93 - GLUTAMINE SUPPLEMENTATION AND METABOLIC RESPONSES IN TRAINED RATS

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INTRODUCTION

The physiological and biochemical responses resulting from the exercise are well established, both in humans and in animals. Sales et al. (2005) argue that the probable cause of lactate-induced fatigue associates itself with high concentrations of H^+ protons, resulting from the oxidation of glucose and hydrolysis of ATP, with increasing energy demand. This condition promotes the decline of pH, both in the cell as well as in the plasma environment.

The excessive accumulation of lactate occurs when the mitochondrial pathways (Krebs cycle and oxidative phosphorylation) become insufficient to regenerate ATP and energy to meet the demand of the cell. That is, the lactate (LAC) blood increases in the extent that intensity of the exercise increases in humans, which can also be observed in rats (CAMARGO et al., 2006; VOLTARELLI, GOBATTO and MELLO, 2002). Snow et al. (2000) found the same behavior with regard to circulating levels of ammonia in response to prolonged exercise.

The synthesis of ammonia (NH3) also presents its own kinetic and its implications for the muscular fatigue and consequently on the physical performance. Its increase during prolonged exercise is probably related to deamination of nucleotides (AMP) and the catabolism of amino acids (DUARTE, DIAS and MELLO, 2008). With regard to AMP which occurs when the use of ATP exceeds its renewal and involves the action of AMP deaminase (Murray et al., 2003). Meanwhile the catabolism of amino acids provides a source of ATP resynthesis, as well as meet a likely depletion of the reserves of glycogen needed for the demands of prolonged muscle contraction (OWEN, KALHAN and Hanson, 2002; SNOW et al., 2000).

It is established that high levels of ammonia promotes a large consumption of oxoglutarate for the synthesis of glutamate (GRAHAM et al., 1997). This condition reduces the rate of synthesis of ATP, to the central nervous system (CNS), via oxidation of glucose, which can cause, among other diseases, a state of coma (GIBALA et al., 2002). Over the year, the central fatigue can manifest due to toxicity induced by hyperammonaemia, which although reversible, may be harmful to critical regions of the CNS (BANISTER and CAMERON, 1990).

Amino acid conditionally essential, glutamine is an important nutritional supplement among high-level athletes and in various diseases. Considering the possibility of the metabolism of amino acids fill the pool of intermediaries in the Krebs cycle and expanding the glycogen storage (Bruce et al., 2001; GIBALA et al., 2002), it has been suggested that the supplementation with glutamine in rats inhibit the hyperammonemia, thus minimizing the muscular fatigue caused by metabolic stress of exercise.

From this discussion originated the proposal of this study to assess the effects of chronic supplementation of glutamine on the levels of lactate and ammonia plasma in rats subjected to exhaustive aerobic exercise, swimming.

MATERIALS AND METHODS

24 Wistar rats were used, male, adult (6-8 months of age), sedentary, from the Tiradentes University (UNIT) *vivarium* - Aracaju/SE. The separation of animals was carried out at random and divided into four groups of six rats, namely: sedentary (SED), sedentary supplemented with glutamine (GSED), trained (TRE) and trained supplemented with glutamine (GTRE).

The animals remained in collective cages (4 mice per cage) and were fed with commercial diet (Purina ®) for rodents and water *ad libitum* and kept under photo-period, clear and dark cycle of 12 hours in average temperature of 25 ± 2° C. All experiments with animals were conducted with the approval of the Research Ethics Committee of Tiradentes University (registration: 090307).

The animals were supplemented with glutamine (Probiótica Laboratory ®) through probe gavage using a needle probe model of IC-800, during the two weeks of the experimental protocol. The animals trained (GTRE) received supplementation immediately after daily training in swimming until the last day of the trial like Castell, Poortmans and Newsholme (1996).

The glutamine supplementation was calculated with reference to the body mass of the animal so that the total number of amino acid would be the same as it administered alone. For this step, was used to the protocol of Barbosa et al. (2003), they believes that the quantity of glutamine in 2 mL of solution corresponding to 0.5 $g.Kg^{-1}$. The SED and TRE groups not received any kind of supplementation beyond your daily diet.

The training of animals was conducted in a system of swimming in individual tanks with dimensions 1 m x 0,8 m x 0,8 m, with temperature maintained at around 31 ± 2° C. The period of training consisted of three weeks, five days a week, totaling 60 minutes per day during 15 days. In the first week, the animals began an exercise sessions aimed adapting them to the liquid medium that was to keep the animal in contact with shallow water for 20 minutes. The purpose of the adjustment was to reduce the stress of the rat however this not promotes physiological changes resulting from physical training.

