Resumo Foi investigado o efeito da suplementação de glutamina no aparelho secretor de peptídeos natriuréticos dos cardiomiócitos do átrio de ratos idosos submetidos a treinamento de resistência. Foram estudados dois grupos: grupo de treinamento de resistência e grupo de treinamento de resistência suplementado com glutamina. Os ratos de ambos os grupos escalaram uma escada vertical de 1,1 m com pesos progressivamente maiores atrelados à cauda.

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PALABRAS CLAVE

Células del músculo del atrio;
Suplementación de aminoácidos;
Entrenamiento anaeróbico;
Péptidos natriuréticos

Os resultados mostraram que os cardiomiócitos de ratos do grupo treinado e suplementado apresentaram maior densidade e maior área de seção de grânulos de peptídeos natriuréticos, maiores volumes relativos de mitocôndrias, retículo endoplasmático, complexo de Golgi e eucromatina nuclear e maior densidade de poros nucleares em comparação com ratos do grupo de treinamento de resistência. Em conclusão, a suplementação com glutamina causou hipertrofia do aparelho secretor dos cardiomiócitos de ratos idosos submetidos ao treinamento de resistência.

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La suplementación de glutamina influye en el aparato secretor de los cardiomiocitos de la aurícula derecha de ratones viejos sometidos a entrenamiento de resistencia

Resumen Se investigó la influencia de la suplementación de glutamina en el aparato secretor de péptidos natriuréticos de cardiomiocitos auriculares de ratones viejos sometidos a entrenamiento de resistencia. Se formaron dos grupos: grupo de entrenamiento de resistencia y grupo de entrenamiento de resistencia suplementado con glutamina. Los ratones escalaron una escalera vertical de 1,1 m con pesos atados a la cola. Los resultados mostraron que los cardiomiocitos de ratones del grupo de entrenamiento de resistencia suplementado presentaron mayor densidad y área de sección de gránulos de péptidos natriuréticos, mayores volúmenes relativos de mitocondrias y de eucromatina nuclear y mayor densidad de poros nucleares en comparación con los ratones del grupo de entrenamiento de resistencia. En conclusión, la suplementación de glutamina causó hipertrofia del aparato secretor en los cardiomiocitos de ratones viejos sometidos al entrenamiento de resistencia.

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Introduction

It is well known that feeding athletes before and/or immediately after training with nutritional supplements such as creatine, L-carnitine and L-glutamine positively affects strength development and resistance training recovery (Raastad et al., 2000; Williams et al., 2002; Volek and Rawson, 2002; Rawson and Volek, 2003; Volek, 2004; Kraemer et al., 2007; Bent et al., 2011). Among nutritional supplements, glutamine is one of the most used by athletes (Novelli et al., 2007). Glutamine supplementation increases muscle cell volume and stimulates protein and glycogen synthesis (Varnier et al., 1995; Low et al., 1996; Antonio and Street, 1999; Fontana et al., 2003; Gleeson, 2008; Coqueiro et al., 2017).

Atrial muscle cells (cardiomyocytes) produce, store, and secrete natriuretic peptide hormones (NP). NP plays an important role in the normal regulation of arterial blood pressure (Debold, 1999; Daniels and Maisel, 2007; Casserly, 2010; De Vito, 2014). Previous studies showed that specific atrium granules contain NP pro-hormones (O'Donnell et al., 2003). The production and secretion of NPs into the bloodstream depends on the structural components of the cardiomyocyte, named the secretory apparatus of the cardiomyocyte (Maksimov et al., 2004; De Souza et al., 2014).

Previous studies have shown that the function of the secretory apparatus of cardiomyocytes is influenced by several factors such as fatty acids, hypertension, protein deprivation, training, hormones, hydrogen peroxide and glutamine (Jing et al., 2000; Maksimov et al., 2004; Gama et al., 2007; Pan, 2008; Firmes et al., 2012; De Vito et al., 2013; Ferro et al., 2013; De Souza et al., 2014; De Souza et al., 2015).

The purpose of the present study was to extend previous findings in two important ways. First, it is known that during aerobic training, blood pressure rises, which causes an increase in the secretion of NPs in the bloodstream (Marie et al., 2004). A previous study of young animals demonstrated that glutamine significantly enhances cardiac ANP, thus implicating the beneficial effects of glutamine supplementation to the ANP system (De Souza et al., 2015). However, there are no studies of NP system, where aged animals undergoing resistance training and supplemented with glutamine are compared with non-supplemented trained controls. The first objective of the present study was to observe the influence of glutamine supplementation on the effects of resistance training on the number and size of NP granules of aged rats.

