



ORIGINAL ARTICLE

Influence of glutamine on the effect of resistance exercise on cardiac ANP in rats



Romeu Rodrigues de Souza^{a,b,*}, Cleomara Angélica Vieira Caldeira^c,
Patrícia Oliva Carbone^c, Eduardo Victor Pianca^d

^a Department of Anatomy, Universidade São Judas Tadeu, São Paulo, SP, Brazil

^b Postgraduate Program in Anatomy of Domestic and Wild Animals, Faculty of Veterinary Medicine and Zootechny, Universidade de São Paulo, São Paulo, SP, Brazil

^c Universidade São Judas Tadeu, São Paulo, SP, Brazil

^d Department of Anatomy, Centro Universitário Sant'Anna, São Paulo, SP, Brazil

Received 29 August 2011; accepted 7 March 2013

Available online 27 January 2015

KEYWORDS

Glutamine;
Resistance exercise;
ANP;
Rat

Abstract Various nutritional supplements (herbs, vitamins, and micronutrients) improve responses and adaptations to resistance exercise. ANP is a heart hormone that contributes to fluid, electrolyte and blood pressure homeostasis through its natriuretic and vasodilative actions. In the present study, the adaptation of ANP in response to resistance exercise was investigated in rats supplemented with glutamine for five weeks. The results showed that supplementation with glutamine did not influence the number of ANP granules per atrial cardiocyte in sedentary animals. In exercised-trained rats, the number and diameter of the granules was significantly higher in comparison with the control group and in exercised animals supplemented with glutamine there was significant increase in the number and diameter of ANP granules compared with controls. Altogether, these data indicated that in resistance exercise rats, glutamine significantly enhances cardiac ANP thus implicating the beneficial effects of glutamine supplementation to the ANP system.

© 2015 Colégio Brasileiro de Ciências do Esporte. Published by Elsevier Editora Ltda. All rights reserved.

PALAVRAS-CHAVE

Glutamina;
Treinamento resistido;
ANP;
Rato

Influência da glutamina nos efeitos do treinamento resistido no ANP cardíaco em ratos

Resumo Vários suplementos nutricionais (ervas, vitaminas e micronutrientes) melhoram as respostas e adaptações ao exercício resistido. O ANP é um hormônio cardíaco que contribui para a homeostase de líquidos, eletrólitos e controle da pressão arterial através de suas ações natriurética e vasodilatadora. No presente estudo, a adaptação do ANP em resposta ao treinamento resistido foi investigada em ratos suplementados com glutamina durante cinco semanas.

* Corresponding author.

E-mail: souzarrd@uol.com.br (R.R. de Souza).

Os resultados mostraram que a suplementação com glutamina não influenciou o número de grânulos de ANP por cardiomiócito em animais sedentários. Em ratos treinados, o número e o diâmetro dos grânulos foi significativamente maior em comparação com o grupo controle e nos animais treinados e suplementados com glutamina houve um aumento significativo no número e diâmetro dos grânulos de ANP comparativamente com os controles. Em conjunto, estes dados indicam que, nos ratos submetidos ao treinamento resistido, a glutamina aumentou significativamente os níveis de ANP cardíaco, mostrando assim, os efeitos benéficos da suplementação com glutamina para o sistema de ANP.

© 2015 Colégio Brasileiro de Ciências do Esporte. Publicado por Elsevier Editora Ltda. Todos os direitos reservados.

PALABRAS CLAVE

Glutamina;
Entrenamiento
de resistencia;
ANP;
Ratón

Influencia de la glutamina sobre los efectos del entrenamiento de resistencia sobre la ANP cardíaca en ratas

Resumen Varios suplementos nutricionales (hierbas, vitaminas y micronutrientes) mejoran las respuestas y adaptaciones al ejercicio de resistencia. El ANP es una hormona cardíaca que contribuye a la homeostasis de fluidos, electrolitos y de control de la presión arterial a través de sus acciones vasodilatadora y natriurética. En el presente estudio, se investigó la adaptación de la ANP en respuesta al entrenamiento de resistencia en ratones suplementados con glutamina durante cinco semanas. Los resultados mostraron que la suplementación de glutamina no influyó en el número de gránulos de ANP por cardiomiócito en animales sedentarios. En ratas entrenadas, el número y el diámetro de los gránulos fue significativamente mayor en comparación con el grupo control y en los animales entrenados y suplementados con glutamina hubo un aumento significativo en el número y diámetro de los gránulos de ANP en comparación con los controles. En conjunto, estos datos indican que en ratones sometidos a entrenamiento de resistencia, la glutamina aumentó significativamente el nivel de ANP cardíaco mostrando de esta manera los efectos beneficiosos de la administración de glutamina para el sistema de ANP. © 2015 Colégio Brasileiro de Ciências do Esporte. Publicado por Elsevier Editora Ltda. Todos los derechos reservados.

