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Basic nutritional investigation

Effects of oral supplementation with glutamine and alanyl-glutamine on glutamine, glutamate, and glutathione status in trained rats and subjected to long-duration exercise

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Abstract

Objective: We investigated the effect of supplementation with the dipeptide L-alanyl-L-glutamine (DIP) and a solution containing L-glutamine and L-alanine, both in the free form, on the plasma and tissue concentrations of glutamine, glutamate, and glutathione (GSH) in rats subjected to long-duration exercise.

Methods: Rats were subjected to sessions of swim training. Twenty-one days before sacrifice, the animals were supplemented with DIP (1.5 g/kg, $n = 6$), a solution of free L-glutamine (1 g/kg) and free L-alanine (0.61 g/kg; GLN + ALA, $n = 6$), or water (CON, $n = 6$). Animals were sacrificed before (TR, $n = 6$) or after (LD, $n = 6$) long-duration exercise. Plasma concentrations of glutamine, glutamate, glucose, and ammonia and liver and muscle concentrations of glutamine, glutamate, and reduced and oxidized (GSSG) GSH were measured.

Results: Higher concentrations of plasma glutamine were found in the DIP-TR and GLN + ALA-TR groups. The CON-LD group showed hyperammonemia, whereas the DIP-LD and GLN + ALA-LD groups exhibited lower concentrations of ammonia. Higher concentrations of glutamine, glutamate, and GSH/GSSG in the soleus muscle and GSH and GSH/GSSG in the liver were observed in the DIP-TR and GLN + ALA-TR groups. The DIP-LD and GLN + ALA-LD groups exhibited higher concentrations of GSH and GSH/GSSG in the soleus muscle and liver compared with the CON-LD group.

Conclusion: Chronic oral administration of DIP and free GLN + ALA before long-duration exercise represents an effective source of glutamine and glutamate, which may increase muscle and liver stores of GSH and improve the redox state of the cell.

Keywords: Glutamine; Alanine; L-alanyl-L-glutamine; Glutathione; Long-duration exercise

Introduction

The use of glutamine as a nutritional supplement is well known within the field of clinical nutrition. However, interest in sports-related glutamine supplementation appears to have decreased, probably because studies with substantial amounts of exogenous glutamine in athletes did not appear to enhance aspects of immune function or performance [1]. Glutamine is classified as a non-essential amino acid, because it can be synthesized by the body [2]. Further, glutamine is the most abundant free amino acid in the human body [3] and plays an essential role in promoting and maintaining the functions of various organs and cells, including cellular proliferation, acid–base balance, transport of ammonia between tissues, and antioxidant synthesis [2,4,5]. However, studies have demonstrated that in some catabolic situations, such as prolonged starvation, sepsis, and long-duration physical exercise, glutamine deficiency can occur [6–9].

A decreased availability of glutamine may depress the synthesis of several key molecules, such as the tripeptide γ-L-glutamyl-L-cysteinylglycine (GSH), which is involved

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in cellular resistance to lesions, oxidative stress, apoptotic processes, and detoxification of xenobiotics [3,10,11]. Moreover, GSH is the most abundant antioxidant inside the cell, and the most important non-enzymatic antioxidant in the body, reacting directly with the reactive oxygen species as an electron donator for peroxide reduction [3,12]. Experimental evidence has shown that high reactive oxygen species synthesis may promote lipid peroxidation, DNA lesions, and oxidation of essential proteins [12,13].

Supplementation with glutamine may serve as an alternative way to increase bodily stores of GSH and attenuate the oxidative stress that occurs in catabolic situations [14,15]. Administration of oral free L-glutamine to humans or animal models has shown minimal effects in the maintenance of physiologic levels of plasma glutamine [16–21]. The use of glutamine dipeptides such as L-alanyl-L-glutamine (DIP) has provided an alternative non-invasive way to increase the concentration of glutamine [20–22]. The greater efficiency of supplementation rendered by DIP is a result of a more hydrolytically stable molecular structure and mitigated use by enterocytes [23]. Our primary hypothesis was that L-glutamine can attenuate the depression in GSH concentration and this may reduce the oxidative stress induced by long-duration exercise. However, most studies have not tested solutions containing the same amino acids in the same quantities as DIP. Thus, this study investigated the effects of supplementation with DIP and a free L-glutamine/L-alanine (GLN + ALA) solution on plasma and tissue glutamine and glutamate levels and the tissue status of reduced and oxidized GSH (GSSG).

