

Effect of swimming training on stress-related metabolic parameters of diabetic and non-diabetic rats

Efeito do treinamento de natação sobre estresse metabólico de ratos diabéticos e não diabéticos

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ABSTRACT

Physical training can be an useful strategy to attenuate the stress markers concentrations and hyperglycemia on type 1 diabetic rats. Thus, the purpose of the present study was to investigate the effects of swimming training on metabolic parameters and stress markers in diabetic and non-diabetic rats. Twenty-four adult male Wistar rats were recruited. The rats were randomly divided into four groups, sedentary control (SC), trained control (TC), sedentary diabetic (SD), and trained diabetic (TD). The diabetic rats received alloxan monohydrate and the trained rats swam for 60 minutes five times per week. The training load was 3.5% and 5% of body weight to diabetic and non-diabetic rats, respectively. After the euthanasia, blood samples for determination of corticosterone, insulin, and glucose levels were collected, and the adrenal weight, adrenal cholesterol concentration, gastrocnemius glycogen concentration, and muscle total protein content were also determined. To compare the groups, a variance analysis was used with Tukey's post-hoc. The significance level of 5% was adopted. The adrenal weight was higher in SD (17.2 ± 0.6 g) than the other groups (SC: 12.2 ± 0.8 g; TC: 13.7 ± 0.6 g; TD: 14.3 ± 0.8 g), and TD had similar values of TC. The corticosterone level of SD was higher than SC and TC. The serum glucose was higher in the diabetic groups and insulin was lower in these groups. Six weeks of swimming training was efficient to reduce stress markers concentration in type 1 rats. Training may be a good tool to avoid the reduction on muscle protein content in type 1 diabetic rats.

KEYWORDS

Stress; Diabetes; Exercise; Metabolism.

RESUMO

Treinamento físico pode ser uma estratégia útil para atenuar as concentrações de marcadores de estresse e hiperglicemia em ratos diabéticos tipo 1. Assim, objetivo do estudo foi investigar o efeito do treinamento de natação sobre os parâmetros metabólicos e marcadores de estresse em ratos diabéticos e não diabéticos. Foram selecionados vinte e quatro ratos machos Wistar divididos em quatro grupos, controle sedentário (SC), controle treinado (TC), diabético sedentário (SD) e diabético treinado (TD). A indução de diabetes foi por aloxana monoidratada. Os ratos foram submetidos a 60 minutos de natação cinco vezes por semana com carga de 3,5% e 5% do peso corporal para ratos diabéticos e não diabéticos, respectivamente. Depois da eutanásia, foram determinados corticosterona, insulina, glicose, peso da adrenal, concentração de colesterol da adrenal e glicogênio do músculo gastrocnêmio e conteúdo proteico total muscular também foram determinados. Foi aplicada análise de variância e Post Hoc de Tukey, com significância estatística menor que 5%. Peso da adrenal foi maior em SD (17.2 ± 0.6 g) que nos outros grupos (SC: 12.2 ± 0.8 g; TC: 13.7 ± 0.6 g; TD: 14.3 ± 0.8 g) e TD apresentou valores similares de TC. A corticosterona de SD foi maior do que SC e TC. Glicose sérica foi elevada no grupo diabético e a insulina foi menor neste grupo. Seis semanas de treinamento de natação foram eficientes para reduzir a concentração de marcadores de estresse em ratos diabéticos tipo 1. O treinamento de natação pode ser uma ferramenta útil para evitar a redução do conteúdo proteico muscular em ratos diabéticos tipo 1.

PALAVRAS-CHAVE

Estresse, Diabetes Mellitus Tipo 1, Exercício, Metabolismo

INTRODUCTION

Diabetes Mellitus is considered a worldwide health problem that affects about 347 millions of people, and there is an estimative that the prevalence of this disease will increase to 366 million people in 2030¹. In 2004, about 3.4 millions of people died in consequence of hyperglycemia, and it is estimated that diabetes will be the seventh leading cause of death in 2030².

This endocrine-metabolic disturbance is characterized by increased concentration of blood glucose, resulting from decreased or stoppage in insulin synthesis and secretion by pancreas, or from insulin resistance in peripheral tissues³, which defines type 1 (DM1) and type 2 (DM2) diabetes, respectively. The absence or reduction of insulin action causes a sustained state of hyperglycemia, and increases serum concentration of free fatty acids and lipoproteins³. Moreover, it alters the secretion and liberation of several hormones, resulting in changes in energetic metabolism³. Experimental models of diabetes, induced by chemical substances that damage the pancreatic cells, have been frequently used to simulate disease carriers³.