The second objective of this work was to evaluate the effects of glutamine supplementation on the secretory apparatus of the atrial cardiomyocytes of resistance trained rats, including the number of pores per 10 µm of nuclear

membrane, the relative volumes (%) of euchromatin, mitochondria, endoplasmic reticulum and Golgi complex. We hypothesize that supplementation could enhance the effects of resistance training by promoting hypertrophy of the secretory apparatus in cardiomyocytes and influencing positively the synthesis of NPs.

Materials and methods

Sixteen aged male Wistar rats (15 months old) were obtained from the Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil. Rats were maintained at 23 °C under a cycle of 12 h light/12 h darkness. At the end of the 15th month, rats were randomly assigned to the resistance trained group (RT, $n=8$) or resistance trained supplemented with glutamine (RTG, $n=8$) group. An aqueous solution of L-glutamine was given to rats from the RTG group by gavage (1 g kg^{-1} body weight in 3 mL saline) 1 h before the training session according to [Shewchuk et al. \(1997\)](#) and [Lagranha et al. \(2004\)](#). Glutamine solution was freshly prepared before administration to avoid glutamine hydrolysis. Rats from RT group received 3 mL of saline (0.1 mol/L citrate, pH 4.5) as placebo. The University Ethics Committee approved handling of animals (Ethical Protocol number 015/2006), in accordance with the International Guiding Principles for Biomedical Research involving Animals.

Training protocol

All animals underwent a pre-adaptation to the training protocol and equipment for five days. The equipment used to carry out the strength-training program with rats was a vertical ladder made of wood with iron steps. The equipment (ladder) height is 110 cm (43.3 inches) with an inclination angle of 80°. On the top of the equipment, there is a plastic box lined with newspaper for the rats' accommodation in the interval between sets ([Campbell et al., 1998](#); [Hornberger and Farrar, 2004](#)). The training protocol was progressive, and the load was adjusted every week. The load was composed of lead weights attached to the rats' tails with a Velcro tape. Animals were supposed to climb the ladder to reach the resting area at the top, which was considered one repetition. The adaptation process lasted one week with six repetitions every day. The training of animals included six continuous repetitions per day, with a rest interval of 45 s between repetitions, five days a week for 12 weeks. The load increase was established from Heyward's proposal ([Heyward, 1998](#)). After measurement of the maximum weight lifted (1 maximum carried load test), the training load was set at 60% of 1 maximum carried load test. As the load was related to the weight of animals, every week all animals were weighed, and their loads were adjusted according to their body weight.

Body weight and Systolic Blood Pressure measurement

At the moment of the sacrifice, (i.e., 18 months of age) rats were anesthetized (ketamine-xylazine 80:40 mg/kg i.p.), weighed, and blood pressure was evaluated by indirect measurement using the tail-cuff method ([Britto et al., 1997](#)).

Determination of the number and sizes of NP granules, and the relative volumes of the endoplasmic reticulum, mitochondria, and Golgi complex

Under anesthesia, trained animals were heparinized prior to fixation to optimize perfusion-fixation. The right and left atria were perfused through the left and right ventricles at a constant pressure of 80 mmHg, using 0.1 M cacodylate buffer (3 min) followed by 2.5% glutaraldehyde solution diluted in the same buffer. Next, the right atrium was isolated and divided into slices approximately 3 mm wide and 5 mm long. These tissue slices were post-fixed in osmium tetroxide in sodium cacodylate buffer for 1 h. The tissue was dehydrated in graded alcohols and embedded in Epon resin, and sectioned so that myocytes were cut in longitudinal section. Thin sections for transmission electron microscopy were stained with uranyl acetate, and lead citrate ([Mifune et al., 2004](#)). Two randomly chosen blocks from each atrium, in which the myocytes were sectioned longitudinally were used for quantitative analysis. The ultrathin sections were placed on a copper grid and randomly chosen fields were selected for micrographs taken with a Jeol transmission electron microscope.

Ten electron micrographs per animal were taken, then chosen by systematic random sampling of squares at a final magnification of 15,000 \times , and were used to obtain the following: the number of granules per 96 μm^2 , the area of granules (μm^2) and the relative volume of mitochondria, endoplasmic reticulum, and Golgi complex. The number of granules present in each field was determined and its area measured using an image analysis program (Axio Vision, Zeiss).

Determination of the number of pores in the nuclear membrane

Ten electron micrographs per rat, examined with a final magnification of 15,000 \times were used to determine the number of pores per 10 μm of nuclear membrane.