Materials and methods

Animals and diet

Thirty-six adult male Wistar rats with average weight (229 ± 16 g) were provided by the animal house of the University of São Paulo for use in this study. The animals were housed in individual cages in a controlled environment at 22 ± 2°C and relative air humidity of 60 ± 10% under a 12-h light/12-h dark cycle (lights off from 0700 to 1900 h) for a period of 6 wk. All animals were allowed to adapt to the experimental conditions for 1 wk before the beginning of the experimental protocol. Throughout the experiment, animals had free access to water and an ad libitum diet, prepared according to the 1993 recommendations of the American Institute of Nutrition for adult rats (AIN-93) [24]. All animal procedures were approved by the ethics committee on animal experimentation of the University of São Paulo. Weight and food intake were monitored three times per week, and the final weight was determined immediately before sacrifice in all groups. The method of sacrifice was decapitation.

Supplementation

Animals were supplemented with DIP (1.5 g/kg of body weight per day), manufactured by Cláris, Pharmaceutical Products of Brazil Ltd. (São Paulo, Brazil) and distributed by Fórmula Medicinal Ltd. (São Paulo, Brazil), or free L-glutamine (1 g/kg of body weight per day) and free L-alanine (0.67 g/kg of body weight per day; GLN + ALA). Both free amino acids were supplied by Ajinomoto Interamerican Industry and Commerce Ltd. (São Paulo, Brazil). The animals received supplementations through gavage for a period of 21 d before sacrifice. The amount of DIP was calculated such that the total amount of glutamine was the same as that of glutamine administered in its free form (1 g of glutamine/kg of body weight per day). The amount of glutamine was chosen because studies have found effects with this dosage on plasma and tissue glutamine concentration [20,21]. Control (CON) animals received water at the same volume by gavage. Animals were divided into groups of similar mean body weight 1 d before the initiation of nutritional intervention.

Training protocol and long-duration exercise

The training protocol has been described by Rogero et al. [20]. Two days after the last session of training, the animals were subjected to 2 h of exercise, twice as long as the maximum time of the training protocol. The tail weight in the long-duration exercise was the same as that of the last exercise session. All the sessions and long-duration exercise were in the dark cycle. Animals were subdivided into groups TR and LD of similar mean body weight 1 d before the initiation of nutritional intervention. The sacrifice of each animal subjected to long-duration exercise (LD) was concomitant to the sacrifice of an animal of its respective group not subjected to long-duration exercise (TR). To exclude the influence of the food variables, all animals of the TR groups were fasted. All animals were sacrificed between 1040 and 1440 h.

Biochemical analysis

After sacrifice by decapitation, blood was collected and centrifuged for plasma and serum separation, which were stored at −80°C for subsequent determination of glutamine, glutamate, ammonia, and glucose concentrations. Immediately after death, the liver and gastrocnemius and soleus muscles were removed and frozen in liquid nitrogen for subsequent determination of protein, glutamine, glutamate, GSH, and GSSG concentrations.

Plasma glutamine and glutamate concentrations were determined with a commercial kit (Sigma-Aldrich Diagnostics Inc., Saint Louis, MO, USA) as described by Lund [25], and plasma ammonia concentration was measured with a commercial kit (Raichem Diagnostics, San Diego, CA, USA) as described by Neeley and Phillipson [26]. Plasma glucose was measured using a commercial kit obtained from Labtest (São Paulo, Brazil), as described by Bergmeyer [27].

Tissue glutamine and glutamate was extracted as described by Sahlin et al. [28] and was determined with a commercial kit (Sigma-Aldrich Diagnostics Inc.) as described by Lund [25]. Mean values are reported as micromoles of glutamine per gram of fresh tissue and as nanomoles of glutamine per milligram of protein. Muscle and liver protein concentrations were determined according to the method of Lowry et al. [29].

Muscle and liver GSH and GSSG concentrations were determined according to the method of Nogueira et al. [30]. A standard Shimadzu high-performance liquid chromatographic system (Shimadzu Corp.,Toquio, Japan) equipped with a Shim-Pack CLC-NH_2 (6.0 × 150 mm) column (Shimadzu Corp.) was used for analysis, and retention times were determined according to standard curves of GSH and GSSG.

