ORIGINAL ARTICLE

Effect of maltodextrin supplementation on blood glucose, lactate, insulin, and cortisol levels in horses subjected to submaximal incremental exercise test

Efeito da suplementação com maltodextrina sobre as concentrações sanguíneas de glicose, insulina e cortisol em cavalos submetidos a teste incremental submaximo

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Abstract

Riders and trainers believe that oral maltodextrin (MD) supplementation prior to exercise increases blood glucose availability and delay the onset of fatigue in horses, although there is no evidence supporting this claim. Therefore, we aimed to evaluate the effect of MD supplementation on blood glucose, L-lactate, insulin, cortisol levels, and heart rate in horses subjected to an incremental exercise test. A randomized placebo-controlled crossover trial was designed to test the effect of three doses of MD (50, 100 and 200 g) on blood glucose, L-lactate, insulin, cortisol levels, and heart rate of four Purebred Lusitanian geldings. A seven-step incremental field exercise test was used; the initial speed was set at 2.5 m/s, followed by 3.3 m/s, 4.1 m/s, 5.0 m/s, 6.6 m/s, and 8.4 m/s. There was a significant influence of exercise itself on plasma glucose and L-lactate concentrations and on heart rate ($p < 0.05$), whereas the amount of MD administered changed only the cortisol levels ($p < 0.05$). During rest and exercise, the variables remained within the normal reference range for stalled and exercising horses, respectively. Blood glucose levels in the placebo group at rest, 5.0 m/s, and 8.4 m/s were 87.63 ± 3.25 mg/dL, 78.33 ± 10.08 mg/dL, and 95.18 ± 14.73 mg/dL, respectively, whereas those for the group supplemented with 200 g MD were 99.25 ± 12.19 mg/dL, 79.55 ± 13.81 mg/dL, and 97.30 ± 16.46 mg/dL, respectively. The mean cortisol levels in the placebo and 200 g MD groups at 8.4 m/s were 1.20 ± 0.53 mmol/L and 5.54 ± 4.73 mmol/L,

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respectively. In conclusion, MD supplementation increased serum cortisol levels and showed no effect on heart rate, blood glucose, L-lactate, and serum insulin levels in horses submitted to an incremental exercise test.

Kewyords: Carbohydrates. Exercise test. Glycolysis.

Resumo

Treinadores e cavaleiros acreditam que a suplementação oral com maltodextrina (MD) nos momentos que antecedem sessões de treinamentos e competições equestres pode elevar a disponibilidade de glicose e aumentar a tolerância ao exercício, entretanto não há evidência de que MD efetivamente atue sobre a glicemia durante o exercício em equinos. O objetivo do presente estudo foi avaliar o efeito da suplementação com MD sobre os níveis sanguíneos de glicose, lactato, insulina e cortisol em cavalos submetidos a teste físico a campo. Um ensaio clínico randomizado, controlado por placebo, do tipo "crossover" foi conduzindo para testar o efeito da suplementação com MD. Os tratamentos diferiram entre si quanto a quantidade de MD administrada (50 g, 100 g, 200 g e placebo). Conduziu-se então um teste escalonado incremental submáximo, composto por sete estágios (2,5 m/s, 3,3 m/s, 4,1 m/s, 5,0 m/s, 6,6 m/s e 8,4 m/s). Houve efeito significativo do protocolo do teste sobre as médias de glicose, lactato e frequência cardíaca ($p < 0,05$), e além disso observou-se que a quantidade de MD administrada influenciou as concentrações séricas de cortisol ($p < 0,05$). As concentrações médias de glicose no grupo placebo em repouso, 5,0 m/s, e 8,4 m/s foram 87,63 ± 3,25 mg/dL, 78,33 ± 10,08 mg/dL, e 95,18 ± 14,73 mg/dL, respectivamente, enquanto que no grupo suplementado com 200 g MD as médias foram 99,25 ± 12,19 mg/dL, 79,55 ± 13,81 mg/dL e 97,30±16,46 mg/dL, respectivamente. As concentrações séricas médias de cortisol do grupo placebo e no grupo 200 g MD à velocidade de 8,4 m/s foram 1,20 ± 0,53 mmol/L e 5,54 ± 4,73 mmol/L, respectivamente. Em conclusão, a suplementação oral com MD aumentou os níveis séricos de cortisol, mas não apresentou efeito sobre frequência cardíaca, concentração sanguínea de glicose, lactato e insulina em cavalos submetidos a teste incremental de esforço.