Calculation of the relative volumes

The relative volume of a structure corresponds to the area occupied by the structure presented as a %. It was obtained using a test system equipped with 110 points (considered as 100%), which was allocated on each electronic image where the points for each component were counted ([Mandarim-De-Lacerda, 2003](#)) (Fig. 1).

The following formula was used to obtain the relative volumes: $VV_{[\text{struct}]} = \Sigma P_{[\text{struct}]} 100 / PT$; where $VV_{[\text{struct}]}$ = relative volume, $\Sigma P_{[\text{struct}]}$ = number of points that hit the structure in question, and PT = total number of points (110) of the test system ([Gundersen, 1986](#); [Mayhew and Lucocq, 2008](#)). The measurements were performed using the image analysis program, Axio Vision (Zeiss, 2009).

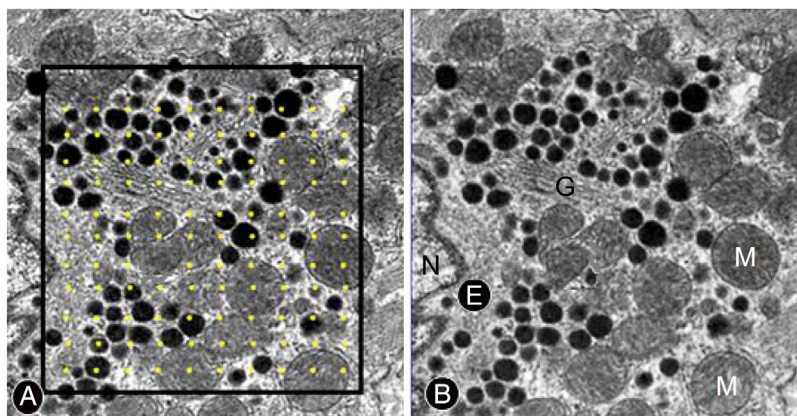


Figure 1 Shows the test system (A) equipped with 110 points (considered as 100%), which was allocated on the electronic image where the points for each component were counted (B). N, nucleus; M, mitochondria; ER, endoplasmic reticulum; G, Golgi complex.

Table 1 Body weight and systolic blood pressure in the two studied group of rats after 12-wk of training.

Parameters	RT	RTG	<i>p</i> -Value
BW (g)	408 ± 42	412 ± 38	>0.05
SBP (mmHg)	112 ± 10	115 ± 14	>0.05

Data are expressed as means ± SE.

Student's *t* test was used for the statistical analysis.

BW, body weight; SBP, systolic blood pressure.

Statistical analysis

All results are means ± SE. Data from the two groups were compared using unpaired Student's *t*-test with $p < 0.05$ as the level of significance. The GraphPad Prism 5 program (Graph Pad Software, San Diego, CA, USA) for Windows was used for data analysis.

Results

The body weight and the mean systolic blood pressure measured at the end of the experiment at rest showed no significant differences between groups ($p > 0.05$) (Table 1).

One repetition maximum

Fig. 2 shows an increase in the absolute values of weight lifted by the trained group that were obtained during the repetition maximum test. RT and RTG rats had similar values for repetition maximum at the beginning of the experiment (day 0). After 6 weeks, the load lifted by rats in the RT group was 19% higher than the load lifted in the first test, and that lifted by rats in the RTG group was 22% higher than that in the first test ($p < 0.05$). At the end of 12 weeks, the load lifted by RT rats was 24% higher than that in the first test, and that lifted by rats in the RTG group was 26% higher than that in the first test ($p < 0.05$). In summary, at all stages of training, the load lifting capacity was always higher in the RTG group than in the RT group.

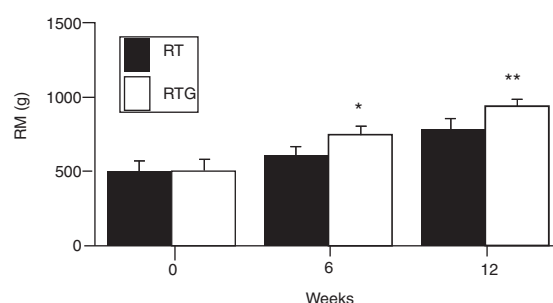


Figure 2 Values for 1 repetition maximum test. Results are mean ± SD. * $p < 0.05$, compared with the RT group at 6 mo. ** $p < 0.05$, compared with the RT group at 12 mo. Groups were compared using one-way ANOVA and Student's *t* test.