Statistical analyses

Results were subjected to multivariate analysis of variance to track type I errors through an array of univariate tests. Comparisons of nutritional treatment and measuring time were performed in series. One-way analysis of variance was conducted at each of the two time points, with the nutritional treatment as an independent variable. Whenever the analysis of variance resulted in a P value less than 0.05, statistically significant differences were identified by the multiple comparison of Tukey's procedure (honestly significant difference). The comparisons between the time points for each nutritional treatment were conducted with t tests. All tests were processed by SAS 9.1.3 (SAS Institute, Cary, NC, USA).

Results

Food intake (CON-TR 20.9 ± 1.5 g/d, DIP-TR 20.9 ± 0.9 g/d, GLN + ALA-TR 20.5 ± 1.5 g/d, CON-LD 20.1 ± 0.8 g/d, DIP-LD 20.6 ± 1.0 g/d, GLN + ALA-LD 21.0 ± 0.7 g/d) and body weight at the end of the experiment (CON-TR 317.1 ± 16.2 g, DIP-TR 305.0 ± 14.5 g, GLN + ALA-TR 307.1 ± 16.3 g, CON-LD 307.5 ± 14.6 g, DIP-LD 311.1 ± 19.3 g, GLN + ALA-LD 308.2 ± 11.1 g) did not differ across groups. The tail weight across groups subjected to the long-duration exercise session was the same (6% of body weight).

Plasma parameters

Plasma glutamine concentrations were higher in the DIP-TR and GLN + ALA-TR groups compared with the CON-TR group (Table 1). After long-duration exercise, plasma ammonia levels were higher in the CON-LD and DIP-LD groups compared with the CON-TR and DIP-TR groups, respectively (Table 1). The DIP-LD and GLN + ALA-LD groups had lower plasma ammonia concentrations compared with the CON-LD group. There was no significant difference in plasma glutamate and glucose concentrations among groups due to nutritional intervention or long-duration exercise.

Tissue glutamine and glutamate

In the soleus muscle higher glutamine concentration and glutamine/protein were found in the supplemented groups, DIP-TR and GLN + ALA-TR, compared with the CON-TR group (Table 2). In addition, soleus muscle glutamate concentrations were higher in the DIP-TR and GLN + ALA-TR groups compared with the CON-TR. The DIP-TR group had higher glutamine and glutamate concentrations in the gastrocnemius muscle than the CON-TR group (Table 2). In the liver, glutamate concentrations were higher in the DIP-TR group than in the CON-TR group. Supplementation with DIP in the

Table 1
Concentrations of plasma ammonia, glutamine, glutamate, and glucose*

	CON	DIP	GLN + ALA	P
Plasma ammonia (μmol/mL)				
TR	5.79 ± 1.47[a]	4.87 ± 0.78[a]	6.26 ± 2.09[a]	0.314
LD	11.76 ± 0.59[a†]	6.94 ± 0.60[b†]	6.22 ± 0.66[b]	0.001
%	103.09	42.28	−0.64	
Plasma glutamine (mmol/L)				
TR	0.41 ± 0.10[a]	0.61 ± 0.11[b]	0.64 ± 0.16[b]	0.040
LD	0.69 ± 0.38[a]	0.75 ± 0.33[a]	0.60 ± 0.32[a]	0.743
%	66.81	22.95	−6.79	
Plasma glutamate (mmol/L)				
TR	0.29 ± 0.04[a]	0.26 ± 0.05[a]	0.33 ± 0.09[a]	0.193
LD	0.31 ± 0.08[a]	0.28 ± 0.08[a]	0.23 ± 0.06[a†]	0.184
%	7.86	6.00	−32.56	
Plasma glucose (mg/dL)				
TR	96.06 ± 7.61[a]	90.58 ± 8.96[a]	94.66 ± 8.11[a]	0.505
LD	99.87 ± 4.45[a]	92.52 ± 9.25[a]	89.14 ± 6.59[a]	0.011
%	3.97	2.14	−5.83	

CON, control; DIP, dipeptide; GLN + ALA, free L-glutamine and L-alanine; LD, long-duration exercise; TR, trained; %, difference between TR rats and LD rats ([LD − TR]/TR)] × 100

* Results presented as mean ± SD (n = 6 per group). Values in the same row with different letters are significantly different (Tukey's honestly significant difference).