Physical exercise seems to be an important strategy to attenuate hyperglycemia in type 1 diabetic rats⁴ and promotes physiological and metabolic adjustments that ameliorate homeostasis⁵. These benefits of physical exercise can be observed after acute and chronic practice of exercise. Acute exercise can translocate the glucose transporter to the cell membrane independently of insulin action, which is mediated by muscle contraction⁶. This and other responses reflect a reaction to stress. Among these responses, the activation of the hypothalamic-pituitary-adrenal axis, that stimulates the releasing of adrenocorticotrophic hormone (ACTH) deserves attention. The release of cortisol or corticosterone, the main glucocorticoid secreted by the cortex of the adrenal gland, is stimulated by ACTH and is directly involved in the homeostasis of energy metabolism^{7,8}. However, even though physical training acutely increase catecholamine and cortisol, it seems that chronically, aerobic training decreases the releasing of ACTH⁸, and attenuates the hyperglycemia and other cardiometabolic alterations, even in rats treated with dexamethasone⁹.

Previous studies have shown higher basal level of corticosterone in streptozotocin-diabetic rats compared with control group. However, this effect can be prevented by insulin replacement¹⁰. It is known that physical exercise can modulate the hormonal response of cortisol, and some authors consider this an important reaction in the adaptive response to stress¹¹. Thus, exercise might be useful to promote a non-pharmacological control of glucose homeostasis and corticosterone release¹¹. However, the potential effect of swimming training on hormonal (corticosterone) concentration, glucose metabolism and adrenal weight, and the relation between the stress markers concentration and metabolic parameters has not been assessed yet. Thus, the aim of the study was to investigate the effects of swimming training on metabolic parameters and stress markers in diabetic and non-diabetic rats and their relationships.

METHODS

Animals

Twenty-four adult male Wistar strain rats were recruited from the Central

Animal Breeding House of São Paulo State University - UNESP, Botucatu, SP; and they were maintained at the Department of Physical Education, UNESP, Rio Claro, SP, Brazil. The rats were approximately 90 days old at the beginning of the experiment. The animals were housed four per cage in a temperature-controlled room ($25 \pm 1^\circ\text{C}$) with a 12/12 h light/dark cycle and free access to food and water.

Body weight was recorded at the beginning of the training and once a week until the day before the sacrifice. All experiments were performed in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes¹². Study was approved by the research ethics committee of the Universidade Estadual Paulista “Julio de Mesquita Filho”, Rio Claro/SP Campus, Brazil.

Experimental groups

The rats were randomly divided into four groups with 6 animals per group, as follows: sedentary control (SC), consisting of euglycemic rats that have not trained; trained control (TC), consisting of euglycemic rats that trained five days per week for 60 min; sedentary diabetic (SD), consisting of alloxan induced diabetic rats that have not trained; and trained diabetic (TD), consisting of alloxan-induced diabetic rats that trained five days per week for 60 min.

Induction of diabetes

For the induction of experimental diabetes, rats received alloxan monohydrate (Sigma) (32 mg/kg body weight) dissolved in a 0.01 M citrate buffer, pH 4.5, and injected into the penile vein (20). After this procedure, the rats were relocated in cages, where they received glucose and water solution (15%) and food ad libitum (19) for the first 24 hr. Five days after drug administration, a blood glucose test was made to confirm the diabetic state; and animals with blood glucose level equal to or greater than 150 mg/dL were identified as diabetic. The control group was injected with citrate buffer to be submitted to the same stress as the animals that received alloxan.

Training Protocol

Before the training period, the trained rats were adapted to the training environment to reduce stress levels. Rats were placed in individual 100x80x80 cm water tanks (water temperature $31 \pm 1^\circ\text{C}$) for 1–10 min daily over a period of 10 days before alloxan administration. Ten rats were adapted for time. Physical training began one week after alloxan administration¹³. The TC and TD were submitted to six weeks of aerobic training. The training consisted of 60 minutes of swimming, five times per week. The training load was equivalent to 3.5% of body weight for the diabetic rats⁵ and 5% for the non-diabetic rats¹¹.