***Palavras-chave**: Carboidrato. Teste físico. Glicólise.*

Introduction

Oral carbohydrate (CHO) supplementation is a nutritional strategy widely adopted across many equestrian disciplines. Trainers and riders believe that increased blood glucose availability leads to enhanced endurance performance, and rapid post-exercise recovery. This belief is probably an erroneous extrapolation from the study of Farris et al. (1995), who showed that intravenous glucose infusion increased blood glucose availability and delayed the onset of fatigue in horses during submaximal exercise on a treadmill. Furthermore, oral supplementation with combined glucose and fructose effectively increased glucose availability during low-intensity exercise in horses (Bullimore et al., 2000).

In addition to simple carbohydrates, products of starch hydrolysis, known as maltodextrins (MD), which are classified as complex carbohydrates with a high glycemic index, have attracted attention within the field of human sports nutrition (Chronakis, 1998). MD supplementation prior and during biathlons was shown to prevent exercise-induced hypoglycemia, without increasing blood insulin and cortisol levels (Mamus et al., 2006).

Although the effect of riding exercise on equine glucose, insulin, and cortisol levels is well documented (Gordon et al., 2007; Strzelec et al., 2011), there is limited evidence to suggest that MD increases glucose availability in exercising horses without altering cortisol and insulin levels. A previous study showed that MD is well absorbed and may replace starch content in equine diets (Nunes Gil et al., 2012), but this study was performed in horses at rest and did not measure insulin and cortisol responses during exercise.

Although commercial MD products are currently available on the market, neither the efficacy nor the biosafety of these products have been demonstrated. Therefore, the aim of this study was to evaluate the effects of MD supplementation on blood glucose, L-lactate, insulin, cortisol levels, and heart rate in horses subjected to an incremental exercise test. We hypothesized that MD supplementation will increase glucose availability and enhance the horses' ability to maintain its power output during exercise.

Material and methods

Study design

This was a randomized, placebo controlled, crossover trial evaluating the effects of three doses of MD (50, 100 and 200 g) against a placebo (water), which were orally administered prior to an incremental exercise test. The variables heart rate, blood glucose, lactate, insulin, and cortisol levels were evaluated. The order of the treatments was stochastically defined and the washout period was defined as 14 days.

Animals and ethics

Four Purebred Lusitanian geldings were used for this study (each aged 3 - 4 years old, weighing 480 ± 20 kg). The animals were housed in individual boxes (3 × 3 × 5 m), and regularly fed hay and commercial feed. The horses were clinically sound at the onset of the experiment, and conditioned for dressage competitions. The study was approved by the Animal Research Ethics Committee of the University of São Paulo, School of Veterinary Medicine and Animal Science (protocol n. 7256300114).

Nutritional intervention and exercise testing

Maltodextrin-water solution was orally administered 30 min before a standardized exercise test. Three doses of pure MD (Galena Quimica e Farmaceutica© - Campinas, Brazil) (50, 100 and 200 g) were diluted in warm water (60 mL) prior to administration. Placebo solution (only water) was also used. The tip of a 20 mL-syringe was cut to enable the administration of maltodextrin-water solution, which viscosity implies certain resistance.

After oral administration, the horses completed an incremental exercise test in a field comprising seven discrete steps; the initial speed was set at 2.5 m/s, followed by 3.3 m/s, 4.1 m/s, 5.0 m/s, 6.6 m/s, and 8.4 m/s, where the same rider was used. Each step lasted 3 min (initial 2 min and 45 s of exercise and 15 s at rest for blood collection). The first step (2.5 m/s) was performed at walk, followed by 3 steps at trot (3.3 m/s, 4.1 m/s and 5.0 m/s), and the last two steps (6.6 m/s and 8.4 m/s) were completed at gallop.

Sound markers were used to alert the rider to maintain a specific velocity at each step. The interval between consecutive sound markers was calculated, and outcomes were processed using audio mixer software (MixPad Recording Software© - Greenwood Village, USA).

The racetrack had a squared design: four straight lines, each one measuring 40 m with curved lines at each vernicle. The racetrack surface was covered with 20 cm of dry sand, and smoothened prior to the exercise test.

The exercise test and sample collection were performed in the morning (9 - 12 AM).