† Significantly different between TR and LD groups ($P < 0.05$, t test).

Table 2
Skeletal muscle and liver concentrations of glutamine and glutamate*

	CON	DIP	GLN + ALA	*P*
Soleus muscle glutamine (μmol/g fresh tissue)				
TR	4.63 ± 0.62[a]	6.37 ± 0.73[b]	8.68 ± 0.77[c]	0.001
LD	4.27 ± 0.48[a]	7.95 ± 0.26[b†]	8.23 ± 0.88[b]	0.001
%	−7.88	29.24	−8.47	
Soleus muscle glutamine (nmol/mg protein)				
TR	28.8 ± 3.8[a]	40.3 ± 1.9[b]	52.1 ± 6.9[c]	0.001
LD	28.4 ± 5.9[a]	46.5 ± 6.5[b]	45.1 ± 6.4[b]	0.001
%	−1.6	15.3	−13.5	
Soleus muscle glutamate (μmol/g fresh tissue)				
TR	1.37 ± 0.06[a]	2.71 ± 0.27[b]	2.57 ± 0.05[b]	0.001
LD	0.95 ± 0.17[a†]	2.38 ± 0.22[b]	2.42 ± 0.45[b]	0.001
%	−31.12	−7.45	−10.62	
Gastrocnemius muscle glutamine (μmol/g fresh tissue)				
TR	2.29 ± 0.38[a]	3.16 ± 0.31[b]	2.67 ± 0.63[ab]	0.018
LD	2.48 ± 0.65[a]	3.34 ± 0.56[b]	3.01 ± 0.44[b]	0.050
%	8.50	5.91	12.53	
Gastrocnemius muscle glutamine (nmol/mg protein)				
TR	16.4 ± 2.8[a]	17.6 ± 7.3[a]	17.1 ± 5.0[a]	0.919
LD	16.9 ± 4.8[a]	20.9 ± 7.3[b]	22.3 ± 4.4[b]	0.050
%	3.6	18.5	30.1	
Gastrocnemius muscle glutamate (μmol/g fresh tissue)				
TR	0.53 ± 0.02[a]	0.74 ± 0.03[b]	0.49 ± 0.03[a]	0.001
LD	0.38 ± 0.04[a†]	0.65 ± 0.06[b†]	0.59 ± 0.01[b†]	0.001
%	−28.64	−20.67	34.76	
Liver glutamine (μmol/g fresh tissue)				
TR	2.84 ± 0.48[a]	3.18 ± 0.56[a]	3.05 ± 0.46[a]	0.519
LD	2.16 ± 0.44[a†]	3.11 ± 0.44[b]	2.17 ± 0.46[a†]	0.003
%	−23.81	−2.25	−28.67	
Liver glutamine (nmol/mg protein)				
TR	17.0 ± 2.9[a]	19.6 ± 4.9[a]	18.7 ± 3.5[a]	0.521
LD	12.3 ± 2.1[a†]	15.6 ± 3.1[a]	12.5 ± 2.1[a†]	0.069
%	−27.5	−20.4	−33.4	
Liver glutamate (μmol/g fresh tissue)				
TR	1.37 ± 0.13[a]	2.18 ± 0.05[b]	1.56 ± 0.18[a]	0.001
LD	1.73 ± 0.18[a†]	1.92 ± 0.11[a†]	1.88 ± 0.08[a†]	0.053
%	−26.47	−11.76	20.55	

CON, control; DIP, dipeptide; GLN + ALA, free L-glutamine and L-alanine; LD, long-duration exercise; TR, trained; %, difference between TR rats and LD rats ([LD − TR]/TR)] × 100

* Results presented as mean ± SD ($n = 6$ per group). Values in the same row with different letters are significantly different (Tukey's honestly significant difference).

† Significantly different between TR and LD groups ($P < 0.05$, *t* test).

DIP-TR group increased the concentration of glutamate in the gastrocnemius and liver compared with the GLN + ALA-TR group (Table 2).

