



## Acute exercise and caffeine improve insulin-induced hypoglycemia in normal and malnourished rats

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**ABSTRACT.** In food restriction, hypoglycemic episodes can be more severe and persistent. This study assessed the influence of acute exercise and caffeine on the insulin-induced hypoglycemia in freely-fed or malnourished (50% food restriction) young rats. At the age of 60 days, rats under overnight fasting received an insulin injection to cause an episode of hypoglycemia. In some animals, hypoglycemia was preceded by an acute session of exercise, in others, caffeine was orally given 15 minutes after insulin injection; or exercise and caffeine were combined. Blood samples were collected at regular intervals for five hours after insulin injection. A beneficial effect of both exercise or caffeine on the hypoglycemic episode in the malnourished rats was found. In the control rats, the association of exercise+caffeine was more beneficial than either intervention alone. It is discussed that exercise and caffeine, alone or combined, can be used as exogenous anti-hypoglycemic resources, but considering the nutritional status of the subject.

**Keywords:** food restriction, glycemia, methylxanthine, physical activity.

## Exercício agudo e cafeína melhoram a hipoglicemia induzida por insulina em ratos normais e desnutridos

**RESUMO.** Na restrição alimentar, os episódios de hipoglicemia podem ser mais severos e persistentes. Este trabalho avaliou a influência do exercício agudo e da cafeína sobre a hipoglicemia induzida por insulina em ratos jovens alimentados livremente ou desnutridos (50% de restrição alimentar) desde o nascimento. Aos 60 dias de idade, os ratos em jejum noturno receberam injeção de insulina para causar um episódio de hipoglicemia. Em alguns animais, a hipoglicemia foi precedida por sessão aguda de exercício; em outros foi administrada a cafeína via oral, 15 min. após a injeção de insulina; ou foi associada a exercício e cafeína. Amostras de sangue foram coletadas em intervalos regulares por 5h desde a aplicação da insulina. Registrou-se efeito benéfico do exercício e da cafeína isolados sobre o episódio hipoglicêmico nos ratos desnutridos. Nos ratos controle, a associação de exercício+cafeína teve efeito benéfico mais acentuado do que as intervenções separadas. É discutido que exercício físico e cafeína, isolados ou combinados, podem ser explorados como recursos anti-hipoglicêmicos exógenos, levando-se em consideração porém o estado nutricional do indivíduo.

**Palavras-chave:** restrição alimentar, glicemia, metilxantinas, atividade física.

### Introduction

Food restriction can be defined as an amount of energy and nutrients incapable of meeting the body needs and providing appropriate growth, maintenance and functioning (MARTINS et al., 2011). When present since childhood, it compromises physical growth and functional development, and the ability to cope with illnesses and other types of internal and environmental stress, characterizing malnutrition (MARTINS et al., 2011; METHA et al., 2013). Without nutritional recovery, the effects of caloric deprivation persist during growth and even in adulthood (WINICK; NOBLE,

1966; VISMARA; FURLAN, 2007; MALTA et al., 2010; VITORIANO et al., 2011).

As glucose is a primary energy substrate for the Central Nervous System, the maintenance of adequate plasma glucose levels (glucose homeostasis), regardless the nutritional status of the individual, is essential. Several mechanisms and processes, both neural and hormonal, aim to reach glucose homeostasis under any circumstance. The major ones are insulin and its antagonistic or counterregulatory hormones: glucagon, catecholamines, glucocorticoids and growth hormone (GH). The hypothalamus and the vagal and sympathetic visceral nerves also have an important role

(CRYER, 1993; AHRÉN, 2000; TIRONE; BRUNICARDI, 2001; LEIBOWITZ; WORTLEY, 2004).