Introduction

Several factors contribute to the beneficial effects of exercise in maintaining cardiovascular homeostasis (Gutkowska et al., 2007; Agarwal, 2012; Rowland and Unnithan, 2013). ANP is a heart hormone that contributes to fluid, electrolyte, and blood pressure homeostasis through its natriuretic and vasodilative actions. It was shown in humans and animals that exercise provokes increased synthesis of ANP thus maintaining adequate levels for the optimal control of blood pressure (Tanaka et al., 1986; Guezennec et al., 1989; Barletta et al., 1998; Ohba et al., 2001; Edwards, 2012; Gutkowska et al., 2007; Wiesner et al., 2010; Endlich et al., 2011).

Because of the critical functions of hormones, researchers have investigated various methods to enhance the exercise–endocrine interaction. For instance, feeding subjects before and/or immediately after resistance exercise alters hormone response (Kraemer and Volek, 1998; Kraemer et al., 2007; Gulli et al., 2012). In addition to feeding, various nutritional supplements (herbs, vitamins, and micronutrients) improve responses and adaptations to resistance exercise. Nutritional supplements such as creatine, L-carnitine and L-glutamine positively affect strength development and resistance exercise recovery (Williams et al., 2002; Kraemer and Volek, 1998; Kraemer

et al., 2007; Rawson and Volek, 2003; Volek and Rawson, 2002; Cermak et al., 2012; Wax et al., 2012). One of these nutritional supplements, glutamine have been widely used by athletes (Mero et al., 2009). However, the effects of this supplement in ANP levels in practitioners of resistance exercise are not known. Therefore, utilizing the rat as an animal model the following study was undertaken to test the hypothesis that glutamine stimulates the cardiac hormone ANP in chronic resistance exercise practitioners.

Materials and methods

Animals

Male Wistar rats weighing 290 ± 20 g (3 months old) were obtained from the Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil. The rats were maintained at 23°C under a cycle of 12 h light/12 h darkness. The animals fed with a standard diet were divided into the following groups of five animals each: sedentary (S), exercised (E), sedentary supplemented with glutamine (SG) and exercised supplemented with glutamine (EG). Non-supplemented rats received 3 ml of saline (0.1 mol/l citrate, pH 4.5) as placebo.

Oral L-glutamine supplementation

An aqueous solution of L-glutamine was given to rats by gavage (1 g kg^{-1} body weight in 3 ml saline) 1 h before the exercise session according to [Shewchuk et al. \(1997\)](#) and [Lagranha et al. \(2004\)](#). Glutamine solution was freshly prepared before administration to avoid glutamine hydrolysis.

Exercise training

Rats in the E and EG groups were trained to climb a 1.1 m vertical (80° incline) ladder with weights tied to their tail ([Hornberger and Farrar, 2004](#)), five days per week, for five weeks. Each training session consisted of six climbs. The weight carried during each session was progressively increased. Over the course of five weeks, the maximal weight carried by the rats was 50% of their body weight. This load was maintained throughout the rest of the period of experiment. The rats in the S and SG groups were placed on a stationary treadmill for 10 min daily. The body weight (BW) was measured at the beginning and at the end of the experiment. Handling of animals was approved by the University Ethics Committee, in accordance with the International Guiding Principles for Biomedical Research involving Animals.

Preparation for electron microscopy

At the end of the experiment, each animal was anesthetized with intraperitoneal Pentobarbital sodium (3 mg/100 g body weight) and then killed. The animals were heparinized prior to fixation to optimize perfusion-fixation. The 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 cacodylate buffer. Next, the heart was isolated and weighed. 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, embedded in Epon resin, and sectioned so that the cardiocytes were cut in longitudinal section. Thin sections for transmission electron microscopy were stained with uranyl acetate, and lead citrate ([Mifune et al., 2004](#)).