Animal euthanasia and biological material collection

At the end of the experiment, the animals were sedated in a CO_2 chamber and euthanized by cardiac blood puncture, in accordance to previous studies¹⁰. The euthanasia in the trained groups was performed ~48h after the last exercise session.

The collected blood was centrifuged and the serum was used to determine the levels of glucose, insulin and corticosterone. Gastrocnemius muscle aliquots were weighed. These samples were used to determine the total protein

content of gastrocnemius (TPG) and the concentration of glycogen (GG) of the gastrocnemius muscle. The subcutaneous adipose tissue was completely removed for the analysis.

Analytical methods

Glucose, insulin, and corticosterone levels were measured. The concentration of serum glucose (SGlu) was determined through the glucose oxidase method using commercial kits (Laborlab®, Guarulhos, Brazil). The serum insulin levels (SIns) were assessed using radioimmunoassay (RIA, Kit Coat-A-Count, Los Angeles, USA). Serum corticosterone (SCort) was measured by the radioimmunoassay (RIA) with the use of the commercially available kit (DPC, Los Angeles, USA). All these procedures were conducted 48 hours after the euthanasia.

Tissue analyzes

Once removed and dissected on a filter paper moistened with saline the adrenal glands were weighed into an analytical balance. Then, the adrenal gland was removed and macerated for determining the content of ascorbic acid. The ascorbic acid concentration was measured 520 nm against a standard curve of ascorbic acid⁶.

For determination of the concentrations of total cholesterol in the adrenal gland, samples were homogenized in 1.5 mL of cooled chloroform/methanol mixed solution (2:1). Furthermore, 2.5 mL of cooled chloroform/methanol mixed solution (2:1) was added, and homogenized again and vortex-mixed. Lipid was extracted from homogenized samples overnight under refrigeration (4°C). Total and free cholesterol in the adrenal gland were determined enzymatically using a commercially available Cholesterol-E test Wako kit (Wako Pure Chemical Industries, Osaka, Japan) and Free Cholesterol-E test Wako kit (Wako Pure Chemical Industries, Osaka, Japan), respectively. The amount of cholesterol ester was assumed to be the difference between total and free cholesterol. The total corticosterone levels were measured using RIA kits (DRG-Instruments, Marburg, Germany), according to the manufacturer's instructions.

The gastrocnemius sample was rapidly obtained, weighted, and frozen for glycogen and TPG analysis. For GG, after digestion in 1 mL of 30% KOH for 60 min in a warm bath, precipitation of glycogen was performed in 0.1 mL saturated Na₂SO₄ solution and 3.5 mL ethanol, following the dosage according to the method of DuBois et al¹⁴. The dosage of total protein content of gastrocnemius (TPG) was performed according to the method of Bradford¹⁵.

Statistical analysis

The numerical variables are presented as mean and standard deviation (SD). Firstly, Levene's test was applied, and afterwards, the one-way variance analysis (ANOVA) was used to compare the sample groups (SC; TC; SD and TD). If necessary, the post-hoc of Tukey was used. The association between stress markers and metabolic parameters was assessed by Pearson correlation. A significance level of 5% was adopted. Statistical analyses were carried out using the software Statistical Package for Social Science (SPSS®: version 13.0).

RESULTS

The sample consisted of 24 male Wistar rats (60 days life) and 6 animals were

allocated in each group. Table 1 presents metabolic and hormonal parameters measured after six weeks of study in the four groups. The adrenal weight was higher in SD compared with all the other groups, and the TD had an adrenal weight similar to the TC.

After six weeks, the SD presented lower weight, and GG than other groups and lower TPG than TC. Both control groups showed higher Peri-epididymal concentration than diabetic groups (Table 1).

Serum glucose was elevated in the diabetic groups (SD: 299.5 ± 25.2 mg/dL and TD: 265.4 ± 26.6 mg/dL) compared with the control groups (SC: 119.9 ± 5.0 mg/dL and TC: 113.8 ± 7.6 mg/dL) (Figure 1A). Furthermore, serum insulin of the SD and TD (9.3 ± 0.9 and 9.5 ± 0.8 μ UI/mL, respectively) were significantly lower than the SC and TC (19.55 ± 0.6 μ UI/mL and 17.6 ± 0.8 μ UI/mL, respectively) (Figure 1B). Serum corticosterone of the SD (283.5 ± 41.1 ng/mL) was significantly elevated than the control groups (SC: 220.3 ± 14.2 ng/mL; TC: 225.9 ± 18.8 ng/mL), while serum corticosterone of the TD (249.5 ± 31.2 ng/mL) did not differ from all the other groups (Figure 1C).