Hypoglycemia is an acute reduction in plasma glucose, below the physiologically acceptable range (CRYER et al., 2003). The systemic response to hypoglycemia encompasses many events hierarchically organized; the first line of defense is the release of glucagon and adrenaline, followed by cortisol and GH when hypoglycemia persists (LAGER, 1991; MITRAKOU et al., 1991; CRYER, 1993; HOFFMAN, 2007). In addition, under severe hypoglycemia, liver autoregulation of plasma glucose, possibly together with neural regulation, has a substantial role, and involves enhancement of glycogenolysis and gluconeogenesis (MOORE et al., 1998).

Under food restriction, insulin sensitivity is enhanced (OKITOLONDA et al., 1987; LEON-QUINTO et al., 1997; MALTA et al., 2010). Impaired responses of several counterregulatory hormones were also observed during severe hypoglycemia in malnourished rats (LEON-QUINTO et al., 1997). Along with increased insulin sensitivity, the altered counterregulatory response can favor more persistent, poorly recovered, hypoglycemic episodes (OKITOLONDA et al., 1987; LEON-QUINTO et al., 1997; MALTA et al., 2010).

Physical activity is an additional challenge to glucose homeostasis because of the increase in skeletal muscle uptake of energy substrates. During exercise, there is an increased availability of plasma glucose to meet the increased transport of glucose into the muscle fiber. The relative magnitude of these processes is affected by the intensity and duration of the exercise; for instance, if exercise intensity increases, plasma glucose reaches higher levels, indicating that glucose release to the bloodstream is greater than tissue uptake (SONNE; GALBO, 1985; CONSTABLE et al., 1988; KJAER et al., 2001; AFONSO et al., 2003; CIOLAC; GUIMARÃES, 2004; SUH et al., 2007; WAHREN; EKBERG, 2007). The acute release of catecholamines, the liver and kidney production of glucose, and the turnover of alternative energy substrates, especially fatty acids, were listed as important participants in these observations (KJAER et al., 2001; SUH et al., 2007; WAHREN; EKBERG, 2007). At the same time, the diminished release of insulin during exercise favors the release of glycogen-derived glucose by the liver and of free fatty acids from the adipose tissue (AFONSO et al., 2003; CIOLAC; GUIMARÃES, 2004).

Caffeine is ergogenic and can interfere with the actions of insulin related to glucose homeostasis (CRIST et al., 1998; PIZZIOLO et al., 1998; GREER et al., 2001; KEIJZERS et al., 2002; THONG et al., 2002). The antagonism to adenosine receptors in the tissues seems to be the major mechanism of action of caffeine as far as glucose homeostasis is concerned, although the caffeine-stimulated release of adrenaline also contributes to antagonize insulin (KEIJZERS et al., 2002; JOHNSTON et al., 2003).

Once exercise and caffeine interfere with glucose homeostasis, and the food restriction favors more persistent episodes of hypoglycemia, this study compared the effects of acute exercise and caffeine on the insulin-induced hypoglycemia in freely-fed or malnourished rats.

## Material and methods

**Experimental groups:** Rats were kept under controlled conditions of light (12:12 light dark cycle<sup>-1</sup>) and temperature ( $22 \pm 2^\circ\text{C}$ ). All the procedures were approved by the Ethics Committee on Animal Experimentation (Statement 050/2010).

Pregnant Wistar rats were housed in individual boxes, where they gave birth. The newborn litters were arranged so that each dam had either six (control group, GC) or 12 puppies (restriction or malnourished group, GR). The dams had free supply of water and chow (Nuvilab CR1; Nuvital, Colombo-PR, Brazil) during gestation and lactation.

Puppies remained with their mothers until the age of 21 days (weaning), when they were placed in plastic boxes in groups of four or five with free access to water. The GC rats received chow ad libitum. The amount of chow ingested by the GC group was recorded daily and each GR was given an amount 50% less than the average ingestion of the GC of corresponding age (VISMARA; FURLAN, 2007; MALTA et al., 2010; VITORIANO et al., 2011; BABATA et al., 2014). No supplementation was added to the chow.