Ultrastructural morphometry

Two randomly chosen blocks from each atrium, in which the cardiocytes were cut in longitudinal section were used for quantitative analysis. The ultra thin sections were placed on a copper grid and 10 randomly chosen fields per block were selected for micrographs taken with a Jeol transmission electron microscope. The number and sizes of secretory granules were obtained according to the method of [Cantin et al. \(1979\)](#). Five electron micrographs per animal, chosen by systematic random sampling of squares were taken at a final magnification of $7500\times$ and the number of granules/cardiocyte was determined. Diameters of all granules

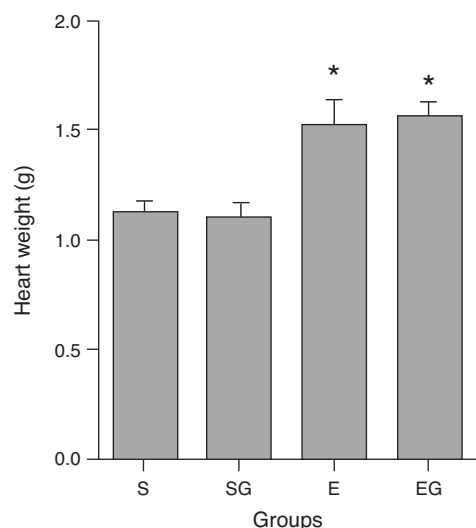


Figure 1 Heart weight of S, SG, E and EG rats. *Significant vs. S and SG.

present in each field were determined in another five micrographs per animal at a final magnification of $15,000\times$. In both cases, a computerized program (Axio Vision, Zeiss) was used.

Statistical analysis

All results are means \pm SE. Data was performed using one-way analysis of variance (ANOVA) and multiple comparison procedure was performed using Tukey's test with $p < 0.05$ as the level of significance.

Results

Heart weight

As shown in [Fig. 1](#), no difference in heart weight was seen between the groups S (1.1 ± 0.2 g) and SG (1.1 ± 0.1 g) and between the groups E (1.5 ± 0.2 g) and EG (1.5 ± 0.1 g). However, in E and EG the heart showed significant hypertrophy compared to S ($p < 0.05$).

Ultrastructure

The ANP granules were mainly located in the perinuclear region and were variable in number ([Fig. 1](#)) and size ([Fig. 2](#)). The number of granules was increased in E and EG compared with S and SG rats ([Figs. 2 and 3](#)).

Ultrastructural morphometry

The number of granules/cardiocyte is shown in [Fig. 4](#) and the diameter of the granules is shown in [Fig. 5](#). The number of granules/cardiocyte was significantly higher in E (62 ± 3) and in EG (72 ± 4) compared to SG (56 ± 4) and S rats (50 ± 3) (in all cases, $p < 0.05$). The number of granules/cardiocyte was significantly higher in EG than in E rats ($p < 0.05$). No significant difference was observed in