After long-duration exercise, gastrocnemius and soleus muscle glutamine and glutamate concentrations were higher in the DIP-LD and GLN + ALA-LD groups than in the CON-LD group (Table 2). Higher concentrations of glutamine/protein in the gastrocnemius and soleus muscles were found in the DIP-LD and GLN + ALA-LD groups than in the CON-LD group. Lower glutamate concentrations in the gastrocnemius and soleus muscles were found in the CON-LD group compared with the CON-TR group. Long-duration exercise resulted in lower concentrations of glutamate in the gastrocnemius muscle of the CON-LD and DIP-LD groups compared with the CON-TR and DIP-TR, groups, respectively. Glutamine in the liver (milligrams of fresh tissue and nanomoles of glutamine/protein) was lower in the CON-LD and GLN + ALA-LD groups than in the CON-TR and GLN + ALA-TR groups, respectively (Table 2). The DIP-LD group had a higher glutamine concentration in the liver than the CON-LD and GLN + ALA-LD groups. All groups subjected to long-duration exercise (LD) had lower glutamate concentrations in the liver compared with their respective groups maintained at rest (TR, $P < 0.05$; Table 2).

Tissues GSH status

As presented in Table 3, soleus muscle GSH concentrations and GSH/GSSG values were higher in the DIP-TR group compared with the CON-TR group. Soleus muscle GSH/GSSG values were also higher in the GLN + ALA-TR group compared with the CON-TR group. Gastrocnemius muscle GSH concentrations and GSH/GSSG were higher in the DIP-

Table 3
Skeletal muscle and liver concentrations of GSH and rate of GSH/GSSG*

	CON	DIP	GLN + ALA	*P*
Soleus muscle GSH (μmol/g fresh tissue)				
TR	0.27 ± 0.05[a]	0.45 ± 0.08[b]	0.32 ± 0.06[a]	0.001
LD	0.29 ± 0.10[a]	0.55 ± 0.08[b]	0.52 ± 0.08[b†]	0.001
%	6.57	20.59	63.22	
Soleus muscle GSH/GSSG				
TR	6.35 ± 0.42[a]	10.52 ± 0.89[b]	9.75 ± 0.38[b]	0.001
LD	6.67 ± 0.50[a]	9.50 ± 1.09[b]	10.29 ± 1.03[b]	0.001
%	5.06	−2.24	−2.55	
Gastrocnemius muscle GSH (μmol/g fresh tissue)				
TR	0.24 ± 0.01[a]	0.39 ± 0.07[b]	0.28 ± 0.05[a]	0.008
LD	0.18 ± 0.04[a†]	0.31 ± 0.05[b]	0.22 ± 0.02[a]	0.005
%	−24.33	−20.15	−20.21	
Gastrocnemius muscle GSH/GSSG				
TR	6.18 ± 0.40[a]	8.65 ± 1.78[b]	5.08 ± 1.29[a]	0.009
LD	5.57 ± 1.26[a]	6.44 ± 1.65[a]	6.20 ± 1.65[a]	0.699
%	−9.81	−25.62	22.15	
Liver GSH (μmol/g fresh tissue)				
TR	0.65 ± 0.05[a]	0.89 ± 0.08[b]	0.90 ± 0.07[b]	0.001
LD	0.54 ± 0.09[a†]	0.94 ± 0.06[b]	0.88 ± 0.02[b]	0.001
%	−17.96	6.06	−2.17	
Liver GSH/GSSG				
TR	6.43 ± 0.57[a]	9.33 ± 0.66[b]	7.50 ± 0.14[c]	0.001
LD	6.59 ± 0.90[a]	9.19 ± 0.70[b]	9.01 ± 1.39[b†]	0.001
%	2.54	−1.48	20.13	

CON, control; DIP, dipeptide; GSH, reduced glutathione; GLN + ALA, free L-glutamine and L-alanine; GSSG, oxidized glutathione; LD, long-duration exercise; TR, trained; %, difference between TR rats and LD rats ([LD − TR]/TR)] × 100

* Results presented as mean ± SD (n = 6 per group). Values in the same row with different letters are significantly different (Tukey's honestly significant difference).