For biometric characterization of the groups, animals from the GC and the GR were weighed at the ages of 21 and 45 days. Animals were killed by iv. injection of thiopental ( $120 \text{ mg kg}^{-1}$  body weight) and the weight of some organs and abdominal fat depots was recorded.

Only male rats were used. All the experiments were carried out at the age of 60 days, that is, young sexually mature rats, in the morning after overnight fasting (approx. 14 hours). The following protocols were employed: a) insulin-induced hypoglycemia (IIH) without exercise or caffeine (subgroups GC and GR); b) IIH with exercise and no caffeine (subgroups GC+E and GR+E); c) IIH without

exercise and with oral caffeine (subgroups GC+C and GR+C); d) IHH with exercise and oral caffeine (subgroups GC+E+C and GR+E+C).

The protocols of insulin-induced hypoglycemia, oral administration of caffeine and acute session of exercise described below were established in previous investigations (MALTA et al., 2010; BABATA et al., 2014).

**Insulin-induced hypoglycemia (IHH):** Animals were injected ip. with insulin at the dose of 1 U.kg<sup>-1</sup> body weight (Novolin; Novo Nordisk, Montes Claros-MG, Brazil). Blood samples (approx. 800 µL) were collected in heparinized Eppendorf tubes through an incision at the tip of the tail, at 0, 15, 30, 45, 60, 120, 180, 240 and 300 min., time 0 being the moment immediately before the insulin injection. After each blood collection, an equal volume of 0.9% saline solution was given ip. After centrifugation (3,000 rpm for 5 min.), plasma was used to determine glucose concentration, as described below.

**Acute exercise:** The exercise session consisted of a run on an adapted treadmill (KT3000, Inbramed, Porto Alegre, Rio Grande do Sul State, Brazil). The treadmill speed increased 0.2 km h<sup>-1</sup> every two minutes from an initial speed of 0.4 km h<sup>-1</sup> and exercise continued until exhaustion, characterizing an acute session. Next, insulin was injected and the plasma glucose was monitored for 300 min.

**Administration of caffeine:** Rats were orally given caffeine (gavage) at the dose of 5 mg kg<sup>-1</sup> body weight (Farmácia São Paulo Manipulação, Maringá, Paraná State, Brazil), dissolved in distilled water, 15 min. after insulin injection. Plasma glucose was recorded for 300 min. after insulin injection.

**Determination of plasma glucose:** Plasma glucose was determined through enzymatic-colorimetric method (GoldAnalisa; Belo Horizonte, Minas Gerais State, Brazil). Plasma samples (10 µL) were added to the reactive medium (1 mL) and incubated for 5 min. at 37°C. Absorbance was read in Bio-2000 spectrophotometer (Bioplus, Barueri, São Paulo State, Brazil). Plasma glucose levels were expressed as mg dL<sup>-1</sup>.

**Statistical analysis:** Data are shown as mean ± standard deviation (SD). After confirmation of data normality, statistical comparisons were carried out.

The GC and GR subgroups of the same protocol were compared through the unpaired Student's t-test. The protocols of the same group were compared through one-way ANOVA with Bonferroni post-test. Values of  $p < 0.05$  were considered statistically significant. The software Prism 5.0 (GraphPad Software; San Diego-CA, EUA) was used for the obtention of the quantitative data (Tables 1 and 2), statistical analysis and construction of the plasma glucose profiles (Figures 1 to 6). The number of data in each set ranged from 6 to 12.

## Results and discussion

**Comparison of control and malnourished groups:** Table 1 shows the biometric data of GC and GR. Both during lactation and after weaning the body weight and the relative weight of the adipose tissue were significantly lower in the GR. The relative weight of the liver and small intestine, representative of the visceral mass, did not reduce in this group.