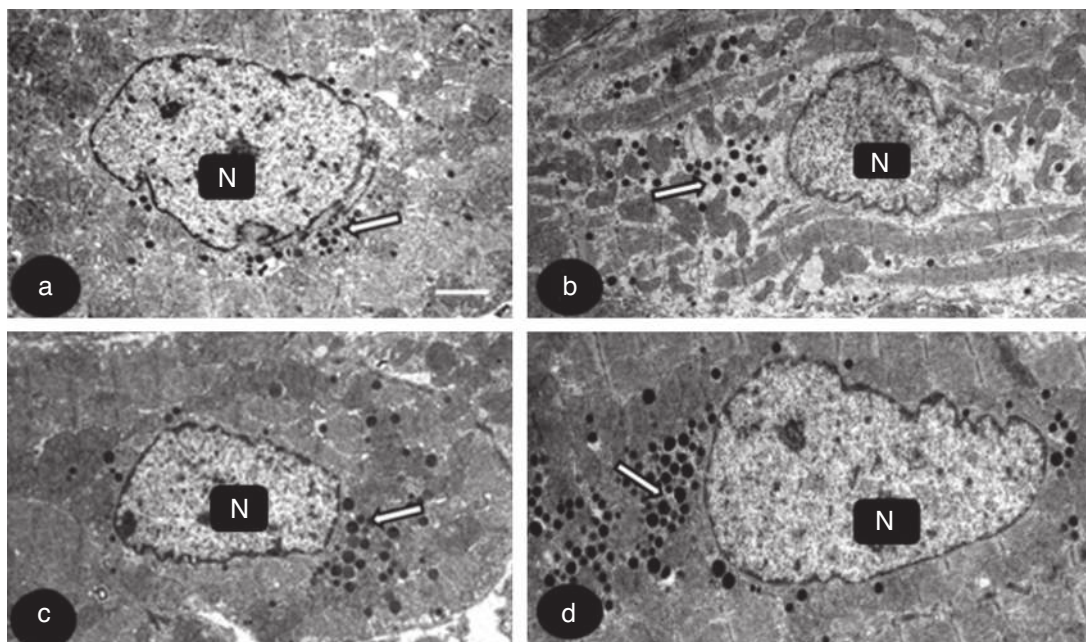


Figure 2 Electron micrographs of the right atrial cardiocytes in S (a), SG (b), E (c) and EG (d) rats. In atrial cardiocytes, ANP-granules (arrows) are variable in number and are mainly located in the perinuclear region. The number of granules in E (c) and EG (d) rats is higher than in S (a) and SG (b). Bar: 2 μ m. N – Nucleus.

the number of granules/cardiocyte between SG and S rats ($p > 0.05$). The diameter of granules was significantly higher in EG (320 ± 24 nm) and E (260 ± 10 nm) rats compared to S rats (212 ± 12 nm) and SG (218 ± 8 nm) rats ($p < 0.05$). The diameter of granules was significantly higher in EG than in E rats ($p < 0.05$).

Discussion

There are three major findings in this work. First, glutamine supplementation for five weeks had no influence on the levels of ANP in atrial cardiocytes. Second, chronic resistance exercise increased significantly the levels of ANP in atrial cardiocytes and third, glutamine enhances the effects of resistance exercise on the levels of ANP in atrial cardiocytes.

Glutamine is an important energy source (nitrogen and carbon) for synthesis of other molecules such as nucleotides, adenosine triphosphate (ATP) and other amino acids (Fontana et al., 2003). Glutamine is also necessary for the absorption of fluids and electrolytes and for the regulation of nitrogen balance (Walsh et al., 1998). In the present study, animals receiving glutamine did not exhibit any significant enhancement of cardiac ANP levels compared with sedentary controls, indicating that glutamine had no effect on the number of ANP granules by cardiocytes.

The present study demonstrated a significant increase in the number of cardiac ANP granules in rats with exercise-training compared to the S group. This result is in agreement with that of Gutkowska et al. (2007) in showing that chronic exercise augmented ANP expression in the right atrium. It is possible that resistance exercise promotes an increase in levels of ANP in cardiomyocytes due to the necessity

of greater release of this hormone to the plasma during exercise. According to Gutkowska et al. (2007) the beneficial effects of exercise may be due, at least in part, to activation of cardiac oxytocin peptide receptors and subsequent enhancement of ANP synthesis and release although a direct effect of exercise on the ANP cannot be excluded. This conclusion emerged from studies showing that activation of cardiac oxytocin receptor is coupled with ANP release (Gutkowska et al., 1997). The physiological action of ANP is conveyed by binding to particulate GC-coupled cell surface functional receptor GC-A which activation promotes the intracellular generation of cGMP (Potter et al., 2006; Gutkowska et al., 2007).

Although several studies have been conducted showing the positive effects of ingesting supplements on resistance exercise (Kerksick et al., 2006) we did not find in literature studies showing the effects of glutamine on the ANP cardiocytes. Glutamine is especially abundant in skeletal muscle tissue. Following intense exercise, intramuscular glutamine decreases and there is an increased uptake of glutamine in the liver, kidney and intestine, to ensure synthesis of glucose and buffering acidosis. Thus, the plasma glutamine concentration decreased significantly, causing the blood amount is not sufficient for uptake and utilization by the various tissues that depend on (Walsh et al., 1998). Glutamine supplementation promotes the maintenance of its plasmatic concentration, improve the hydration of skeletal muscle and contribute as a substrate for gluconeogenesis resulting in increased muscle cell volume (Antonio and Street, 2003; Fontana et al., 2003; Waddell and Fredricks, 2005). Possibly, these effects also occur in heart muscle which may have influenced the increase of the levels of ANP in cardiocytes from rats supplemented with glutamine and submitted to exercise.

