INTERVAL TRAINING IS INSUFFICIENT TO ATTENUATE METABOLIC DISTURBANCES IN DIABETIC RATS

TREINAMENTO INTERVALADO É INSUFICIENTE PARA ATENUAR DISTÚRBIOS METABÓLICOS EM RATOS DIABÉTICOS

ENTRENAMIENTO A INTERVALOS ES INSUFICIENTE PARA ATENUAR TRASTORNOS METABÓLICOS EN RATAS DIABÉTICAS

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ABSTRACT

Introduction: Type 1 diabetes is a metabolic disease associated to blood disturbances and disorder of the innate immune system functionality. Objective: This study investigated the effect of two weeks interval training on blood biochemistry and immunological parameters in rats with type 1 diabetes. Methods: Male Wistar rats were divided into three groups: sedentary (SE, n = 10), diabetic sedentary (DI, n = 10), diabetic interval training (DIT, n = 10). IV injection of streptozotocin (45 mg/kg) induced diabetes. Interval training consisted of swimming exercise for 30 seconds with 30 seconds of rest for 30 minutes three times a week during two weeks, with an overload of 15% of the total body mass. The evaluations performed were fasting blood glucose, triglycerides, very low-density lipoprotein cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol and total cholesterol concentrations, phagocytic capacity, cationic vesicles content, superoxide anion, and production of hydrogen peroxide of blood neutrophils and peritoneal macrophages. Results: The results showed that two weeks interval training did not attenuate the hyperglycemic state at rest and did not decrease blood lipids in the DIT group. Diabetes increased the functionality of blood neutrophils and peritoneal macrophages in the DI group. Interval training increased the content of cationic vesicles and the phagocytic capacity of blood neutrophils and peritoneal macrophages in the DIT group. Conclusion: It was found that two weeks of interval training increased the functionality parameters of innate immune cells, although this has been insufficient to attenuate the biochemical disorders caused by diabetes.

Keywords: diabetes mellitus; immune system; hyperglycemia.

RESUMO

Introdução: O diabetes tipo 1 é uma doença metabólica associada a alterações sanguíneas e distúrbios da funcionalidade do sistema imunológico inato. Objetivo: Este estudo investigou os efeitos de duas semanas de treinamento intervalado sobre a bioquímica sanguínea e os parâmetros imunológicos em ratos com diabetes tipo 1. Métodos: Ratos Wistar machos foram divididos em três grupos: sedentário (SE, n = 10), sedentário diabético (SD, n = 10), treinado diabético (TD, n = 10). O diabetes foi induzido por uma injeção IV de estreptozotocina (45 mg/kg). O treinamento intervalado de natação consistiu em 30 segundos de exercício, com 30 segundos de recuperação, por 30 min., três vezes por semana, durante duas semanas, com sobrecarga de 15% da massa corporal total. Foram avaliados: glicemia de jejum, triglicerídeos, frações de lipoproteínas de muito baixa densidade, baixa densidade e alta densidade do colesterol, concentrações do colesterol total, capacidade fagocítica, conteúdo de vesículas catiônicas, produção de ânion superóxido e produção de peróxido de hidrogênio pelos neutrófilos sanguíneos e macrófagos peritoneais. Resultados: Os resultados mostraram que duas semanas de treinamento intervalado não atenuaram o estado hiperglicêmico em repouso e não diminuíram os lipídeos sanguíneos do grupo TD. O diabetes aumentou a funcionalidade dos neutrófilos sanguíneos e dos macrófagos peritoneais no grupo SD. O treinamento intervalado aumentou o conteúdo das vesículas catiônicas e a capacidade fagocítica dos neutrófilos sanguíneos e macrófagos peritoneais no grupo TD. Conclusão: Em duas semanas de treinamento intervalado verificou-se aumento dos parâmetros de funcionalidade das células do sistema imunológico inato, que foi, porém, insuficiente para atenuar os distúrbios bioquímicos causados pelo diabetes.

***Descritores**: diabetes mellitus; sistema imunológico; hiperglicemia.*

RESUMEN

Introducción: La diabetes Tipo 1 es un trastorno metabólico asociado con alteraciones de la sangre y trastornos de la funcionalidad del sistema inmunológico innato. Objetivo: Este estudio investigó los efectos de dos semanas de entrenamiento a intervalos sobre la bioquímica de la sangre y los parámetros inmunológicos en ratas con diabetes tipo 1. Métodos: Ratas Wistar machos fueron divididos en tres grupos: sedentario (SE, n=10), sedentario diabético (SD, n = 10), entrenado diabético (ED, n = 10). La diabetes se indujo por medio de inyección IV de estreptozotocina (45 mg/kg). El entrenamiento a intervalos consistió en ejercicios de natación de 30 seg. con 30 seg. de recuperación por 30 minutos, tres veces por semana durante dos semanas, con una sobrecarga del 15% de la masa corporal total. Se evaluaron: glucemia en ayunas, triglicéridos, las fracciones de lipoproteínas de muy baja densidad, de lipoproteínas de baja densidad, de lipoproteínas de alta densidad del colesterol, las concentraciones de colesterol total, la capacidad fagocítica, contenido