The training itself started in the second week which consisted of daily exercises, swimming with overload sets (weights tied to the base of the animal´s tail). The load was calculated based on total mouse body mass up to 1% and 2% in the first and second weeks, respectively, according to Meneguello (2002). The signs of exhaustion was demonstrated when the mouse remained submerged more than 10 seconds, which according to Rogero et al. (2002), it's made like a subjective criterion for stopping the session of exercise in order to avoid the loss of the sample.

At the end of the experiment the animals were submitted to a submaximal bout of exercise, after anesthetized with Thiopentax; 50 mg/kg; via i.p (Cristália Lab ®) to then be held to collect blood by cardiac puncture. For biochemical analysis was used diagnostic kits of Randox Brazil Ltda. in Spectrophotometer. The entire testing procedure was performed at the Laboratory of Biochemistry at the Tiradentes University (UNIT-Se). The data were analyzed by descriptive statistics and non-parametric, through the test H Kruskal-Wallis, assuming a significance level of 5% ($p < 0.05$).

RESULTS AND DISCUSSION

The effect of glutamine supplementation on the level of muscle fatigue was assessed by the response of blood lactate levels ($mmol.L^{-1}$) and ammonia ($\mu mol.L^{-1}$) among the 4 groups of rats distributed as shown in table 1.

Table 1. Values of lactate (LAC) and ammonia (NH_3), rationed after the end of the trial.

	TRE	SED	GTRE	GSED
LAC ($mmol.L^{-1}$)	4,26 ± 0,18	4,42 ± 0,04	4,36 ± 0,08	4,40 ± 0,04
NH_3 ($ymol.L^{-1}$)	26,75 ± 10,24	27,71 ± 8,08	37,69 ± 14,41	44,32 ± 32,11

The experiment sought to evaluate whether chronic supplementation with glutamine carries interference on the levels of blood lactate and ammonia in rats when in aerobic exercise from swimming. It was found that glutamine promoted a modest control over the accumulation of these metabolites. According Carvalho-Peixoto et al. (2007), this increase is due to the unbalance between the release by skeletal muscle and clearance by different tissue as kidneys, liver, lungs and muscle fiber at rest.

The values of LAC and NH_3 showed no significant differences between the four groups confronted each other, p = 0087 (LAC, figure 1) and p = 0282 (NH_3, Figure 2). Nevertheless, levels of LAC demonstrated that the exercise had to be in a condition above the anaerobic threshold. In that sense, Manchado, and Gobatto Contarteze (2005) and Beneke (1995), reported that the level of 4.0 mmol/L is consistent with a proportional relationship between the accumulation of lactate and their removal in response to a given load of exercise. The results found here are related to the findings of Bruce et al. (2001), when they suggest the possibility of energy synthesis via oxidative phosphorylation, has not been stimulated with the supplementation of GLN, probably due to failed delivery of oxygen to the muscle or of acetyl groups to the cycle of tricarboxylic acid.

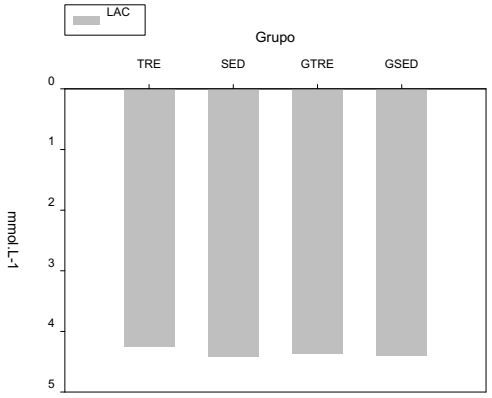


Figure 1. Concentrations of lactate after the effort in each group of rats.

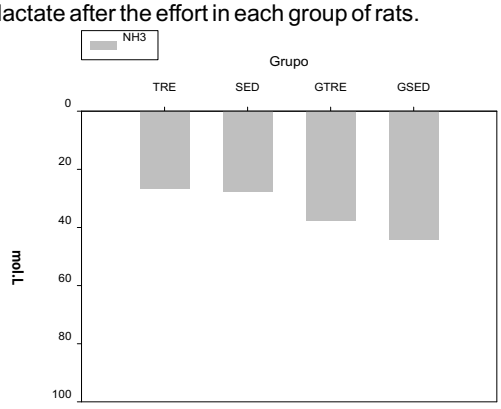


Figure 2. Concentrations of ammonia after the effort in each group of rats.