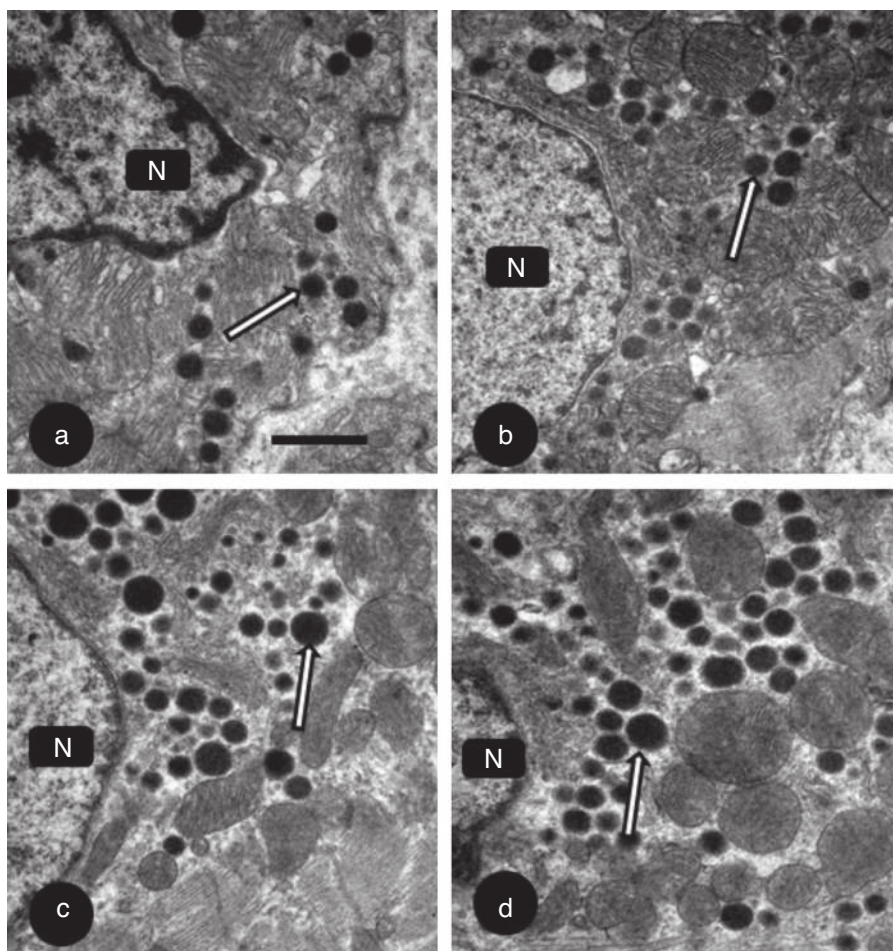


Figure 3 Electron micrographs of the right atrial cardiocytes in S (a), SG (b), E (c) and EG (d) rats. It can be seen that in all groups ANP-granules (arrows) are variable in size. In E and EG rats, the sizes of the granules are higher than in S and SG. Bar: 1 μ m. N – Nucleus.

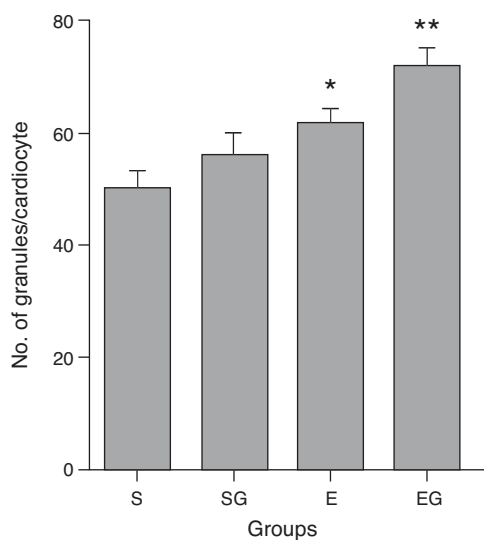


Figure 4 Number of ANP-granules/cardiocyte in the right atria from S, SG, E and EG rats. *Significant vs. S, SG and EG groups ($p < 0.05$). ** Significant vs. S and SG groups ($p < 0.05$).