Serum glucose concentration presented a positive correlation with corticosterone and adrenal weight, while serum insulin concentration presented a negative correlation with corticosterone and adrenal weight (Table 2).

TABLE 1 – Metabolic and hormonal parameters measured after six weeks of the study in male Wistar control and diabetic rats evaluated after 48 hours rest.

	SC (n=6)	TC (n=6)	SD (n=6)	TD (n=6)	F	p-value
Body weight (g)	359.6 \pm 8.7	332.5 \pm 22.8	281.3 \pm 30.1 ^{a,b}	334.6 \pm 25.3 ^c	12.6	0.00*
Adrenal weight (g)	12.2 \pm 0.8	13.7 \pm 0.6 ^a	17.2 \pm 0.6 ^{a,b}	14.3 \pm 0.8 ^{a,c}	36.0	0.00*
Adrenal chol (mg/100g)	1.4 \pm 0.3	1.6 \pm 0.2	1.2 \pm 0.2	1.8 \pm 0.2 ^c	4.6	0.01*
Adrenal AA (μ g/mg)	8.1 \pm 0.6	6.6 \pm 0.5 ^a	5.7 \pm 0.4 ^a	6.0 \pm 0.6 ^a	21.0	0.00*
GG (mg/100g)	0.6 \pm 0.0	0.7 \pm 0.0 ^a	0.5 \pm 0.0 ^{a,b}	0.7 \pm 0.0 ^{a,c}	19.2	0.00*
TPG (mg/100g)	8.7 \pm 0.7	9.3 \pm 0.6	7.6 \pm 0.5 ^b	8.6 \pm 0.7	5.8	0.00*
Peri-epididymal (mg/100g)	307.3 \pm 29.2	285.5 \pm 15.0	75.0 \pm 20.6 ^{a,b}	84.6 \pm 15.3 ^{a,b}	216.1	0.00*

#Mean \pm SD; SC – sedentary control; TC – trained control; SD – sedentary diabetic; TD – trained diabetic; Chol – total cholesterol; AA – ascorbic acid; GG – gastrocnemius glycogen; TPG – total

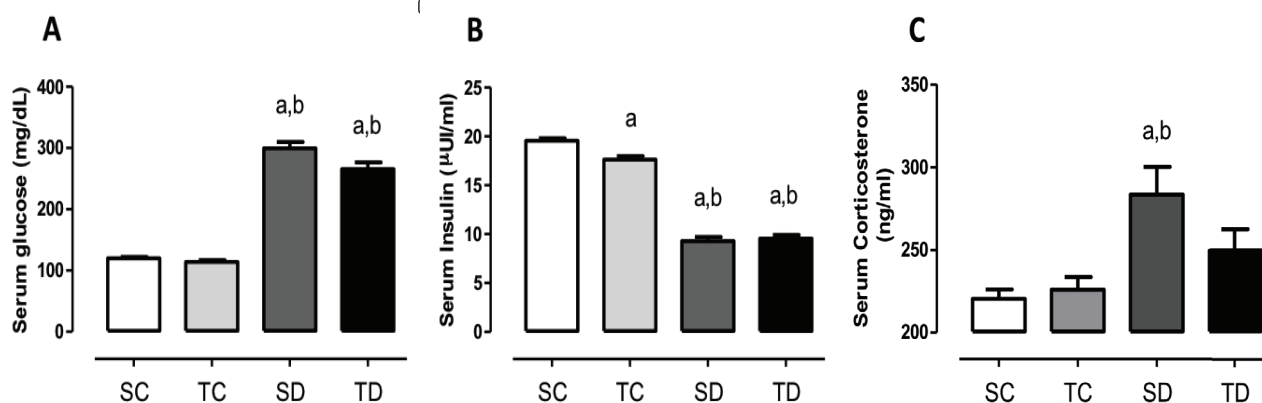


FIGURA 1 – Serum glucose (panel A), serum insulin (panel B) and serum corticosterone (panel C) measured in the 4 groups: sedentary control (SC), trained control (TC), sedentary diabetic (SD) and trained diabetic (TD), a \neq SC; b \neq TC; c \neq SD.

