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Whey protein hydrolysate enhances the exercise-induced heat shock protein (HSP70) response in rats

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ABSTRACT

Whey protein has been suggested to be potential protective agent against various forms of stress. The heat shock protein HSP70 confers greater cellular tolerance against stressors. The present study evaluated the effects of whey protein intake on HSP70 expression. Forty-eight male Wistar rats were divided into sedentary and exercised groups, and each group was fed as a protein source casein (CAS), whey protein (WP) or whey protein hydrolysate (WPH) for 3 weeks. Exercise on a treadmill was used as the source of stress in the animals from the exercised group. The results showed a larger increase in HSP70 expression in the soleus, gastrocnemius and lung of the WPH-fed rats than WP or casein-fed rats. HSP70 expression in the sedentary rats was very low, independent of the diet or tissue. Protein carbonyls were lower in the group that consumed WPH. These data suggest that the consumption of WPH enhances HSP70 expression.

1. Introduction

Whey protein (WP) represents approximately 20% of the proteins present in bovine milk, and has been recognised for its high nutritive value, high digestibility, fast absorption and appearance as plasma amino acids. WP has been the subject of many investigations focused on properties such as the modulation of the enzymatic antioxidant system (Gad et al., 2011) and the maintenance of muscle mass (Lollo, Amaya-Farfan, & Carvalho-Silva, 2011) as well as the contributions of WP to an anti-stress effect (Nery-Diez et al., 2010).

Heat shock proteins (HSPs) were discovered by Feruccio Ritossa in 1962, in the chromosomes of *Drosophila melanogaster* submitted to heat shock treatment resulting from exposure to near-lethal temperatures (Ritossa, 1962). HSPs are a natural endogenous defence system that is capable of protecting against and repairing damage. The system is activated during alterations in homoeostasis, including temperature changes, reactive oxygen species (ROS) generation, ischaemia, hypoxia and glucose deprivation, as well as various other types of physiological stress, such as those commonly associated with physical exercise (Silver & Noble, 2012). Exercise causes heat shock and oxidative stress and promotes HSP response; thus exercise could be a practical method to induce and study organic alterations such as heat stress (Salo, Donovan, & Davies 1991).

HSPs confer greater cell tolerance and resistance against a variety of stressors. They serve to maintain cell integrity, structure and function and promote cell survival during periods of stress. Under stressful conditions, these proteins are responsible for repairing or correctly folding damaged cellular proteins, or assisting in the elimination of irreversibly damaged proteins. The heat shock protein 70 (HSP70) family is easily inducible, highly active, considered to be a complementary antioxidant system and has been well studied (Silver & Noble, 2012).

The administration of glutamine has been shown to promote an increase in HSP70 as a protecting agent against various forms of injury, in a dose-dependent manner (Wischmeyer et al., 2001). Whey protein contains glutamine as well as abundant amounts of branched-chain amino acids (BCAAs), which are a source of nitrogen for the endogenous synthesis of glutamine catalysed by glutamine synthetase (Lollo et al., 2011; He et al., 2010).

We hypothesise that the consumption of whey protein hydrolysate enhances the production of HSP70 in rats subjected to exercise as source of stress. We also hypothesise that the glutamine synthetase enzyme could be involved in the mechanism of enhanced HSP70 production.

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2. Material and methods

2.1. Animals

Forty-eight male Wistar rats (21 days old, specific-pathogen free) reared in the Multidisciplinary Centre for Biological Research, University of Campinas, SP, Brazil, were housed (~22 °C, 55% RH, inverted 12-h light cycle) in individual growth cages with access to commercial feed (Labina, Purina, Brazil) and water *ad libitum*, until they reached 150 ± 8.7 g. The study was approved by the Ethics Committee on Animal Experimentation of the University of Campinas (CEEA-UNICAMP, protocol 2297·1).

2.2. Diets

The diets were based on the AIN93-G diet (Reeves, Nielsen, & Fahey, 1993), except that the protein content was 12% and whey protein (WP), whey protein hydrolysate (WPH) or casein (CAS) was the only protein source. Tables 1 and 2 show the nutrient compositions of the diets and the amino acid compositions of the protein sources, respectively. The molecular weight distribution of the WPH peptides was 40.5% <1 kDa, 26.7% between 1 and 5 kDa, and 15.6% between 5 and 20 kDa.

2.3. Experimental procedures

When the animals reached 150 ± 8.7 g of body mass, they were randomly assigned to six groups, corresponding to the three diets (CAS, WP and WPH) and two exercise regimes (S and E, for sedentary (unstressed) and exercised (stressed), respectively). The experimental diets were provided for 3 weeks.