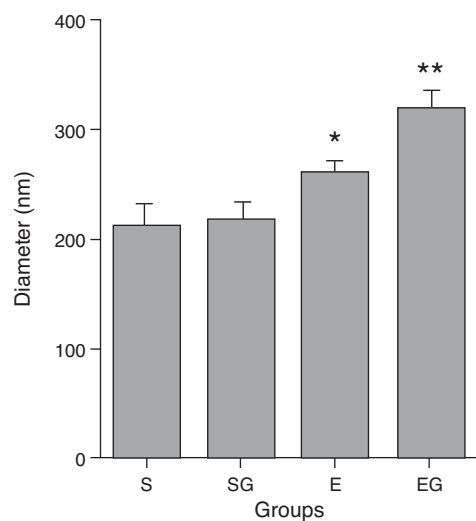


Figure 5 Diameter of ANP-granules (nm) in the right atrial cardiocytes in S, SG, E and EG rats. *Significant vs. S, SG and EG groups ($p < 0.05$). ** Significant vs. S and SG groups ($p < 0.05$).

Conclusion

Glutamine supplementation did not improve ANP production by the cardiocytes but when associated with resistance-training it potentiates the increased cardiac ANP levels promoted by exercise.

Conflicts of interest

The authors declare no conflicts of interest.