Figure 1 illustrates the plasma glucose profile during IHH in the GC and GR not subjected to exercise or caffeine. The basal plasma glucose (at 0 min.) did not differ ( $p > 0.05$ ). Hypoglycemia was already present 15 min. after the insulin injection and glucose level continued to drop during the first hour. From 60 min. onwards, plasma glucose in the GR was significantly lower than in the GC, and so remained until 300 min. The GC began to recover the plasma glucose at 240 min., while the GR showed a slight recovery only at 300 min. Plasma glucose recovery was not complete in neither of the groups: at the end of the recording period (300 min.), it was about 38 mg dL<sup>-1</sup> lower than at 0 min. in the GC, while in the GR it was 54 mg dL<sup>-1</sup> lower.

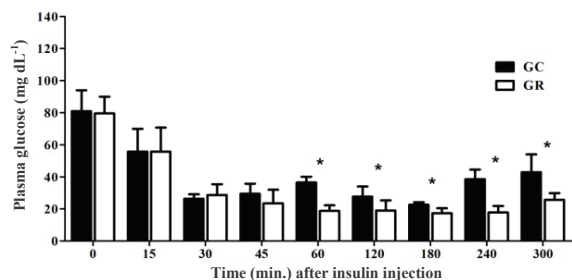
Running time until exhaustion was significantly higher in the GR ( $13.82 \pm 2.23$  min.) than in the GC ( $9.99 \pm 2.07$  min.,  $p < 0.05$ ).

Significant differences were detected in plasma glucose levels of the GC and GR that received insulin after the acute exercise session (Figure 2). At 0 min., i.e. immediately after exercise and before the insulin injection, the malnourished animals (GR+E) had plasma glucose significantly higher than the control rats (GC+E).

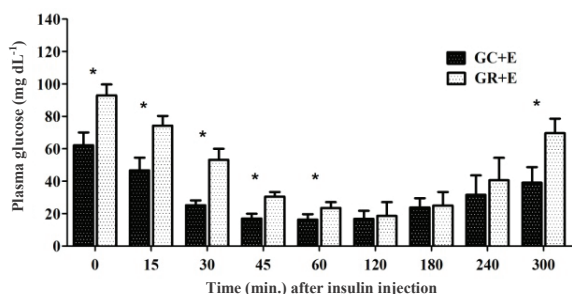
**Table 1.** Biometric parameters of freely-fed (GC) and malnourished (GR) rats.

Parameter	GC (n = 6-10)	GR (n = 6-10)
Body weight at 21 days (g)	36.67 ± 3.78	27.50 ± 2.03*
Body weight at 45 days (g)	161.43 ± 2.62	89.33 ± 11.67*
Periepididymal fat (g.100 g <sup>-1</sup> body weight)	0.99 ± 0.18	0.51 ± 0.11*
Retroperitoneal fat (g.100 g <sup>-1</sup> body weight)	1.14 ± 0.22	0.24 ± 0.11*
Liver (g.100 g <sup>-1</sup> body weight)	3.80 ± 0.22	3.93 ± 0.46
Small intestine (g.100 g <sup>-1</sup> body weight)	2.52 ± 0.45	3.29 ± 0.26*

Data presented as mean ± SD. \*p < 0.05 of GR compared with GC, Student's t test.



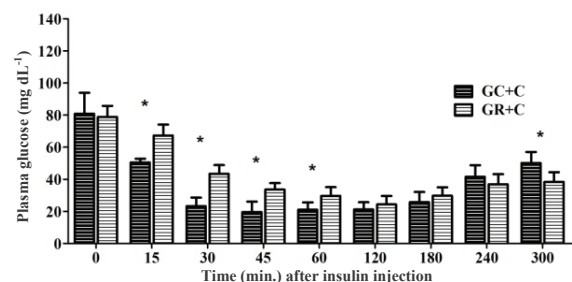
**Figure 1.** Plasma glucose profile of freely-fed (GC) and malnourished (GR) rats during insulin-induced hypoglycemia. Data presented as mean $\pm$ SD. \* $p < 0.05$  between groups, Student's  $t$  test;  $n = 10-12$ .