Number and sizes of NP granules

Cytoplasmic secretory granules were predominantly arranged in groups among the mitochondria (Fig. 3) and near the endoplasmic reticulum and Golgi complex (Fig. 4). The number of NP granules/cardiomyocyte was significantly higher in RTG rats (64 ± 3) compared to RT rats (53 ± 4) ($p < 0.05$). The number and size (areas) of granules was significantly higher in RTG rats (0.09 ± 0.01) μm^2 compared to RT rats (0.13 ± 0.02) ($p < 0.05$) (Fig. 5).

Relative volume of Golgi complex, mitochondria, and endoplasmic reticulum

RTG rats showed higher relative volumes of the Golgi complex, mitochondria, and endoplasmic reticulum (%) compared with the RT group (in all cases, $p < 0.05$) (Table 2).

Number of pores in the nuclear membrane

In the two groups, the euchromatin appeared disperse, whereas the heterochromatin appeared as a peripheral band near the nuclear membrane (Fig. 4). The ultrastructural sections showed the nuclear membrane as two ultrathin parallel membranes showing pores as spaces where the inner and outer nuclear membranes join (Fig. 4). The density of pores

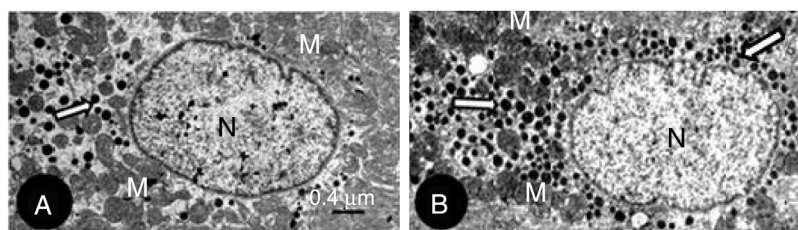


Figure 3 Electron micrographs of left atrium cardiomyocytes in RT (A) and RTG (B) rats. In the RTG rat (B) more granules are observed in the cytoplasm than in the RT. N, nucleus; M, mitochondria.

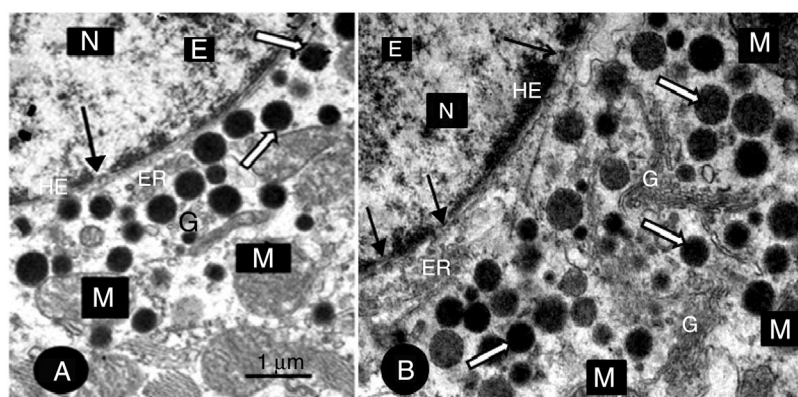


Figure 4 Electron micrographs of left atria cardiomyocytes in the RT (A) and RTG (B) rats observed with higher magnification. NP granules with varied sizes (white arrows) are dispersed among mitochondria (M), endoplasmic reticulum (ER) and Golgi complex (G). The nucleus (N) shows the euchromatin disperse (E) and the heterochromatin (HE) as a band near the nuclear membrane. The nuclear membrane appears as two thin membranes showing the nuclear pores (black arrows).

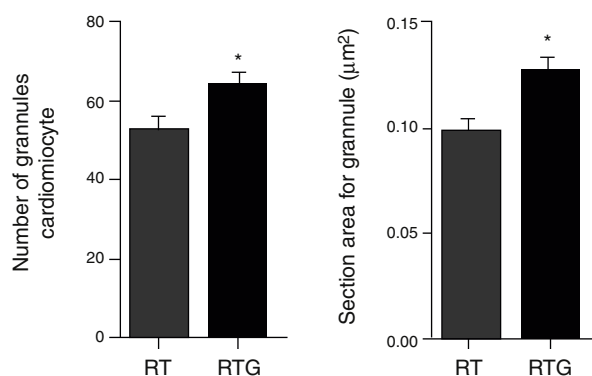


Figure 5 Mean data showing the effects of glutamine supplementation on the number (A) and size (B) of NP granules in RA cardiomyocytes of rats. RT, resistance trained group; RTG, resistance trained and supplemented with glutamine group. * $p < 0.05$ vs. RT. Values are means \pm SD. Student's *t*-test was used for statistical analysis.

in the nuclear membrane was significantly higher in the RTG group compared to the RT group ($p < 0.05$) (Table 2).