2.4. Exercise protocol

The animals in the exercised groups were subjected to five intense exercise sessions on a treadmill at a speed of 22 m/min for 30 min during the last week of treatment. The exercise on a treadmill is an effective form to promote HSP response (Salo et al. 1991). After the last exercise session, the rats were allowed to recover for 6 h and were then killed by decapitation (Wischmeyer et al. 2001). Immediately after sacrifice, the gastrocnemius, soleus, spleen, lung, kidney and heart were collected and stored in liquid nitrogen until analysis.

2.5. Protein extraction and immunoblotting

The protein content of the supernatants was determined by the Lowry method. For immunoblotting, tissue homogenates were subjected to SDS–PAGE and transferred to a nitrocellulose membrane. The blots were probed with the appropriate antibodies to assess the protein level of the HSP70 (Stressgen, Victoria, BC, Canada; Ref SPA810 diluted 1:3000 and 1:500 for exercised and sedentary rats, respectively), glutamine synthetase (GS) (Abcam, Cambridge, Ref. ab64613 diluted 1:1000) and tubulin (Abcam, Cambridge, Ref. ab44928 diluted 1:1000). The appropriate secondary mouse antibody conjugated to peroxidase and the BM chemiluminescence blotting system (Abcam) were used for detection. The bands were visualised using a GE ImageQuant, model LAS 4000 instrument. Specific protein bands present in the blots were quantified using the digital program ImageJ (v. 1.44 for Windows).

Table 1
Diets composition (g/kg of diet).

Ingredient	CAS (g)	WP (g)	WPH (g)
Corn starch	437.92	427.31	425.00
Dextrinised starch	145.42	141.90	141.13
Sucrose	110.16	107.50	106.92
WPH	–	–	156.41
WP	–	152.77	–
CAS	135.96	–	–
Vegetable oil	70.00	70.00	70.00
Fibre (cellulose)	50.00	50.00	50.00
Mineral mixture	35.00	35.00	35.00
Vitamin mixture	10.00	10.00	10.00
L-Cystine	3.00	3.00	3.00
Choline bitartrate	2.50	2.50	2.50
tert-Butylhydroquinone	0.014	0.014	0.014

Table 2
Amino acid profile of the protein sources.

Amino acid	CAS	WP	WPH
Asparagine	5.96	11.52	11.16
Glutamate	19.00	18.82	17.99
Serine	4.68	5.31	5.04
Glycine	1.39	1.74	1.75
Histidine	2.12	1.31	1.27
Arginine	3.03	2.66	2.31
Threonine	3.56	7.64	7.40
Alanine	2.30	5.11	4.89
Proline	8.85	5.89	5.68
Tyrosine	4.57	2.88	2.78
Methionine	2.32	2.51	2.52
Cystine	0.16	1.48	1.60
Isoleucine	4.51	6.88	6.97
Leucine	7.62	10.14	10.15
Valine	5.36	5.68	5.81
Phenylalanine	3.89	2.86	2.78
Lysine	6.62	9.20	9.48
Total BCAA	17.49	22.70	22.93

2.6. Amino acid composition of the protein sources and plasma free amino acids

The protein sources were hydrolysed at 110 °C in 6 M HCl for 24 h. The hydrolysed samples (wet basis) were then diluted in deionised water; α-aminobutyric acid was added as the internal standard (Sigma–Aldrich Corp., St Louis, MO), and the amino acids were derivatised with phenylisothiocyanate. The PTH-derivatives were chromatographed using a Luna C-18, 100 Å; 5 μm, 250 × 4.6 mm column (Phenomenex, Torrance, CA), at 50 °C. The plasma free amino acids were extracted with trichloroacetic acid and then derivatised and chromatographed as described above.

2.7. Blood parameters

Blood samples were collected in Vacutainers, kept at 4 °C, and centrifuged at 3000*g* (4 °C, 15 min) to obtain the serum and plasma. The sera were assessed for uric acid, creatine kinase (CK), lactate dehydrogenase (LDH), total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine and urea using spectrophotometric (Beckman-Coulter DU 640, Palo Alto, CA) determinations employing Laborlab kits (São Paulo, Brazil). Glucose in the blood was measured using an Accu-Chek Active glucometer (Roche Diagnostics, Mannheim, Germany). Skin temperature was measured both before and after the last exercise session with an infrared thermometer (Luong & Carrive, 2012) (Geratherm Medical Diagnostic Systems, Geschwenda, Germany). Corticosterone (CORT) was determined using an enzyme immunoassay kit (Assay Designs – Stressgen, catalogue 900.097; Enzo Life Sciences, Exeter, UK).