**Figure 2.** Plasma glucose profile of freely-fed (GC) and malnourished (GR) rats during insulin-induced hypoglycemia after an acute exercise session. Data presented as mean  $\pm$  SD. \* $p < 0.05$  between groups, Student's  $t$  test;  $n = 6-8$ .

This difference was maintained at 15, 30, 45 and 60 min. At 180 min., plasma glucose began to rise in both groups, but the recovery in the GR was significantly better than in the GC, with a difference of 30 mg dL<sup>-1</sup> in the final plasma glucose (300 min.) between the groups.

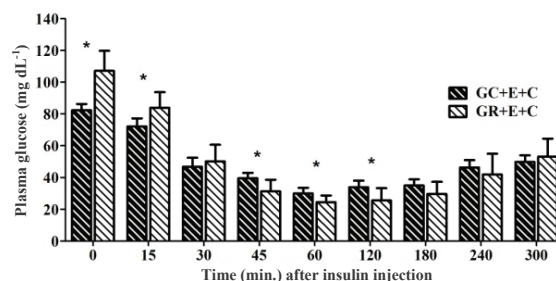
The groups given oral caffeine 15 min. after insulin injection showed significant differences at 15, 30, 45 and 60 min., with less severe hypoglycemia in the GR at these times (Figure 3); however, at 300 min. the final plasma glucose in the GR was significantly lower than in the GC.



**Figure 3.** Plasma glucose profile of freely-fed (GC) and malnourished (GR) rats during insulin-induced hypoglycemia and oral administration of caffeine at 15 min. Data presented as mean $\pm$ SD. \* $p < 0.05$  between groups, Student's  $t$  test;  $n = 8-12$ .

The plasma glucose profile of GC and GR animals subjected to IIH after exercise and given

oral caffeine 15 min. after insulin injection is shown in Figure 4. At 0 and 15 min., malnourished rats exhibited plasma glucose values significantly higher than the controls. From 45 to 120 min., however, the malnourished group had a significantly lower plasma glucose level compared with the controls, with values close to 25 mg dL<sup>-1</sup> at 60 and 120 min. During plasma glucose recovery, both groups had similar values ( $p > 0.05$ ). Plasma glucose recovery was slight in both groups, not exceeding 15 mg dL<sup>-1</sup> from 180 to 300 min.



**Figure 4.** Plasma glucose profile of freely-fed (GC) and malnourished (GR) rats during insulin-induced hypoglycemia after an acute exercise session and oral administration of caffeine at 15 min. Data presented as mean $\pm$ SD. \* $p < 0.05$  between groups, Student's  $t$  test;  $n = 6-10$ .

Table 2 presents the decrease and recovery of plasma glucose relative to the basal value (0 min.) in the different experimental protocols. The decrease of plasma glucose ranged from 64 to 80%. There was a greater variation in the percentages of recovery: the lowest value was found for GR (32%); the highest, for GR + E (75%). It should be noticed the low recovery in the GR given caffeine (GR+C and GR+E+C) compared with their corresponding control groups.

**Table 2.** Decrease and recovery of plasma glucose as percentages of the basal plasma glucose (0 min.), during insulin-induced hypoglycemia, in freely-fed (GC) and malnourished (GR) rats.

Subgroup	Plasma glucose decrease (% of basal)	Plasma glucose recovery (% of basal)
GC	72.13	52.91
GR	78.21	32.25
GC+E	73.75	62.92
GR+E	79.99	75.00
GC+C	75.75	62.08
GR+C	69.06	48.73
GC+E+C	63.58	60.51
GR+E+C	77.21	49.55

GC and GR: IIH without exercise or caffeine; GC+E and GR+E: acute exercise session before IIH; GC+C and GR+C: IIH with oral caffeine at 15 min.; GC+E+C and GR+E+C: IIH after acute exercise session and oral caffeine at 15 min.