TABLE 2 – Correlation coefficients (r) between stress markers with metabolic parameters in non-diabetic and diabetic rats exposed or not to swimming training (n = 24).

Metabolic parameters	Stress Markers	
	Corticosterone (ng/mL)	Adrenal Weight (g)
Glucose (mg/dL)	0.59*	0.73*
Insulin (μUI/mL)	-0.62*	-0.72*

* p-value < 0.05

DISCUSSION

The purpose of the present study was to analyze the effect of six weeks of training on stress-related metabolic and hormonal parameters in controlled and diabetic rats, and their relationships.

Figures A and B show that the alloxan was effective in inducing type 1 diabetes, as has already been shown by Arantes et al.¹¹. The serum glucose of the diabetic groups was significantly higher than both SC and TC. These results agree with the study of Repetto et al.⁶. The SD and TD groups had decreased insulin concentrations compared with SC and TC. Training did not attenuate hyperglycemia in diabetic groups.

The GG was significantly higher on the training groups (TC and TD) compared with the sedentary groups (SD and SC). Our finding is consistent with the literature that points out reduced muscular glycogen concentration due to reduced glucose uptake in type 1 diabetes¹⁶. However, training stimulus seemed to promote a positive adaptation on glucose uptake even in diabetic rats. This finding is in line with Bak et al.¹⁷ who demonstrated increase of glycogen synthase activity in type 1 diabetes after training, even without enhancing insulin receptor function. Probably, the role of exercise in increasing glucose uptake¹⁸ may be the reason for this result. Manabe et al.¹⁹ have shown that training increases both the level of GLUT4 protein (major glucose transporter in skeletal muscle) and the glycogen synthase activity. The explanation for the increase of glycogen content is the augmentation of the major signaling proteins that regulate glycogen synthase (i.e. protein phosphatase 1 and glycogen synthase kinase)²⁰.

The total protein content in gastrocnemius (TPG) was significantly reduced in SD compared with the other groups. Due to the association between type 1 diabetes and lower levels of insulin growth factor²⁰ and growth hormone²¹, and their close relationship with muscle hypertrophy²², the SD presented a reduced TPG. However, the training was effective in increasing the TPG in TD. The increase in glycogen content and the swimming stimulus during the training could be a reason for the increase of TPG in TD, since mRNA of genes implicated in the regulation of hypertrophy are reduced when muscle glycogen content is low²²⁻²⁴. It is known that even aerobic swimming stimulates growth hormone/insulin growth factor-1 in type 1 diabetic rats¹⁹. Although this study did not attempt to study molecular mechanisms, the effect of increased insulin growing factor activity after training could account for the muscle modifications.

The main findings of the study were the significant lower adrenal weight of the trained groups (TC and TD) and SD compared with the SC, and the sig-

nificant higher serum corticosterone of the SD group compared with SC and TC. The adrenal gland plays a central role in the pathophysiology of stress by releasing cortisol (human) or corticosterone (rodents) into the circulatory system. Normally, glucocorticoids inhibit the hypothalamic-pituitary-adrenal axis (negative feedback)²⁴. In the present study, the SD adrenal weight was higher than all other groups, suggesting a hypertrophy of the gland due to the diabetes. This result agrees with others authors²⁴. The reason for this might be that in diabetics rats, the glucocorticoids negative feedback is impaired, increasing the action of the hypothalamic-pituitary-adrenal axis²⁵, and leading to adrenal hypertrophy.

The increased values of serum corticosterone in SD might be related to the inflammatory status of diabetic rats. Regarding this aspect, Martín-Cordero et al.²⁶ have stated that the elevation of interleukin-6 (IL-6) stimulates corticosterone release. Despite a non significant difference between corticosterone in TD and SD, the mean corticosterone value was 12% lower in TD than SD, suggesting that previous training had some effect reducing stress markers. Previous studies showed that IL-6 concentration was higher in obese rats after training²⁶, however, muscle-derived IL-6 seems to be a true anti-inflammatory “exercise factor”, protecting against insulin resistance, and enhancing glucose infusion rate²⁷. Thus, aerobic training may reduce systemic inflammation and, consequently, the corticosterone concentration^{26,27}.