Regarding the serum levels of ammonia, to see if the results of TRE there are the lowest levels of this metabolite (26,75 $\mu mol.L^{-1}$ ± 10,24) reflecting the close relationship between trainability and concomitant decline of production and release of ammonia. In a study of Graham et al. (1997), to define this behavior, they argue that trained individuals have lower elevation of ammonemia when compared with not trained, while Yuan et al. (2002) found evidence into trained individuals in anaerobic activities. To justify the decline in levels of ammonia, Graham et al. (1997) say that there´s a possibility of reducing the activity of enzymes AMP desaminase, glutamate dehydrogenase and simultaneous increasing of alanine and glutamine.

The mechanisms of muscular fatigue are multifactorial origin, and may occur at level peripheral or central nervous system (CNS). In both situations the metabolic aspects related to exercise have significant influence. Duarte, Dias and Melo (2008) argue that ammonemia induced by exercise enhances the central fatigue due to the protein degradation and deamination of amino acids. The imbalance between the capture and removal of ammonia in the cerebral spinal fluid (CSF) can cause fatigue and affects the metabolism of neurotransmitters (NYBO et al., 2005).

Some studies have highlighted an increase in the production of ammonia, situation that accelerates the fatigue, the muscles due to the supplemental branched-chain amino acids (ARMADA-DA-SILVA and ALVES, 2005; SNOW et al., 2000), recently Favano et al., (2008) reported greater tolerance to fatigue, in football players supplemented with a solution of carbohydrate and glutamine, than when supplemented with only carbohydrate.

The relationship between the supplementation of glutamine and the genesis of metabolic products over the year is very controversial and where researchers like Newsholme et al. (2002) and Stumvoll et al. (1999) that rank it as a major substrate anaplerotic and neoglicogenic, mainly in both skeletal muscle, but also in organs such as liver and kidneys. This fact leads to resynthesis of glycogen and carrying nontoxic of ammonia (ARMADA-DA-SILVA and ALVES, 2005), thus minimizing the central fatigue possibilities (BANISTER and CAMERON, 1990).

However, in contribution to the controversy about this discussion, the present study, unlike established by some authors (Bruce et al. 2001; COSTER Mc CAULEY and FRACS, 2004; FAVANO et al., 2008; NEWSHOLME et al., 2002), emphasizes that the amino acid supplementation GLN enhanced the accumulation of NH3 to the GTRE compared with the TRE as well as the GSED presented elevation in serum levels of ammonia in relation to the SED, although the results showed no statistically significant at p<0, 05.

CONCLUSION

The association of data of this study with the reviewed in the literature allowed the understanding that the levels of lactate and ammonia can be influenced by a range of variables, ranging from the levels of training, hormone levels including the cell capacity to metabolize energy substrates. However, is important to emphasize that the limitations of this study, such as conducting invasive samples collected during the process of training and the use of an anesthetic that provides immediate sedation of animals with minimal interference in the variables blocked measurements of metabolites at the beginning of training program and before the last bout of exercise.

However, the results of this study possible to say that the chronic supplementation of glutamine did not alter the response of lactate in groups trained and sedentary. With regard to ammonia was observed that supplementation of glutamine not provided protection against amonemia in the sedentary group and trained group, which showed higher levels of this metabolite, although they have not shown significant differences.

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GLUTAMINE SUPPLEMENTATION AND METABOLIC RESPONSES IN TRAINED RATS

Abstract

The blood lactate increases the extent that it increases the intensity of the exercise. The synthesis of ammonia also has its own kinetic and its implications for the muscular fatigue and the physical performance. The proposal of this study was to evaluate the effects of chronic supplementation of glutamine on the levels of blood lactate and ammonia in rats submitted to aerobic exercise in swimming. 24 rats were used, male, in four groups, sedentary, sedentary supplemented with glutamine, trained and trained supplemented with glutamine. Having been familiar with the liquid medium and with weights tied to the tail, the animals were subjected to two weeks of training, and five days a week, 60 minutes per day or until exhaustion. The workload ranged in a ratio of 2% to 5% of body mass. On the last day the animals trained until exhaustion then were anesthetized and had their blood collected. The values of lactate and ammonia did not show significant differences when confronted between the four groups. The results of this study say that enable the addition of chronic glutamine did not alter the response of lactate, while for ammonia observed that the glutamine not provided protection against amonemia in GSE and GTRE, which showed higher levels of this metabolite, but have not made significant differences.

Key-words: Glutamine, lactate, Ammonia, aerobic exercise.