**Comparison of control subgroups:** Figure 5 shows the plasma glucose profiles of the GC subgroups subjected to IIH. Some time points of GC+E, GC+C and GC+E+C were statistically compared with the GC (one-way ANOVA): 0 min.

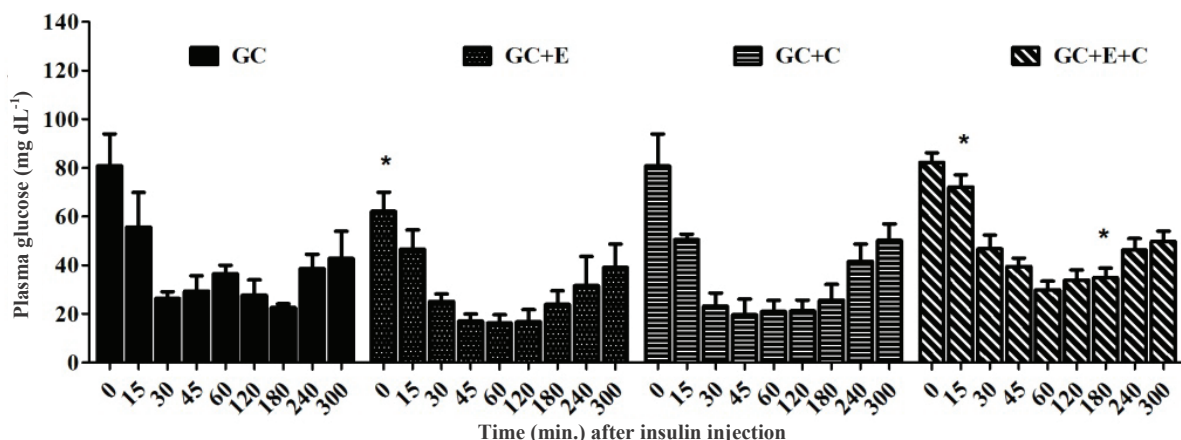
(basal plasma glucose), 15 min. (initial hypoglycemia in the GC), 180 min. (lowest plasma glucose in the GC) and 300 min. (final plasma glucose). Exercise (GC+E) significantly decreased the basal plasma glucose, but when associated with caffeine (GC+E+C) decreased the initial hypoglycemia (15 min.) and the severity of the hypoglycemia at 180 min. Qualitatively, the association of exercise+caffeine resulted in less marked hypoglycemic profile than when analyzed separately. In accordance, the GC+E+C had the lowest decrease in plasma glucose (63.58%, Table 2). The final plasma glucose was not significantly influenced by exercise and/or caffeine, but it was 8-10% better in the groups that received these treatments, compared with the GC (Table 2).

#### Comparison of malnourished subgroups:

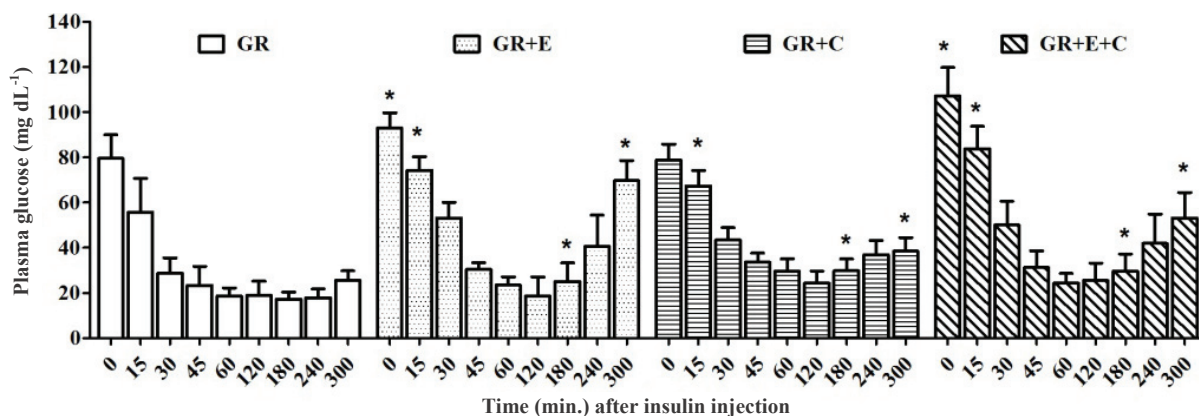
Figure 6 shows the plasma glucose profiles of the GR subgroups subjected to IIH. Some time points of GR+E, GR+C and GR+E+C were statistically compared with the GR (one-way ANOVA): 0 min.