† Significantly different between TR and LD groups ($P < 0.05$, t test).

supplemented group compared with the CON-TR and GLN + ALA-TR groups. In the liver, GSH concentrations and GSH/GSSG were higher in the DIP-TR and GLN + ALA-TR groups compared with the CON-TR group.

Table 3 also demonstrates that after long-duration exercise, the soleus muscle of the DIP-LD and GLN-ALA-LD groups had higher GSH concentrations and GSH/GSSG values than the CON-LD group. In the gastrocnemius muscle, higher GSH concentrations were observed in the DIP-LD group than the CON-LD group. Long-duration exercise reduced the concentration of GSH in the CON-LD group compared with the CON-TR group. In the liver, the GSH concentrations and the GSH/GSSG were higher in the DIP-LD and GLN + ALA-LD groups compared with the CON-LD group. Liver GSH concentrations were lower in the CON-LD group than in the CON-TR group. Furthermore, livers of the GLN + ALA-LD group had higher GSH/GSSG values than livers of the GLN + ALA-TR group (Table 3).

Discussion

This research demonstrates that chronic oral supplementation with free L-glutamine or DIP administered before long-duration exercise is an effective method to provide glutamine to rats.

The measured effect on plasma and muscle glutamine or glutamate for groups that received free L-glutamine may be attributed to the addition of free L-alanine to the solution. In similar studies, despite administering glutamine amounts equivalent to those in the present study, solutions containing the same amino acids in the same quantities among groups were not tested [20–22]. The addition of free L-alanine to the solution likely caused a rapid increase in the plasma concentration, because its transport through the intestinal epithelium cell occurs preferentially by a transporter [31]. Studies evaluating the transport of L-alanine in intestinal epithelial cells have demonstrated that its absorption can be reduced in the presence of other neutral amino acids. However, L-glutamine was not included among these amino acids [32]. The exact method of oral administration of L-glutamine is an important factor in determining glutamine bioavailability. In addition, our results suggest that L-alanine may also have contributed to the effect of the DIP on glutamine availability found in our and other studies [20–22].

The chronic oral supplementation of L-glutamine in the L-alanyl-glutamine form administered before long-duration exercise represents an efficient means to provide glutamine to rats. This has been previously demonstrated in our laboratory in studies conducted in sedentary rats subjected to intense exercise [20,21]. It has also been shown by other researchers in studies done in humans under conditions of high-protein catabolism [22,33]. The effects of the utiliza-

tion of L-glutamine in the form of DIP on glutamine availability has been attributed to the fact that enterocytes have a more efficient transport mechanism for the absorption of dipeptides and tripeptides than for the absorption of free amino acids [34,35]. The glycopeptides transport protein (Pept-1), which is located exclusively in the luminal membrane, has broad substrate specificity and actively transports dipeptides and tripeptides in the intestines of humans and animals [23,36]. Research utilizing radioactively labeled glutamine dipeptides has shown that nearly 90% of the radioactivity accumulates intact in the cytosol [33,37]. In this manner, glutamine can avoid intracellular hydrolysis and subsequent metabolism by enterocytes, proceeding directly to systemic circulation [23,20].

The groups not subjected to long-duration exercise and supplemented with DIP or GLN + ALA had higher plasma glutamine concentrations compared with the CON-TR group. This effect may be attributed to the higher concentration of glutamine found in the soleus muscle of the DIP-TR and GLN + ALA-TR groups and the gastrocnemius of the DIP-TR group. Similar results, using DIP, were observed by Rogero et al. [20] in rats subjected to intense exercise. The skeletal muscle is the primary site involved in the synthesis, storage, and delivery of glutamine. Because skeletal muscle stores more than 60% of total free glutamine in rats, its intracellular metabolism can influence glutamine concentrations in the plasma [2]. The concentration of glutamine in the plasma, however, does not correlate with the concentration in tissues, even under elevated muscular catabolic conditions [38]. In our study, the DIP-TR and GLN + ALA-TR groups exhibited an increase in concentrations of glutamine and glutamate in the soleus muscle, without a concomitant increase in plasma concentrations.