2.8. Protein carbonyls

Samples of the gastrocnemius muscle (150–200 mg) were mixed and homogenised in 3 mL of 50 mM phosphate buffer, pH

7.4, containing 0.1% digitonin, and a cocktail of antiproteases (40 μg/mL phenylmethylsulfonyl fluoride, 5 μg/mL leupeptin, 7 μg/mL, pepstatin, 5 μg/mL aprotinin and 1 mM EDTA). The plasma (100 μL) was directly homogenised in 2,4-dinitrophenylhydrazine (DNPH). The carbonyl groups reacted with the DNPH, and after successive deproteinisation and dissolution procedures in guanidine hydrochloride, the spectra from 355 to 390 nm were read in a spectrophotometer (Epoch micro-plate reader; BioTek, Instruments, Inc., Winooski, VT) according to a previously described method (Reznick & Packer 1994). A standard curve was constructed by dissolving bovine serum albumin (BSA) in guanidine hydrochloride, and the results were expressed in ng/mg of protein.

2.9. Determination of glycogen

Glycogen was determined in the heart and the gastrocnemius muscle (35–50 mg) according to a previously described method (Lo, Russell, & Taylor 1970). The absorbance was read in a spectrophotometer (Beckman Coulter DU 640, Palo Alto, CA) at 490 nm, and the results are expressed as g/100 g of tissue.

2.10. Statistical analysis

Data were analysed by ANOVA, followed by the Duncan post hoc test, using SPSS software, version 17.0. The level for significance was set to $p < 0.05$.

3. Results

3.1. Hsp70

Fig. 1A and B shows the concentrations and patterns of HSP70 in the lungs, soleus, gastrocnemius, kidney, heart and spleen in the sedentary and exercised groups. Compared with either casein or whey protein, the consumption of WPH increased the HSP70 expression response in the lungs, soleus and gastrocnemius skeletal muscles, but not in the spleen, kidney or heart in the exercised group (Fig. 1B). The results for the sedentary animals (Fig. 1A) showed that the concentrations of HSP70 in the different tissues were always very low or undetectable, as described by Rohde et al. (2005).

3.2. Glutamine synthetase

The concentrations of glutamine synthetase (GS) in the soleus and lung for the different treatment groups are shown in Fig. 1. These are among the tissues that have been reported to exhibit substantial levels of GS activity (Huang, Wang, & Watford, 2007). The data revealed that only the WPH diet produced an elevation of this enzyme in the soleus, while no effect was observed in the lung.

3.3. Temperature

The rats' skin temperatures were measured as an indicator of the induction of heat stress associated with the exercise; the rectal temperature measure was avoided because this invasive procedure could influence HSP response. Fig. 2A shows that the mean temperature of all the groups was considerably elevated as a result of the exercise, regardless of the diet.

3.4. Carbonyl proteins

Carbonyl proteins are formed as a result of the action of reactive oxygen species (ROS), thus circulating and tissue proteins tend to become carbonylated in the presence of ROS. The type of dietary proteins influenced the extent of carbonylation. Fig. 2B shows that the plasma of the animals consuming either the WP or the WPH diets exhibited lower concentrations of carbonyl proteins than those consuming the CAS diet, whereas only the animals consuming the WPH diet exhibited lower levels in the gastrocnemius muscle. Carbonyl proteins were measured only in the exercised group because the action of ROS becomes important only in situations of stress.

3.5. Glycogen

The glycogen stores were determined in both the gastrocnemius and heart muscles (Fig. 2C and D). These data showed that the consumption of the whey proteins promoted a greater store of glycogen in both muscles than did casein. In the case of the gastrocnemius muscle (Fig. 2C), the WPH diet produced the greatest amount of glycogen in the sedentary group, while in the exercised group, both the WP and WPH diets caused a substantial increase. For the heart muscle, both whey proteins produced similar responses, surpassing casein in the sedentary animals. Exercise, however, eliminated the diet-associated difference in glycogen in the heart (Fig. 2D).

3.6. Free amino acids in the plasma

Table 3 shows the plasma free amino acid profiles for the three diets in the sedentary and exercised animals. With the exception of aspartic acid, significant differences were observed for all the amino acids as a result of either diet or exercise. The differences found for glutamate, glutamine and the branched-chain amino acids (BCAAs) are of particular interest. In the sedentary animals, the whey proteins (WP and WPH) produced an increase in the levels of glutamate, but only WPH increased the levels of leucine and isoleucine. Exercise tended to cause a decrease in most amino acids. Glutamine concentrations were lower in the casein and most of the WPH exercised animals, whereas valine was lower in the exercised animals, independent of the diet. Finally, exercise caused a decrease in glutamate and leucine in the animals that consumed WPH.