(basal plasma glucose), 15 min. (initial hypoglycemia in the GR), 180 min. (lowest plasma glucose in the GR) and 300 min. (final plasma glucose). Exercise significantly increased the basal plasma glucose, and either alone or combined with caffeine decreased the intensity of the hypoglycemia at 15 and 180 min. and increased the final plasma glucose. Nevertheless, the results in Figure 6 and Table 2 reveal that the plasma glucose profile as a whole and the percent recovery of plasma glucose were better in the GR+E than in the subgroups given caffeine (GR+C and GR+E+C).

The increased litter size was the method chosen for food restriction during lactation in this study. Followed by a 50% restricted supply of chow, it resulted in significant delay of biometric development in the GR (Table 1), thus being considered as a model of malnutrition, as observed in other studies (WINICK; NOBLE, 1966; VISMARA; FURLAN, 2007; VITORIANO et al., 2011).



**Figure 5.** Plasma glucose profile of freely-fed rats during insulin-induced hypoglycemia (IIH) subjected to different experimental protocols. Data presented as mean±SD. \* $p < 0.05$  compared with GC, one-way ANOVA with Bonferroni;  $n = 6-12$ . GC: IIH without exercise or caffeine; GC+E: acute exercise session before IIH; GC+C: IIH with oral caffeine at 15 min.; GC+E+C: IIH after acute exercise session and oral caffeine at 15 min.



**Figure 6.** Plasma glucose profile of malnourished rats during insulin-induced hypoglycemia (IIH) subjected to different experimental protocols. Data presented as mean±SD. \* $p < 0.05$  compared with GR, one-way ANOVA with Bonferroni;  $n = 6-12$ . GR: IIH without exercise or caffeine; GR+E: acute exercise session before IIH; GR+C: IIH with oral caffeine at 15 min.; GR+E+C: IIH after acute exercise session and oral caffeine at 15 min.

Plasma glucose profiles described in this study confirmed previous observations (BABATA et al., 2014; MALTA et al., 2010) that food-restricted/malnourished rats have more persistent episodes of IHH, with little or no recovery in plasma glucose, while freely-fed rats partially recovered plasma glucose levels 5 hours after insulin injection (Figure 1).

Even single exercise sessions (i.e. acute exercise, such as that in this investigation) can have measurable effects on glucose homeostasis (THOMPSON et al., 2001). In this study, malnourished rats exercised until exhaustion had higher post-exercise plasma glucose level and significant improvement in plasma glucose profile during the following hypoglycemia, both when compared with the exercised controls and with the non-exercised malnourished rats (Figures 2 and 6, respectively, and Table 2).

The drop in the post-exercise basal plasma glucose level in the GC+E and the increase in the GR+E (Figure 2) compared with their non-exercised pairs can be related to liver glycogen; when present, it favors relative post-exercise hyperglycemia, while its absence causes relative hypoglycemia (BORBA-MURAD et al., 1998). In malnourished rats, higher levels of liver and muscle glycogen than in control rats were observed (NEIVA et al., 1999). The *in situ* liver perfusion after overnight fasting (MALTA et al., 2010; VITORIANO et al., 2011; BABATA et al., 2014) revealed high liver glucose release in the absence of gluconeogenic substrates, and hence liver glycogenolysis, in these malnourished rats, otherwise it was very low in the control animals.