References

- Agarwal SK. Cardiovascular benefits of exercise. *Int J Gen Med* 2012;5:541–5.
- Antonio J, Street C. Glutamine: a potentially useful supplement for athletes. *Can J Appl Physiol* 2003;24:1–14.
- Barletta G, Stefani L, Del Bene R, Fronzaroli C, Vecchiarino S, Lazzeri C, et al. Effects of exercise on natriuretic peptides and cardiac function in man. *Int J Cardiol* 1998;65:217–25.
- Cantin M, Timm-Kennedy M, El-Khatib E, Huet M, Yunge L. Ultrastructural cytochemistry of atrial muscle cells: comparative study of specific granules in right and left atrium of various animal species. *Anat Rec* 1979;193:55–70.
- Cermak NM, Res PT, de Groot LC, Saris WH, van Loon LJ. Protein supplementation augments the adaptive response of skeletal muscle to resistance-type exercise training: a meta-analysis. *Am J Clin Nutr* 2012;96:1454–64.
- Edwards JG. Swim training increases ventricular atrial natriuretic factor (ANF) gene expression as an early adaptation to chronic exercise. *Life Sci* 2012;70:2753–68.
- Endlich PW, Firmes LB, Gonçalves WL, Gouvea AS, Moisés MR, Bisoli NS, et al. Involvement of the atrial natriuretic peptide in the reduction of arterial pressure induced by swimming but not by running training in hypertensive rats. *Peptides* 2011;32:1706–12.
- Fontana KE, Valdes H, Valdissera V. Glutamina como suplemento ergogênico. *Rev Bras Cienc Mov* 2003;11:91–6.
- Gulli RA, Tishinsky JM, MacDonald T, Robinson LE, Wright DC, Dyck DJ. Exercise restores insulin, but not adiponectin, response in skeletal muscle of high-fat fed rodents. *Am J Physiol Regul Integr Comp Physiol* 2012;303:R1062–70.
- Gutkoswska J, Jankowski M, Lambert C, Mukaddam-Daher S, Zingg HH, McCann SM. Oxytocin releases atrial natriuretic peptide by combining with oxytocin receptors in the heart. *Proc Natl Acad Sci USA* 1997;94:11704–9.
- Gutkowska J, Paquette A, Wang D, Lavoie JM, Jankowski M. Effect of exercise training on cardiac oxytocin and natriuretic peptide systems in ovariectomized rats. *Am J Physiol Regul Integr Comp Physiol* 2007;293:267–75.
- Guezennec CY, Fournier E, Galen FX, Lartigues M, Louisy F, Gutkowska J. Effects of physical exercise and anti-G suit inflation on atrial natriuretic factor plasma level. *Eur J Appl Physiol* 1989;5:500–7.
- Hornberger TAJ, Farrar RP. Physiological hypertrophy of the FHL muscle following 8 weeks of progressive resistance exercise in the rat. *Can J Appl Physiol* 2004;29:16–31.
- Kerksick CM, Rasmussen CJ, Lancaster SL, Magu B, Smith P, Melton C, et al. The effects of protein and amino acid supplementation on performance and training adaptations during ten weeks of resistance training. *J Strength Cond Res* 2006;20:643–53.
- Kraemer WJ, Volek JS. Hormonal responses to consecutive days of heavy resistance exercise with or without nutritional supplementation. *J Appl Physiol* 1998;85:1544–55.
- Kraemer WJ, Hatfield DL, Spiering BA, Vingren JL, Fragala MS, Ho J, et al. Effects of a multi-nutrient supplement on exercise performance and hormonal responses to resistance exercise. *Eur J Appl Physiol* 2007;101:637–46.
- Lagranha CJ, Senna SM, de Lima TM, Silva E, Doi SQ, Curi R, et al. Beneficial effect of glutamine on exercise-induced apoptosis of rat neutrophils. *Med Sci Sports Exerc* 2004;36:210–7.
- Mero A, Leikas A, Knuutinen J, Hulmi JJ, Kovanen V. Effect of strength training session on plasma amino acid concentration following oral ingestion of leucine, BCAAs or glutamine in men. *Med Sci Sports Exerc* 2009;105:215–23.
- Mifune H, Honda J, Takamori S, Sugiyama F, Yagami K, Suzuki S. A-type natriuretic peptide level in hypertensive transgenic mice. *Exp Anim* 2004;53:11–9.
- Ohba H, Takada H, Musha H, Nagashima J, Mori N, Awaya T, et al. Effects of prolonged strenuous exercise on plasma levels of atrial natriuretic peptide and brain natriuretic peptide in healthy men. *Am Heart J* 2001;141:751–8.
- Potter LR, Abbey-Rosch S, Dickey DM. Natriuretic peptides, their receptors, and cyclic guanosine monophosphate-dependent signaling functions. *Endocr Rev* 2006;27:47–72.
- Rawson ES, Volek JS. Effects of creatine supplementation and resistance training on muscle strength and weightlifting performance. *J Strength Cond Res* 2003;17:822–31.
- Rowland T, Unnithan V. Myocardial inotropic response to progressive exercise in healthy subjects: a review. *Curr Sports Med Rep* 2013;12:93–100.
- Shewchuk LD, Baracos VE, Field CJ. Dietary L-glutamine supplementation reduces the growth of the Morris hepatoma in exercise-trained and sedentary rats. *J Nutr* 1997;127:158–66.
- Tanaka H, Shindo M, Gutkowska J, Kinoshita A, Urata H, Ikeda M, et al. Effect of acute exercise on plasma immunoreactive atrial natriuretic factor. *Life Sci* 1986;39:1685–93.
- Volek JS, Rawson ES. Scientific basis and practical aspects of creatine supplementation for athletes. *Eur J Clin Nutr* 2002;56:585–92.
- Waddell D, Fredricks K. Effects of glutamine supplement on the skeletal muscle contractile force of mice. *Am J Undergr Res* 2005;4:11–8.
- Walsh NP, Blannin AK, Clark AM, Cook L, Robson PJ, Gleeson M, et al. The effects of high-intensity intermittent exercise on the plasma concentrations of glutamine and organic acids. *Eur J Appl Physiol* 1998;77:434–8.
- Wax B, Kavazis AN, Webb HE, Brown SP. Acute L-arginine alpha ketoglutarate supplementation fails to improve muscular performance in resistance trained and untrained men. *J Int Soc Sports Nutr* 2012;9:1–6.
- Wiesner S, Birkenfeld AL, Engeli S, Haufe S, Brechtel L, Wein J, et al. Neurohumoral and metabolic response to exercise in water. *Horm Metab Res* 2010;42:334–9.
- Williams AG, Ismail AN, Sharma A, Jones DA. Effects of resistance exercise volume and nutritional supplementation on anabolic and catabolic hormones. *Eur J Appl Physiol* 2002;86:315–21.