3.7. Blood parameters

The data in Table 4 show that consumption of the WPH diet decreased blood glucose levels in the sedentary animals, although the levels remained within the range of normal values. However, in the exercised animals the diet did not affect blood sugar levels. With respect to the uric acid concentrations the exercised animals consuming the WPH diet showed higher levels than those fed the casein diet, whereas there were no differences in uric acid among the sedentary groups. Exercise alone increased the uric acid levels in animals consuming the WP and WPH diets, but not for the casein diet. The remaining parameters, CK, LDH and urea, appeared to be unaltered in all the groups. The animals consuming the WP diet exhibited increased blood creatinine levels regardless of exercise. The protein sources also affected total blood proteins: this parameter was increased by both WP and WPH diets in both the sedentary and exercised groups. Serum albumin responded similarly, except that exercise independently increased serum albumin levels. For the liver marker AST, the data revealed that while the diet had no effect, exercise alone increased the values. The ALT parameter, however, showed a decrease in the sedentary groups that consumed either the WP or WPH diets, but no difference was observed in ALT as a function of diet in the exercised groups. The corticosterone concentration was higher in the exercised group than in the sedentary group, regardless of the diet consumed. However, for the exercised groups, consumption of the WPH diet resulted in the highest levels of this hormone.

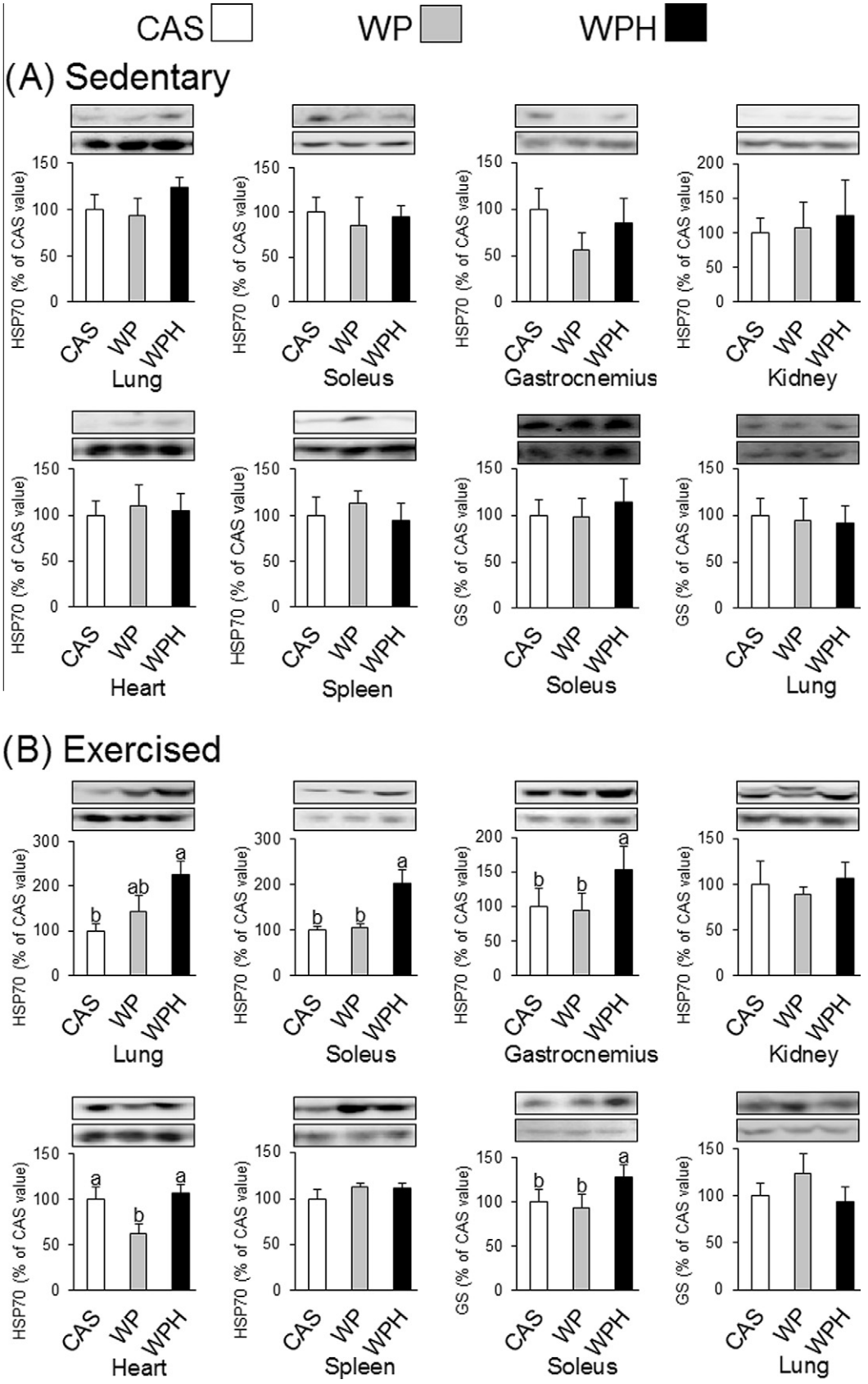

Fig. 1. Mean and standard error for the Western blot analysis of HSP70 and glutamine synthetase in sedentary (A) and exercised (B) groups in different tissues. Diets: Casein (CAS, $n = 8$); whey protein (WP, $n = 8$); whey protein hydrolysate (WPH, $n = 8$); glutamine synthetase (GS). Different letters represent significant differences.