COMPLÉTER AVEC GLUTAMINA ET RÉPONSES DANS METABÓLICAS SOURIS FORMATION

Résumé

Le lactate de sang augmente la mesure où elle augmente l'intensité de l'exercice. La synthèse de l'ammoniac a aussi sa propre cinétique et ses conséquences pour la fatigue musculaire et la performance physique. La proposition de cette étude était d'évaluer les effets chroniques de la supplémentation de glutamine sur les niveaux de lactate et d'ammoniaque plasmatique chez les rats soumis à des exercices aérobiques, la natation. 24 rats ont été utilisés, de sexe masculin, en quatre groupes, sédentaire, sédentaire complétée par la glutamine, la formation et la formation complétée par la glutamine. Ayant été familiarisés avec le milieu liquide et avec des poids attachés à la queue, les animaux ont été soumis à deux semaines de formation, et cinq jours par semaine, 60 minutes par jour ou jusqu'à épuisement. Les accusations allaient dans une proportion de 2% à 5% de la masse corporelle. Le dernier jour, les animaux exercitaram jusqu'à épuisement puis ont été anesthésiés et leur sang collecté. Les valeurs de lactate et d'ammoniac n'a pas montré d'importantes différences face entre les quatre groupes. Les résultats de cette étude que dire de permettre l'ajout de glutamine chronique n'a pas modifié la réponse de lactate, alors que pour l'ammoniac a fait observer que la glutamine pas assuré la protection contre amonemia dans l'ESG et GTRE, qui a montré des niveaux plus élevés de ce métabolite, mais n'ont pas apporté d'importantes différences.

Mots-clés: glutamine, lactate, d'ammoniac exercice aérobie.

SUPLEMENTACION CON GLUTAMINA Y RESPUESTAS METABÓLICAS EN RATOS ENTRENADOS

Resumen

El lactato en sangre aumenta en la medida en que aumenta la intensidad del ejercicio. La síntesis de amoníaco también presenta su propia cinética y sus consecuencias para la fatiga muscular y el rendimiento físico. El objetivo de este estudio fue evaluar los efectos de la crónica de la administración de suplementos de glutamina en el plasma los niveles de lactato y amoníaco en ratas sometidas a ejercicio aeróbico de la natación. Se utilizaron 24 ratas Wistar macho en cuatro grupos, el sedentarismo, sedentarismo suplementada con glutamina, entrenados y capacitados suplementada con glutamina. Habiendo sido familiarizados con el medio líquido y con pesos atados a la cola, los animales fueron sometidos a dos semanas de entrenamiento, cinco días a la semana, 60 minutos por día o hasta el agotamiento. Los cargos iban a una tasa del 2% al 5% de masa corporal. En el último día de los animales ejercido hasta el agotamiento luego fueron anestesiados y tienen su sangre. Los valores de lactato y amoníaco no mostró diferencias significativas entre los cuatro grupos se enfrentan. Los resultados de este estudio es posible decir que la crónica de la administración de suplementos de glutamina no alteró la respuesta de lactato, mientras que para el amoníaco se observó que la glutamina no brinda protección contra amonemia en GTRE y GSE, que mostraron mayores niveles de este metabolito, aunque no han mostrado diferencias significativas.

Palabras clave: Glutamina, lactato, amoniaco ejercicio aeróbico.

SUPLEMENTAÇÃO COM GLUTAMINA E RESPOSTAS METABÓLICAS EM RATOS TREINADOS

Resumo

O lactato sangüíneo aumenta na medida em que se aumenta a intensidade do exercício. A síntese de amônia também apresenta sua própria cinética e suas implicações para a fadiga muscular e sobre o desempenho físico. A proposta do presente estudo foi avaliar os efeitos da suplementação crônica de glutamina sobre os níveis de lactato e de amônia plasmáticos em ratos submetidos a exercício aeróbio de natação. Foram utilizados 24 ratos *Wistar*, machos, em quatro grupos, sedentário, sedentário suplementado com glutamina, treinado e treinado suplementado com glutamina. Após terem sido familiarizados com o meio líquido e com pesos atados à cauda, os animais foram submetidos a duas semanas de treinamento, sendo cinco dias por semana, 60 minutos por dia ou até a exaustão. As cargas variaram em uma proporção de 2% a 5% da massa corporal. No último dia os animais exercitaram até a exaustão em seguida foram anestesiados, e tiveram o sangue coletado. Os valores de lactato e amônia não apresentaram diferenças significativas quando confrontados entre os quatro grupos. Os resultados do presente estudo possibilitam afirmar que a suplementação crônica de glutamina não alterou a resposta do lactato, enquanto que relação a amônia observou-se que a glutamina não propiciou proteção contra a amonemia nos GSE e GTRE, os quais apresentaram maiores níveis deste metabólito, embora não tenham apresentado diferenças significativas.

Palavras-chave: Glutamina, Lactato, Amônia Exercício aeróbio.