Sample collection and processing

Blood samples were collected from the jugular vein at rest, and during the last 15 s of each step of the test (2.5 m/s, 3.3 m/s, 4.1 m/s, 5.0 m/s, 6.6 m/s, and 8.4 m/s). Blood samples were divided into sodium fluoride-containing tubes and tubes without the anticoagulant and centrifuged (5,000 RPM, 10 min.); plasma/serum aliquots were stored at -80 °C.

Plasma L-lactate and glucose concentrations were analyzed through colorimetric and hexokinase methods, respectively, using an automated analyzer (Randox© - Crumlim, United Kingdom) and commercial kits (L-LACTATE© and GLUC-PAP© - Crumlim, United Kingdom).

Serum insulin levels were determined using a species-specific kit (Mercodia© - Uppsala, Sweden), over five steps: 0 m/s, 2.5 m/s, 3.3 m/s, 4.1 m/s, and 8.4 m/s. Serum cortisol levels were analyzed using an enzyme-linked immunosorbent assay (ELISA), multiple species ADI 907-0711 (ENZO© - São Caetano do Sul, Brazil), measured over all steps of the test.

Heart rate was continuously monitored using the Polar System RS800 (Polar Electro) (Polar© - Embu das Artes, Brazil). Data were exported using an infrared device (Polar© - Embu das Artes, Brazil).

Statistical analysis

Assumption of normality was assessed using a Shapiro-Wilk test ($p > 0.15$), and the baseline

differences between the groups were tested for each outcome using a one-way analysis of variance (ANOVA). A repeated measures ANOVA was performed to test the main effects of "group," "speed," and "interaction (group*speed)," using a significance level of 5%. Both "group" and "speed" were set as fixed factors, and "horses" was set as a random factor. Tukey's test was performed for *post hoc* analysis. Data are presented in the table and graphic as mean ± standard deviation. Pearson's correlation analysis was performed for the whole set of variables at a significance level of 1%.

Results and discussion

Blood glucose, blood L-lactate, serum insulin, serum cortisol, and heart rate were within the reference range at rest, and there were no differences among groups. Blood glucose, blood L-lactate, serum insulin levels, and the heart rate did not differ among groups during the exercise test; the serum cortisol concentrations of the supplemented groups (50, 100 and 200 g) were higher than placebo group (0 g) ($p < 0,01$) (Figure 1 and Table 1). Furthermore, horses supplemented with 200g of MD showed the highest serum cortisol levels (5.54 ± 4.73 mmol/L) at the last step of the test (8,4 m/s), which is 4.5 times greater than horses supplemented with placebo, at the same step (8,4 m/s; serum cortisol equal to 1.20 ± 0.53 mmol/L).

The main finding of this preliminary study was that MD supplementation did not significantly affect the blood glucose levels in horses engaged in the incremental exercise test. This observation disagrees with previous studies performed in resting horses (Nunes Gil et al., 2012), and in exercising humans (Mamus et al., 2006), which supports the association between MD supplementation and increased blood glucose availability.