catiónico vesicular, producción de anión superóxido, producción de peróxido de hidrógeno por neutrófilos sanguíneos, y macrófagos peritoneales. Resultados: Los resultados mostraron que dos semanas de entrenamiento a intervalos no atenuó el estado hiperglucémico en reposo ni disminuyó los lípidos sanguíneos en el grupo ED. La diabetes aumentó la funcionalidad de los neutrófilos sanguíneos y de los macrófagos peritoneales en el grupo SD. El entrenamiento a intervalos aumentó el contenido de las vesículas catiónicas y la capacidad fagocítica de los neutrófilos de la sangre y los macrófagos peritoneales en el grupo ED. Conclusión: En dos semanas de entrenamiento a intervalos se verificó el aumento de los parámetros de funcionalidad de las células del sistema inmunológico innato, que sin embargo fue insuficiente para atenuar los trastornos bioquímicos causados por la diabetes.

Descriptores: diabetes mellitus; sistema inmunológico; hiperglucemia.

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INTRODUCTION

Type 1 diabetes (T1D) is a chronic autoimmune disease characterized by the loss of insulin producing cells in the pancreas and affects 10% of the population in the developed world[1]. This metabolic disease is associated with hyperglycemia, which leads to many alterations lipids and lipoprotein metabolism, increasing risk of atherosclerosis and other cardiovascular diseases[2]. Nevertheless, T1D has been associated with various defects in the innate immune system, which in turn may increase the risk of infections[3].

For type 1 diabetes, successful management of the disease is based on individualized insulin therapy, adjusted diet and regular exercise[4]. Regular exercise is associated with significant health benefits for patients with type 1 diabetes, including increased cardiorespiratory fitness, decreased insulin requirements, improved endothelial function, decreased serum cholesterol, and increased vascular health along with improvements in body composition and quality of life[5]. Clinical practice guidelines recommend 150 min of moderate (50–70% of person's maximum heart rate) to vigorous (>70% of person's maximum heart rate) physical activity weekly for adults with T1D[6]. However, more than 60% of adults with T1D do not achieve recommended levels of physical activity[7].

In recent years, there has been renewed interest in the application of interval training (IT) for improving health. A bout of IT involves repeated vigorous-intensity efforts lasting from a few seconds up to several minutes, separated by short periods of rest or recovery[8]. There is no standardized number, length, or intensity of the intervals in a IT session, which makes inherently difficult comparisons between research studies. Nevertheless, there is accumulating evidence that a variety of IT protocols are highly effective at improving cardiorespiratory fitness, endothelial function, glycemic control, lipids profile, blood presure, muscle metabolic capacity, and insulin sensitivity[9].

Many researchers have used the experimental model of DM by streptozotocin (STZ) to investigate metabolic and immunologic alterations of DM. These animals exhibit many changes similar to those seen in humans with DM, such as hyperglycemia and dyslipidemia[10]. Our group have previously shown that, after six weeks of IT, STZ-induced diabetic rats present a significant reduction of glycaemia[11]. However, it is not known if a lower exercise volume will reproduced such findings. Therefore, here we investigate the effect of two weeks of interval training, using low volume and high intensity exercise, on blood biochemical and immune parameters in diabetic rats. We hypothesize that this protocol could attenuate the metabolic and some immune disturbances caused by type 1 diabetes model.

MATERIALS AND METHODS

Animals

All procedures involving animals were approved by the Local Committee of Animal Welfare of *Universidade Federal do Paraná* and were carried out in accordance with the ethical principles established by the Experimental Brazilian Council (COBEA), protocol number 23075.075135/2008-34. Seven-days-old male Wistar rats were kept at constant temperature (23 ± 1°C), under a light/dark cycle (12/12h) with free access to food and water. The animals were randomly divided into three groups: sedentary (SE, n=10), diabetic sedentary (DI, n=10), diabetic interval training (DIT, n=10).

Enzymes and reagents

Buffer reagents were obtained from Vetec Química Fina Ltda (Rio de Janeiro, RJ, BRAZIL). Streptozotocin was purchased from Sigma Chemical Co (St Louis, MO, USA). Bioliquid line kits (Laborclin Laboratory Products Ltda, Pinhais, PR, Brazil) were used for the biochemical assays.

Induction of diabetes

An insulin deficiency state was induced by a single intravenous injection of freshly prepared 45 mg/kg of STZ, dissolved in citrate buffer (pH 4.8), under ether anesthesia. The SE group received an equivalent volume of buffer solution. Blood samples were obtained from the tail 48-h after STZ administration. Animals with fasting blood glucose over 150 mg/dL were considered in a diabetic state[12].