Besides the positive effects of physical training, typical stress-induced responses are also described after physical training, specially its influence over the adrenal activity²⁸. Bartalucci et al²⁸ verified increases in the adrenal gland size of mice after two types of training (high and low intensity). The mice that trained at higher intensity presented 31.04% of increase in adrenal size compared to 10.08% increase in the low intensity group. This result suggests the influence of training intensity on the adrenal weight response to training. The present study observed that the TD had lower adrenal weight than SD, showing a positive effect of exercise training on modulating the adrenal activity, and leading to the same adrenal weight of the TC. Taking this finding together with the absence of difference between the serum corticosterone of SC, TC and TD (Figure 1C) evidence that aerobic swimming training was effective to reduce the stress on the adrenal gland in diabetic rats.

The present study observed a difference on total cholesterol at the adrenal gland between TD and SD, while no difference was verified between the control groups. In rats, cholesterol content after 3-weeks of swimming training associated with dexamethasone supplementation was reduced compared with control animals, however dexamethasone alone reduced even more the cholesterol content²⁹, which suggests an effect of the exercise on the liberation of corticosterone. The cholesterol content might be useful to detect overload on adrenal gland due to increased glucocorticoid release, since cholesterol is a glucocorticoid precursor²⁹. The higher cholesterol content on the TD is not in accordance with recent results²⁹ that verified increased adrenal weight and cholesterol content in psychosocial stressed mice. Thus, training might have a different impact on adrenal cholesterol when diabetes is induced.

Ascorbic acid (AA) is essential for catecholamine production since it was demonstrated that the deficiency of AA in mice induced a reduction of nor-epinephrine and epinephrine³⁰. Padayatty et al.³¹ have demonstrated that ad-

renocorticotrophic (ACTH) hormone stimulation increases Vitamin C concentration at adrenal vein. The present study showed that adrenal AA was significantly higher in the SC than the other three groups (Table 1). Thus, adrenal AA may reflect the stimulation of ACTH on adrenal gland in sedentary rats. On the other hand, mutant mice lacking the plasma membrane AA transporter (SVCT2) have severely reduced tissue levels of AA and die soon after birth. There is a significant decrease of tissue catecholamine levels in the adrenals. In the present research, SD presented reduced adrenal AA beside higher weight of the gland, suggesting a damage in plasma membrane of diabetic rats and minor processes of hormonal synthesis through the AA, but the last might have induced adrenal hypertrophy. On the other hand, physical training may attenuated these effects on diabetic rats.

One of the main findings of the present study is the relationship between stress markers (corticosterone and adrenal weight) and metabolic parameters (glucose and insulin). The higher glucose concentration, the higher is corticosterone level and adrenal weight. Thus, there is a relationship between stress and metabolic parameters in rats. Trained diabetic rats have higher (not significantly different) corticosterone levels than SC and TC, evidencing that, even with training (six weeks), the diabetes still influences his stress-related status (corticosterone). However, although not significantly different, the glucose, insulin, and corticosterone level on TD is lower than SD. Probably, a longer training period could evidence statistical differences between TD and SD, highlighting that trained diabetic rats could have more similar stress-related status than sedentary control or, even, trained control.

This study is limited by the sample size of each group, and the difficulty of extrapolating results directly to humans. Furthermore, even though six weeks can promote benefits on metabolic and stress markers in type 1 diabetic rats, probably, a longer period of training could demonstrate higher differences between the groups.

Thus, we conclude that six weeks of swimming training was able to reduce the stress related metabolic parameters induced by type 1 diabetes in rats. In addition, this training prevents the reduction on muscle protein content and enhances muscle glycogen content of diabetic rats.

Competing interests

The authors declare that they have no competing interests.

Contribution of the Authors

EZC: draft of the article and selection of manuscripts to discuss the results; APJ and HNA: selection of manuscripts to introduce and discuss the results; SUC: acquisition, analysis and interpretation of data; JCN: selection of manuscripts to discuss the results; EL: conception and design of the study, review and approval of the final version to be submitted. All authors read and approved the final manuscript.

Acknowledgements

The authors would like to thank the Rômulo A. Fernandes and Alexandre R. Spagnol from the Post-Graduate Program in Movement Sciences, UNESP, Rio Claro Campus, for their essential support this paper.

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RECEIVED 13/12/2013
REVISED 20/03/2014
27/03/2014
APPROVED 31/01/2014