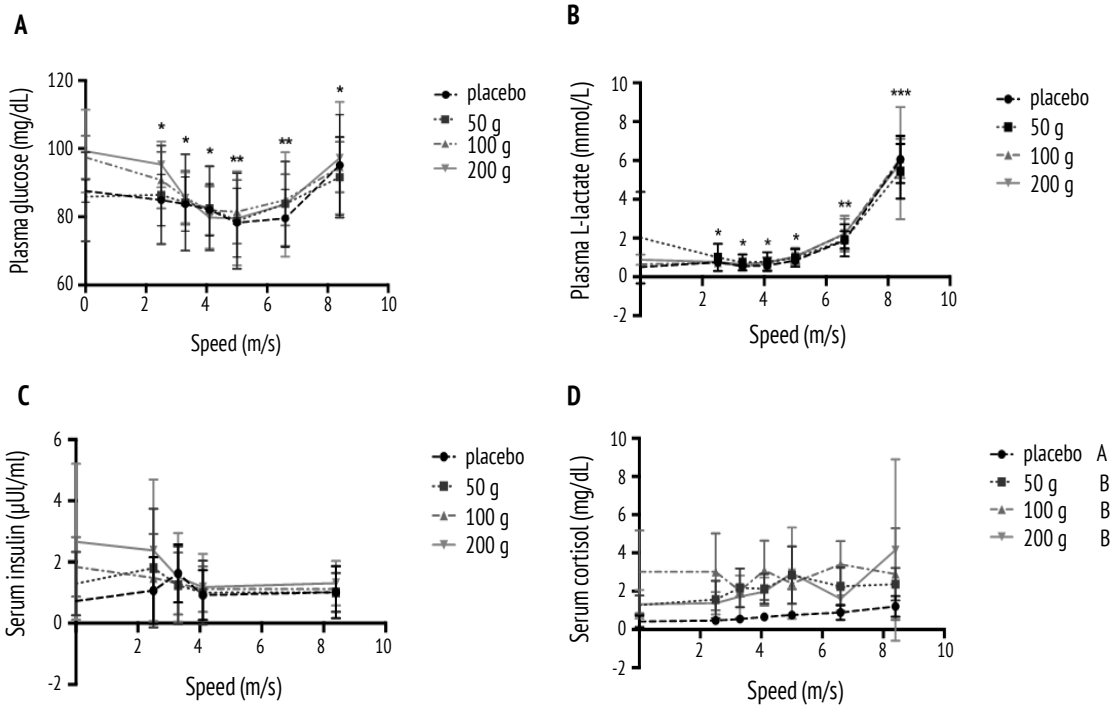

Figure 1 - Plasma glucose (A), L-lactate (B), serum insulin (C) and cortisol (D) levels in horses subjected to submaximal incremental exercise test following supplementation with maltodextrin (50, 100 and 200 g), and placebo (water). * Represents speed effect; $^{A/B}$ Indicates group effect.

Table 1 - Plasma glucose, L-lactate, serum insulin, and cortisol levels in horses subjected to a submaximal incremental exercise test following supplementation with maltodextrin (50, 100 and 200 g), and placebo (water)

Group	Speed	obs	Glucose	Lactate	HR	Cortisol	Insulin
g	*m/s*	*n*	*mg/dL*	*mmol/L*	*beats per min*	*mmol/L*	*µUI/mL*
0	0	4	87.63 ± 3.25	0.45 ± 0.11	47 ± 20	0.41 ± 0.28	0.72 ± 0.08
	2.5	4	84.90 ± 7.51	0.68 ± 0.18	65 ± 16	0.46 ± 0.12	1.06 ±1.09
	3.3	4	83.75 ± 7.90	0.50 ± 0.57	76 ± 16	0.54 ± 0.13	1.63 ± 0.94
	4.1	4	82.08 ± 7.71	0.52 ± 0.76	83 ± 21	0.64 ± 0.21	0.92 ± 0.81
	5.0	4	78.33 ± 10.08	0.76 ± 0.20	101 ± 25	0.74 ± 0.21	-
	6.6	4	79.58 ± 8.51	1.69 ± 0.75	120 ± 18	0.88 ± 0.38	-
	8.4	4	95.18 ± 14.73	5.45 ± 1.10	160 ± 19	1.20 ± 0.53	1.01 0.85
50	0	4	85.95 ± 13.06	0.18 ± 0.21	52 ± 11	1.27 ± 0.50	1.29 ± 1.03
	2.5	4	86.45 ± 14.47	0.90 ± 0.63	55 ± 14	1.56 ± 0.98	1.80 1.94
	3.3	4	84.20 ± 14.16	0.6 ± 0.37	71 ± 16	2.17 ± 1.01	1.24 ± 1.27
	4.1	4	82.50 ± 12.40	0.71 ± 0.43	92 ± 25	2.12 ± 0.78	1.00 ± 1.04
	5.0	4	78.80 ± 14.08	0.88 ± 0.41	103 ± 24	2.84 ± 1.50	-
	6.6	4	83.83 ± 12.40	1.72 ± 0.39	134 ± 76	2.25 ± 0.98	-
	8.4	4	91.63 ± 11.82	4.90 ± 1.27	159 ± 15	2.36 ± 0.85	1.01± 0.63
100	0	4	97.48 ± 6.31	0.58 ± 0.11	45 ± 88	3.01 ± 2.17	1.84 ± 0.97
	2.5	4	90.78 ± 8.32	0.66 ± 0.19	57 ± 12	3.01 ± 2.01	1.48 ± 1.43
	3.3	4	85.65 ± 7.91	0.50 ± 0.16	77 ± 38	2.02 ± 3.07	1.29 ± 1.01
	4.1	4	81.95 ± 7.29	0.60 ± 0.30	86 ± 10	3.09 ± 1.55	1.11 ± 0.75
	5.0	4	81.48 ± 9.36	0.94 ± 0.33	114 ± 12	2.40 ± 3.01	-
	6.6	4	84.98 ± 7.48	1.97 ± 0.71	134 ± 16	3.39 ± 1.22	-
	8.4	3	94.63 ± 7.43	5.50 ± 0.91	173 ± 19	3.85 ± 1.75	1.11 ± 0.74
200	0	4	99.25 ± 12.19	0.80 ± 0.23	44 ± 86	1.31 ± 0.75	2.66 ± 2.55
	2.5	4	95.40 ± 6.74	0.70 ± 0.18	57 ± 11	1.37 ± 0.59	2.38 ± 2.31
	3.3	4	85.58 ± 7.46	0.59 ± 0.20	65 ± 60	1.71 ± 1.11	1.50 ± 1.44
	4.1	4	79.80 ± 9.12	0.66 ± 0.17	91 ± 94	1.97 ± 0.74	1.18 ± 1.08
	5.0	4	79.55 ± 13.81	9.45 ± 4.07	106 ± 16	2.94 ± 2.39	-
	6.6	4	83.70 ± 15.30	2.00 ± 0.84	136 ± 16	1.60 ± 0.62	-
	8.4	3	97.30 ± 16.46	5.28 ± 2.61	161 ± 34	5.54 ± 4.73	1.30 ± 0.72