Among the stress-related factors promoted by prolonged training is the production of plasma ammonia, combined with the deamination of purines, and catabolism of amino acids inside myofibrils [39]. The animals in the CON-LD group displayed hyperammonemia in comparison with the concentration in the CON-TR group. The same effect was found in the DIP-TR group, an observation that may suggest that no beneficial effect resulted from the administration of DIP in the presence of high levels of ammonia induced by long-duration exercise. However, comparisons between the CON and supplemented groups showed that GLN + ALA or DIP supplementations reduced the levels of ammonia in the DIP-LD and GLN + ALA-LD groups compared with the CON-LD group. A similar effect of supplementation with L-glutamine on the reduction of plasma ammonia levels was found in human athletes [40]. Supplementation with glutamine in the free form or in DIP at levels equivalent to 1 g of glutamine per kilogram per day is not regarded as low dose [41,42]. Although higher levels of supplementation of amino acids can cause effects such as toxic hyperammonemia, these effects were not detected in the present study.

Before and after long-duration exercise, higher concentrations of glutamine and glutamate in the soleus muscle were observed in both supplemented groups. The effects of the supplementation were also found in the concentration of glutamine and glutamate of gastrocnemius muscle after long-duration exercise. These results suggest that the ammonia produced by the long-duration exercise served as a substrate for the synthesis of glutamine by the action of the glutamine synthetase enzyme [2]. In this manner, the effect of both supplementations not only decreased the production of ammonia that is induced by long-duration exercise but also increased the availability of muscular glutamine. Intense and prolonged physical exercise and exhaustive or high frequency training promote a reduction in the synthesis and storage of glutamine, which decreases the availability of the amino acid [6,7,17,43].

The effect of chronic oral supplementation with L-glutamine in the free form or as DIP on the synthesis of GSH, the main non-enzymatic antioxidant of the organism, was also evaluated. In our study, the DIP-TR and DIP-LD groups had higher GSH concentrations in the soleus and gastrocnemius muscles and higher GSH/GSSG in the soleus muscle than the CON-TR and CON-LD groups. This may be due, in part, to an increase in tissue glutamine and glutamate concentrations. Experimental evidence has indicated that higher glutamine availability, by means of parenteral supplementation in humans subjected to metabolic stress events (abdominal region surgeries), decreases muscular depletion of GSH, which improves patient recovery [14]. After trauma or catabolic situations, muscle concentrations of glutamine and glutamate are reduced, whereas the other amino acids that comprise GSH (i.e., cystine and glycine) remain at relatively constant levels [5,10,15].

The higher glutamate concentrations provide sufficient substrate for the enzyme γ-glutamylcysteine synthetase, which is the first regulated step in the synthesis of GSH. However, although glutamate in high concentrations is considered neurotoxic [13], the supplementation with glutamine, from the use of DIP or a solution containing GLN + ALA, is an efficient means to increase the availability of glutamate for the synthesis of GSH.

In the liver, the concentration of GSH and the cellular redox state (GSH/GSSG) of the supplemented groups, subjected or not to long-duration exercise, were higher than those in the control groups of the study. This effect is particularly important, because the liver is the primary organ for the de novo synthesis of GSH and is responsible for supplying 90% of circulating GSH in the human body [12,15]. During prolonged physical exercise, the liver exports GSH to the plasma under the influence of several hormones stimulated by cyclic adenosine monophosphate, including glucagon, vasopressin, and catecholamines [13,44], whereas the skeletal muscle tissue is responsible for the elevated concentrations of plasma GSH [13,45].

However, only the DIP-TR and DIP-LD groups showed a relation between liver GSH concentration and glutamine and glutamate availability in the same tissue, which indicates that several mechanisms take part in the synthesis of GSH. It is hypothesized that changes in the cell volume,

induced by an increased influx of sodium ions caused by an increase in the transport of glutamine to the intracellular medium, can favorably influence protein turnover by increasing or maintaining the availability of substrates for the synthesis of compounds such as GSH [46]. Other hypotheses are related to an increase in liver concentrations of adenosine triphosphate induced by glutamine [45]. Decreased hepatic adenosine triphosphate levels after stresses have been correlated with intracellular damage, which can lead to cell injury and death [47].

Conclusion

The present results indicate that when administered before long-duration exercise, oral supplementation with L-glutamine in the dipeptide form (DIP) or in the free form associated with L-alanine represents an effective supplementation to provide glutamine and glutamate to rats, which increases muscle and liver stores of GSH and improves the redox state of the cell.

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