The increased oxidation of fatty acids by the exercising muscles (SONNE; GALBO, 1985; AFONSO et al., 2003), with simultaneous glucose sparing, could explain the reduced insulin sensitivity – as assessed by the attenuated hypoglycemic profile – after acute exercise in the GR compared with the GC. However, taking into account that the lipid stores in malnourished rats must be relatively small, probably a marked glucose release by the liver was more important for the increased post-exercise basal plasma glucose level, the less severe hypoglycemia and the better plasma glucose recovery in the GR+E.

The longer time to exhaustion in the GR compared with the GC found herein was also recorded in similar studies (unpublished results). It is surprising because malnourished animals have a significantly lower body development, in terms of both adipose and lean mass. On the other hand, this could be indeed the determining factor for the better

performance of the GR: the smaller size and weight could reduce the energy cost of locomotion, delay the development of fatigue and prolong the time to exhaustion. Changes in body composition caused by early malnutrition – decreased body weight, less fat and muscle – can be related to the greater resistance, but certainly, other explanations, especially regarding the energy systems that fuel the skeletal muscle contraction, are involved in this result.

The rate of decrease of the hypoglycemia was less pronounced in the GR+C compared with the GC+C, that is, caffeine decreased the severity of hypoglycemia during its installation. Effectively, then, caffeine antagonized the hypoglycemic action of insulin in malnourished rats (Figures 3 and 6). In the controls, no significant effect of caffeine was detected (Figure 5). Moreover, the systemic use of glucose and insulin sensitivity are lower in resting humans after caffeine administration (GREER et al., 2001; KEIJZERS et al., 2002; THONG et al., 2002; SILVEIRA et al., 2004). Phosphodiesterase inhibition and antagonism of muscle adenosine receptors are suggested as responsible for these effects of caffeine. In addition, caffeine stimulates the release of free fatty acids and adrenaline, both of which favor systemic insulin resistance (VAN SOEREN et al., 1993; PIZZIOLO et al., 1998; GREER et al., 2001; KEIJZERS et al., 2002; THONG et al., 2002).

Thong et al. (2002) observed increased muscle uptake of glucose after exercise, but decreased uptake when caffeine was given after exercise. Therefore, these interventions had antagonistic actions in the muscle uptake of glucose. In this study, however, the systemic effect on plasma glucose during IHH in control rats was higher with the association of exercise+caffeine than with each intervention separately (Figure 5). In malnourished animals, the association of exercise+caffeine was not more effective than exercise alone in improving the hypoglycemic episode and the plasma glucose recovery (Figure 6 and Table 2). In addition, hypoglycemia was worsened in the 45-120 min. interval (Figure 4).

In summary, both the acute exercise session and caffeine had a beneficial effect on the profile of insulin-induced hypoglycemia in rats. In freely-fed animals, the association of these interventions led to the best result, with less intense hypoglycemic profile (Figure 5). In malnourished animals, caffeine and especially exercise alone had beneficial effects, but the association (exercise+caffeine) had no relevant additive effect on the hypoglycemic profile (Figure 6).

Therefore, it becomes evident that the long-term nutritional status (i.e. free or restricted feeding)



influences the body glucose dynamics and the response to external agents, in this case exercise and caffeine. This is relevant when considered that food restriction can cause asymptomatic hypoglycemia, is resistant to carbohydrate administration and shows impaired endogenous counterregulation (FIELD, 1989; LEON-QUINTO et al., 1997). Exercise and caffeine could be considered as exogenous factors of potential anti-hypoglycemic action.

## Conclusion

Both the acute exercise session and caffeine had a beneficial effect on the profile of insulin-induced hypoglycemia in normally-fed rats. In malnourished animals, these interventions are more effective when given separately than when associated. Therefore, exercise and caffeine had potential anti-hypoglycemic action, but the nutritional status of the individual is relevant.

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