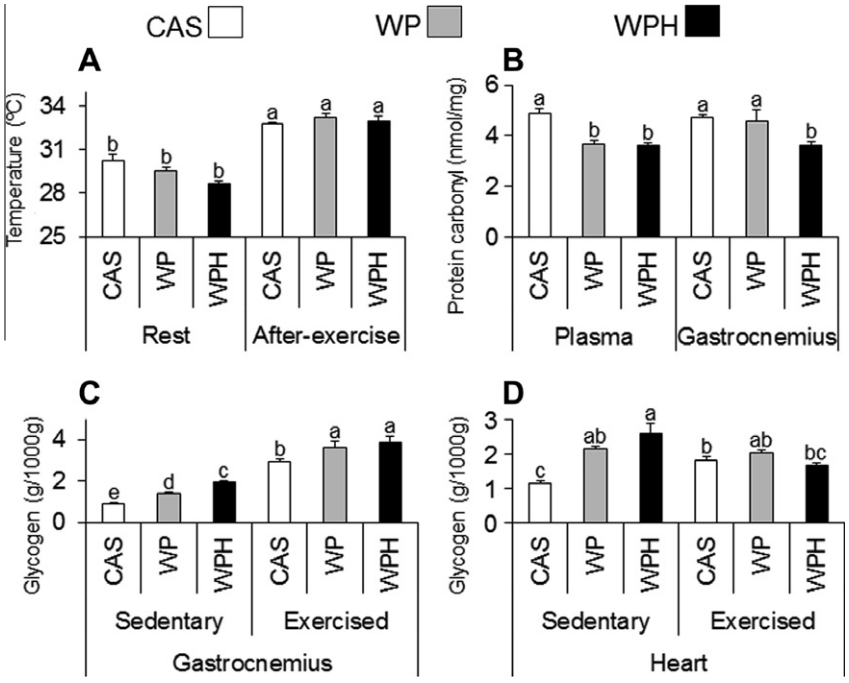

Fig. 2. Mean and standard error for the: (A) skin temperature of the exercised animals at rest and after exercise; (B) plasma and gastrocnemius concentration of protein carbonyls in the exercised groups; (C) gastrocnemius glycogen concentration; (D) heart glycogen concentration. Diets: Casein (CAS, $n = 8$), whey protein (WP, $n = 8$), whey protein hydrolysate (WPH, $n = 8$). Different letters represent significant differences between rest and exercise for each diet.

Table 3
Plasma free amino acid profile (μm/l) (mean values with their standard errors).