The remarkable sympathetic response to intense exercise, which has been previously described in horses (Snow et al., 1992), may play an important role in MD supplementation. Circulating catecholamine acts on blood flow redistribution, muscle and liver glycogen breakdown, GLUT-4 activity, and glucose oxidation rate (Richter and Hargreaves, 2013). These physiological changes indicate that a specific metabolic set takes place during exercise to prioritize muscle demands over other functions such as digestion and absorption. Recently, CHO absorption during exercise has aroused the interest of researchers; studies in athletes indicate that the gut is able to adapt to nutritional training and improve nutrients delivery during exercise (Jeukendrup, 2017).

The reduced amount of MD administered to our experimental units may also account for the reported gap between MD supplementation and glucose availability. Nunes Gil et al. (2012)

administered 800 - 2500 g of MD following an adaptation period, and observed expected effects on blood glucose and insulin levels. According to the authors' knowledge, there is a lack of consensus in the literature regarding the optimum amount of MD supplementation for horses prior to exercise. However, based on previous studies evaluating CHO intake in humans (3.0 g/kg) (Potgieter, 2013), even the upper limits of CHO supplementation (200 g) had negligible effects on glucose levels, and higher doses could still be investigated.

We report an exponential increase in L-lactate concentration following the first half of the test, which was likely due to type II muscle fiber recruitment and increased rate of anaerobic glycolysis. Blood L-lactate and heart rate response to submaximal exercise test, as observed in this study, agree with several studies that used the field exercise protocol to evaluate fitness and performance in Thoroughbred (Evans, 2007), Arabian (Goachet and Julliand, 2015), and Warmblood sport horses (Munsters et al., 2014), as well as in Mangalarga Marchador horses (Bogossian et al., 2017). This agreement suggests that the Purebred Lusitanian breed would also be an interesting model for future investigations in sport nutrition.

Insulin levels were unchanged with speed or nutritional intervention in this study; graphical analyses suggest that speed "grouped" the data, attenuating apparent baseline difference. Starch replacement by MD in resting horses showed a positive effect on insulin (Nunes Gil et al., 2012), although, as cited elsewhere, the smaller amount of MD used in our study might account for this difference.

Finally, previous studies on exercise-induced hypercortisolemia suggested that there was a positive association between high-intensity exercise and increased salivary cortisol levels in horses (Strzelec et al., 2011) and in humans (Jacks et al., 2002). Our exercise protocol involved only submaximal intensities and was unlikely to elicit a stress response. We show that the effects of nutritional intervention on cortisol levels (group effect) in horses fails to conform to the previously accepted consensus that cortisol secretion during exercise in humans functions as a metabolic response for glucose homeostasis (MacLaren et al., 1999; Lane et al., 2010). Future studies will investigate the association between glucose availability and cortisol secretion in horses.

Conclusion

Maltodextrin supplementation increased serum cortisol levels and showed no effect on heart rate, blood glucose, L-lactate and serum insulin levels in horses subjected to an incremental exercise test.

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