Discussion

There are two major findings in the present study. First, the NP levels in the atrial cardiomyocytes were significantly increased in resistance trained aged animals supplemented with glutamine compared to the trained non-supplemented

Table 2 Morphometric indexes of the cardiomyocyte nucleus and cytoplasm in the right atrial cardiomyocytes of RT and RTG rats.

Morphometric indexes	RT	RTG	<i>p</i> -Value
RVGC (%)	2.8 \pm 0.2	3.9 \pm 0.2 ^a	0.05
RVM (%)	25 \pm 4	32 \pm 2 ^a	0.05
RVER (%)	69 \pm 5	78 \pm 3 ^a	0.05
Npo/10 μ m NM	4.6 \pm 0.2	6 \pm 0.2 ^a	0.05

RT, resistance trained group; RTG, resistance trained and supplemented with glutamine group; RVGC, relative volume of Golgi complex; RVM, relative volume of mitochondria; RVER, relative volume of endoplasmic reticulum; NPo/10 μ m NM, number of pores/10 μ m of nuclear membrane.

^a $p < 0.05$ vs. RT rats.

group, which indicates that glutamine enhances the effects of resistance training on the atrial biosynthetic process. Second, resistance trained aged rats supplemented with glutamine exhibited a significant increase in relative volumes for components of the secretory apparatus in RA cardiomyocytes compared to resistance-trained controls. Presented data suggest that glutamine supplementation induced a distinct pattern of the secretory apparatus.

In the present study, resistance trained aged animals supplemented with glutamine showed a significant improvement in the number and size of cardiac NP granules when compared to the RT group. The mechanism by which glutamine associated with resistance training significantly

increased NP levels in cardiomyocytes is unknown. It is known that glutamine is the most abundant amino acid in plasma, as well as in muscle, and accounts for more than 60% of the total intramuscular free amino acid pool (Antonio and Street, 1999; Kreider, 1999; Gleeson, 2008). It is also a precursor for the synthesis of proteins, nucleotides, and many other biological molecules (Smith, 1990; Watford, 2000; Curl et al., 2005). Studies in skeletal muscle showed that glutamine supplementation contributes as a substrate for gluconeogenesis by resulting in increased cell components (Antonio and Street, 1999; Fontana et al., 2003; Coqueiro et al., 2017). It is possible that the same mechanism could be responsible for the results of the present study.

In addition, another mechanism for enhanced cardiomyocyte production of NPs observed in supplemented trained rats might relate to oxytocin. During resistance training, there is an increase in blood pressure that promotes the atrium wall distention and causes cardiomyocytes to secrete NP into the plasma, decreasing NP levels in cardiomyocytes (Schiebinger and Greening, 1992; Seul et al., 1992; Ohba et al., 2001; Chien et al., 2008). After training, as cardiac oxytocin receptor of cardiomyocytes is coupled with NP release (Gutkowska et al., 1997), the higher production of NPs may be due to activation of cardiac oxytocin receptor and subsequent improvement of NP synthesis (Gutkowska et al., 2007). Possibly, this mechanism was enhanced by glutamine, which may have influenced the increase of NP production in supplemented rats.

In the present study, resistance trained rats receiving glutamine exhibited the more significant enhancement of components of cardiomyocyte secretory apparatus (mitochondria, endoplasmic reticulum, Golgi complex and number of pores in the nuclear membrane) compared with the RT group, which indicates that glutamine increases the effects of resistance training on the secretory apparatus of NPs in cardiomyocytes. The effect of supplementation with glutamine on the secretory apparatus of cardiomyocytes in trained animals can be explained in two ways. First, resistance training is associated with significant elevations in anabolic hormones. There are four major anabolic hormones that indirectly or directly affect the secretory apparatus and protein synthesis of cardiomyocyte. They are the growth hormone (GH), insulin-like growth factor-1 (IGF-1) and testosterone (Kraemer and Ratamess, 2005; Kraemer et al., 2007), which increase protein synthesis (Herbst and Bhasin, 2004; Olsen et al., 2006). Second, several studies indicate that glutamine supplementation stimulates protein and glycogen synthesis (Varnier et al., 1995; Low et al., 1996; Antonio and Street, 1999). Probably, the effects of glutamine supplementation on the secretory apparatus of NPs in cardiomyocytes of resistance trained animals observed in the present study could be related to these two mechanisms.

Conclusion

In conclusion, the results of the present work indicate that in aged rats with normal arterial pressure undergoing resistance training, glutamine ingestion promoted hypertrophy of atrial cardiomyocytes and intensive synthesis of NPs.

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Conflicts of interest

The authors declare no conflicts of interest.

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