Amino acid	Sedentary						Exercised					
	CAS		WP		WPH		CAS		WP		WPH	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Aspartic acid	65.30	2.30	68.00	1.22	67.00	1.87	70.00	0.57	67.00	2.90	68.33	2.40
Glutamate	28.50^{b}	4.30	35.00^{a}	2.82	39.50^{a}	2.40	42.50^{a}	4.90	32.00^{ab}	3.70	29.50^{b}	4.60
Hydroxyproline	46.65^{a}	2.10	45.30^{ab}	1.24	47.33^{a}	2.30	44.33^{ab}	1.80	39.30^{bc}	0.23	37.00^{c}	1.70
Asparagine	83.00^{ab}	4.50	77.00^{bc}	4.94	90.66^{a}	1.40	77.33^{bc}	3.40	75.00^{bc}	2.60	66.00^{c}	1.70
Serine	182.50^{a}	2.00	104.50^{c}	3.17	118.33^{bc}	7.80	136.60^{b}	7.70	80.50^{d}	4.90	112.60^{c}	5.50
Glutamine	826.33^{a}	27.10	835.30^{a}	34.40	795.00^{a}	20.00	697.33^{b}	29.80	773.30^{ab}	4.20	651.30^{c}	10.60
Glycine	80.50^{d}	10.70	105.00^{bc}	6.70	90.00^{cd}	4.60	125.50^{ab}	9.50	52.00^{e}	8.00	136.00^{a}	0.50
Histidine	21.00^{c}	2.90	34.66^{a}	0.23	30.00^{ab}	0.50	23.5^{c}	0.80	30.66^{a}	4.30	24.50^{bc}	0.50
Arginine	36.50^{ab}	4.90	26.00^{b}	4.00	24.30^{b}	6.35	50.50^{a}	10.10	48.50^{a}	2.00	49.00^{a}	1.00
Taurine	87.50^{c}	2.00	123.50^{b}	3.10	183.50^{a}	0.28	75.50^{c}	1.40	119.00^{b}	13.20	130.00^{b}	10.00
Threonine	353.50^{b}	9.50	552.00^{a}	18.30	513.00^{a}	3.67	222.00^{c}	4.20	542.00^{a}	11.00	547.50^{a}	6.60
Alanine	551.00^{c}	25.40	607.50^{bc}	6.60	690.00^{a}	2.80	487.50^{d}	33.10	637.00^{ab}	2.80	555.50^{c}	24.50
Proline	430.00^{a}	29.90	186.50^{cd}	7.20	205.60^{c}	6.70	260.50^{b}	3.70	168.30^{cd}	3.00	138.50^{d}	5.40
Tyrosine	34.50^{c}	2.00	46.50^{b}	3.10	37.00^{c}	2.30	15.32^{d}	0.94	54.00^{a}	1.70	52.50^{ab}	2.50
Valine	135.50^{a}	8.40	79.00^{b}	1.00	90.00^{b}	0.57	80.50^{b}	7.70	56.00^{c}	5.70	50.00^{c}	1.00
Methionine	48.00^{ab}	1.20	54.00^{a}	2.80	52.00^{a}	1.63	40.50^{b}	7.20	52.00^{a}	2.00	53.00^{a}	2.00
Cystine	72.00^{a}	3.50	72.00^{a}	1.41	59.66^{b}	1.84	62.00^{ab}	1.00	68.00^{ab}	3.60	65.50^{ab}	4.30
Isoleucine	17.50^{d}	3.80	17.65^{d}	0.54	37.00^{b}	4.20	27.00^{c}	1.15	33.50^{bc}	1.40	49.00^{a}	4.00
Leucine	72.50^{d}	10.20	77.50^{d}	12.40	131.00^{a}	1.00	70.50^{d}	16.4	112.50^{b}	3.60	104.50^{c}	2.00
Phenylalanine	11.00^{d}	3.00	39.50^{b}	0.50	30.00^{c}	2.80	11.00^{d}	0.57	52.00^{a}	2.30	37.00^{bc}	4.00
Tryptophan	149.30^{bc}	7.00	172.50^{ab}	18.70	127.50^{c}	6.00	124.00^{c}	7.5	162.50^{ab}	6.00	189.00^{a}	5.70
Lysine	426.00^{c}	20.1	597.00^{a}	29.40	492.00^{b}	3.40	394.00^{c}	19.6	448.00^{bc}	12.70	485.50^{b}	11.20
BCAAs	225,50	5.70	174,15	4.20	258.00	4.90	178.00	4.10	202.00	3.90	203.00	4.40

Mean and standard error for the blood parameters. Diets: casein (CAS), whey protein (WP), whey protein hydrolysate (WPH). Different letters represent significant differences.

4. Discussion

No reports were found in the literature relating to the effect of whey protein intake on the expression of HSP70. Previous results indicate that the HSP70 is strongly induced by different types of stress while its expression is very low or undetectable under conditions of normal homoeostasis (Rohde et al., 2005), which is consistent with our data for the sedentary animals. In our study, exercise induced an increase in skin temperature independent of the type of diet.

Although the level of exercise was the same for all the exercised groups and the heat stress was indistinguishable among the protein sources, the greatest enhancement of HSP70 for the gastrocnemius, soleus and lung was observed in animals consuming the

Table 4
Blood parameters.

Parameter	Sedentary						Exercised					
	CAS		WP		WPH		CAS		WP		WPH	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Glucose (mg dL^{-1})	156.00	6.11	151.00	3.23	135.00	5.82	141.00	5.02	141.00	5.37	136.00	3.14
Uric acid (mg dL^{-1})	1.62	0.14	1.27	0.16	1.62	0.35	1.99	0.35	2.32	0.25	2.89	0.38
LDH (U L)	726.41	28.64	743.55	17.20	730.67	12.75	756.24	35.13	796.07	42.01	749.70	44.83
CK (U L)	1112.62	91.31	1105.45	47.09	1071.29	51.90	1144.97	90.15	1240.00	49.97	1074.00	93.73
AST (U L)	112.98	10.06	103.36	8.92	104.74	9.50	145.40	7.55	146.09	10.40	136.52	9.89
ALT (U L)	24.11	2.38	19.13	0.92	17.80	1.90	24.46	1.35	27.83	1.59	23.63	1.35
Total protein (g dL^{-1})	5.75	0.20	7.06	0.56	6.62	0.34	6.55	0.17	7.25	0.27	7.27	0.31
Albumin (g dL^{-1})	3.98	0.08	4.71	0.10	4.43	0.08	4.51	0.07	5.10	0.25	5.12	0.18
Urea (mg dL^{-1})	18.23	1.69	17.52	1.71	20.09	1.94	19.14	1.75	18.96	1.79	18.86	1.28
Creatinine (mg dL^{-1})	0.50	0.04	0.60	0.02	0.47	0.08	0.53	0.03	0.63	0.03	0.53	0.02
CORT (ng mL^{-1})	99.00	17.35	87.44	8.79	86.59	9.65	162.75	18.32	161.10	15.05	206.91	22.02

Mean and standard error for the blood parameters. Diets: casein (CAS), whey protein (WP), whey protein hydrolysate (WPH). Different letters represent significant differences. LDH, lactate dehydrogenase; CK, creatine kinase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CORT, corticosterone.

WPH diet. The increase in HSP70 has been reported to protect intestinal epithelial cells, reduce tissue damage, ease recovery from critical illnesses, including the recovery of striated muscle after exercise, promote longevity, reduce cell mortality, protect lung against inflammatory injury induced by sepsis, and increase tolerance and resistance against various kinds of cell injury (Salway, Gallagher, Page, & Stuart 2011; Singleton & Wischmeyer 2007; Wischmeyer et al. 2001). HSP70 expression may protect and exert anti-apoptotic effects in lungs exposed to hypoxia stress. Hypoxia is a stressor for living organisms and many kinds of physiological or pathological processes are induced by hypoxia. Exercise can cause hypoxia in the body, and the lung is the primary organ directly exposed to the hypoxic situation (Kim et al. 2006). Our study suggests that whey protein hydrolysate was a factor that enhanced the exercise-induced HSP70 system.

It is also well documented that the administration of glutamine can promote a dose-dependent increase in HSP70 as a form of protection against various forms of injury (Wischmeyer et al. 2001). The proposed mechanism by which glutamine increases HSP70 appears to be an enhancement of the hexosamine biosynthetic pathway (Hamiel, Pinto, Hau, & Wischmeyer 2009), and this protective effect of glutamine may be related to the increase in the expression of heat shock proteins (HSPs). When Singleton and Wischmeyer (2007) silenced the HSP70 gene, the administration of glutamine did not reduce the damage markers. These findings suggest that HSP70 expression is required for glutamine to affect the survival of injured tissue. Whey proteins contain generous amounts of glutamine and BCAAs, and these amino acids (BCAAs) could be a source of readily available nitrogen for the endogenous glutamine-synthetase-mediated synthesis of glutamine.

The concentrations of the free amino acids isoleucine and leucine were increased in the plasma of the sedentary animals consuming the WPH diet, compared to either casein or whey protein, thus showing the greater availability of these amino acids in the WPH group for the eventual biosynthesis of glutamine. In contrast, the exercised animals in the WPH diet demonstrated other alterations in the free amino acid profiles, including reduced concentrations of leucine and valine, amino nitrogen donors, and glutamate used for glutamine synthesis. Consistent with the abovementioned decreases in the plasma concentration of glutamine precursors, there was also an increase in the GS in the soleus of the animals that also consumed WPH. However, no diet-associated differences in the levels of GS were observed in the lungs. This observation is consistent with the notion that the skeletal muscle, rather than the lung, is responsible for nearly 70% of the total production of this amino acid in the body (He et al. 2010).

The tendency for exercise to reduce glutamine levels (Table 3) is in agreement with reports in the literature, which show that exercise reduced glutamine levels (Santos, Caperuto, & Costa Rosa 2007). However, the decrease found in the exercised WPH group was greater than that found in the animals consuming either casein or whey protein. This result could be related to the observation that the group consuming WPH exhibited the highest production of HSP70, which is consistent with the notion that glutamine is used to increase HSP70 production (Hamiel et al. 2009).

Stress states associated with increased endogenous glucocorticoid release (e.g., exercise), have been shown to increase GS in the muscle as part of the response to the stress (Labow, Souba, & Abcouwer 1999). Regarding the relevance of glucocorticoid hormones on the activation of GS, Mezzarobba et al. (2003) showed that rats unable to produce corticosterone were also unable to respond to stress by increasing the production of GS. In the current study, a large increase in GS and the highest levels of corticosterone were observed in the exercised group consuming the WPH diet. These results are consistent with the influence of corticosterone on GS.

The carbonyl proteins formed as a result of the action of ROS in the gastrocnemius muscle and plasma of the animals fed the whey protein hydrolysate diet were lower than in those of the animals consuming the other diets and the production of HSP70 was also considerably greater in these animals. These observations suggest that HSP70 may be responsible for protecting the gastrocnemius against the modification of tissue proteins caused by ROS. This finding would be consistent with previous evidence that HSP70 may serve as an auxiliary antioxidant. Some authors have suggested that the ROS produced by exercise could be one of the means favouring adaptation of the trained organism, although it is still not completely clear if the decrease in generation of ROS could negatively affect the exercise-induced adaptations.

With respect to the blood parameters, glucose levels were lower in the sedentary animals that consumed WPH. Similar results have been reported in the literature, and Petersen et al. (2009) suggested that whey proteins, or some amino acids, such as the BCAAs, lysine and threonine, exert a dose-dependent insulinotropic effect. Uric acid is the most abundant and powerful serum antioxidant (Waring, McKnight, Webb, & Maxwell 2006) and exercise alone has been reported to increase the levels of uric acid (Kaya et al. 2006). Our data confirmed this increase in uric acid for the rats fed either WP or WPH diets, concurrently with exercise, but not for the casein diet. These results indicate that the protein source may affect the antioxidant protection of uric acid caused by exercise.

Whey proteins have been shown to preserve the levels of serum albumin and total proteins during exercise (Pimenta, Abecia-Soria, Auler, & Amaya-Farfan 2006). Serum albumin has antioxidant capacity, assisting in the transport of antioxidant agents, such as bilirubin and nitric oxide (Quinlan, Martin, & Evans 2005). The present results suggest that the consumption of either form of whey proteins could minimise the losses of serum albumin, thus sparing its functional properties, including its antioxidant capacity. The present results for AST and ALT enzymes and blood urea indicated that none of the protein sources caused any apparent liver or kidney damage.

The CK and LDH are blood indicators related to muscle damage (Cooke, Rybalka, Stathis, Cribb, & Hayes 2010). Ours results for CK and LDH showed no significant alteration in relation to the diet or exercise. This was probably due to the times of the sample collections, since the rise in the levels of CK and LDH can take from 24 to 72 h to occur (Cooke et al. 2010). The consumption of WP favoured an increase in the levels of serum creatinine. Investigations have suggested that creatinine could be used as indirect marker to estimate muscle mass, since there is a strong correlation between serum creatinine levels and the amount of lean mass (Schutte, Longhurst, Gaffney, Bastian, & Blomqvist 1981).

Glycogen is one of the most important forms by which an organism can store energy. Exercise causes a depletion of glycogen stores, which affects performance and the anticipation of fatigue. The speed of restoration of the glycogen stores after exercise is also an important factor in the recovery process. The rate of restoration is variable and can take up to 24 h, depending on the diet and on the extent of glycogen depletion (Jentjens & Jeukendrup 2003).

Both WP and WPH restored the glycogen reserves in the gastrocnemius muscle more effectively than casein. The present results are consistent with the findings of Morifuji, Sakai, Sanbongi, and Sugiura (2005), who also observed that the glycogen concentrations increased in exercised rats that had consumed whey protein. The mechanism by which whey proteins stimulate the accumulation of glycogen is still unknown.

Depending on the diet consumed after exercise, depleted muscle glycogen concentrations can increase to above basal levels, such as those found in the non-exercised muscle, by a process known as glycogen supercompensation (Jentjens & Jeukendrup, 2003). The present results supported this concept in that glycogen levels were higher in the exercised animals than in the sedentary animals. In addition, it has been suggested that increases in HSP70 levels can stimulate lipid oxidation by elevating citrate synthase and β-hydroxyacyl-CoA dehydrogenase levels, thus promoting energy expenditure (Henstridge et al. 2010), which could aid in the preservation of glycogen as a source of energy.

Cardiac glycogen stores appear to be related to a greater tolerance and resistance to hypoxia and anoxia, promoting survival and homoeostasis of the heart muscle (Taegtmeyer 2004). The present findings suggest that the consumption of WP and WPH promoted the accumulation of cardiac glycogen in the sedentary groups, similar to the results reported by Faria, Nery-Diez, Lollo, Amaya-Farfan, and Ferreira (2012), whereas no effect was found in the exercised groups.

In conclusion, the data obtained in this study showed that the consumption of whey protein hydrolysate resulted in a greater increase in the concentration of HSP70, than that produced by the non-hydrolysed whey protein or by casein. This finding was observed in the gastrocnemius and soleus muscles and lung, but not in the spleen, kidney or heart. The data also suggested that the enzyme glutamine synthetase could be modulated by the different sources of protein in the diet. These results suggest that the increase in HSP70 caused by the consumption of whey protein hydrolysate may affect different tissues in response to physical exertion.

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