Interval training protocol

The interval training protocol was based on the model proposed by Braga et al.[13] with minor modifications. The training was carried out in a swimming pool apparatus with temperature maintained between 30 and 32°C. The apparatus was composed by two tubes with a fenestrated inner tube which allowed keeping the animal into the water and removing it at the end of the set. Initially the animals performed 1 week of water adaptation (Monday, Wednesday and Friday), swimming 15 minutes continuously with an overload of 6% of their body mass fastened to them through a vest. The second week the DIT group performed 30-s exercise with 30-s rest during 2 weeks, three times a week, with an overload representing 15% of their body mass. Training sections were performed on Mondays, Wednesday and Fridays; all trained rats get rest in the other days. The SE and DI groups were submitted to the same transportation procedures and kept in a shallow pool during the time dispended by the exercising groups. At the end of 2 weeks of training, rats from all groups were anesthetized and decapitated 72-h after the last session of exercise in order to eliminate the acute effect of exercise on metabolism. Blood was collected to obtain plasma and cells by centrifugation. Resident peritoneal macrophages and blood polymorphonuclear cells were obtained for determination of phagocytic capacity, cationic vesicles content, superoxide anion and hydrogen peroxide production.

Biochemical parameters

Plasma glucose, triacylglycerol (TAG), very low density lipoprotein (VLDL), low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol and total cholesterol (TC) concentrations were

measured through colorimetric enzymatic assays as described by Togni et al.[14], 72-h after the last training section and at least 12 h fast. The results are expressed as mg/dL.

Macrophage isolation

Resident macrophages were obtained by intraperitoneal lavage with 10 mL of sterile phosphate buffered saline (PBS). Peritoneal cells were collected by centrifugation (290 *g* at 4°C for 5 min), washed, and resuspended in PBS or RPMI medium and counted in a Neubauer chamber by optical microscopy using a trypan blue solution (1%); the resulted of viability was 96%. Peritoneal cells were characterized by flow cytometry; purity was about 50%. Macrophages were further purified by incubating peritoneal cells in tissue culture plates for 2 h and washed three times with PBS to remove non-adherent cells[15].

Blood polymorphonuclear cells isolation

Polymorphonuclear cells were isolated from the blood of rats. Blood (10 mL) was diluted with an equal volume of PBS at pH 7.4 containing 100 mM $CaCl_2$–50 mM $MgCl_2$ and carefully layered on 10 mL of a commercial gradient of Ficoll-Paque Plus (density = 1.077). The tube was centrifuged at 1.200 *g* at 18°C for 30 min. The supernatant, rich in mononuclear cells, was discarded. The pellet was submitted to hypotonic treatment with 10 mL of solution containing 150 mM NH_4Cl, 10 mM $NaHCO_3$, and 0.1 mM EDTA to promote lysis of contaminating erythrocytes. The sample was homogenized and maintained for 10 min at 37°C to allow erythrocyte lysis and then centrifuged at 1.200 *g* at 4°C for 10 min. Centrifugation was repeated twice. Polymorphonuclear cells were counted in a Neubauer chamber under optical microscope. The number of viable cells, always >95% neutrophils, was determined by trypan blue exclusion[16].

Phagocytic capacity

Aliquots of peritoneal macrophage or blood neutrophil suspension (0.1 mL) were added to the wells of a 96-well flat-bottomed tissue culture plate (10^5 cells/well) and left to adhere for 60 min. The non-adherent cells were washed twice with PBS. Then 10 µl of neutral-red stained zymosan (1 x 10^8 particles/mL) were added to each well. After incubation for 30 min, the cells were fixed with Baker's formol–calcium (4% formaldehyde, 2% sodium chloride, 1% calcium acetate) for 30 min. Afterwards, the cells were washed two times and centrifuged at 450 *g* for 5 min. The neutral-red stain was solubilized by adding 0.1 mL of acidified alcohol (10% acetic acid, 40% ethanol in distilled water) to each well. After 30 min, the absorbance of each well was read on a plate reader at 550 nm. The results were expressed as percentage of control[15].

Cationic vesicles content

The uptake of the cationic dye neutral red, which concentrates mostly in cell lysosomes, was used to assess the volume of the peritoneal macrophage or blood neutrophil lysosomal system. Peritoneal macrophage or blood neutrophil suspensions (0.1 mL) were added to the wells of a 96-well flat-bottomed tissue culture plate (10^5 cells/well) and left to adhere for 60 min. The non-adherent cells were washed twice with PBS. Twenty microliters of 3% neutral red in PBS were added to the adhered cells per well for 30 min. The cells were then washed twice with PBS by centrifugation (450 *g* for 5 min). Neutral red was solubilized by a 30 min incubation adding 0.1 ml of 10% acetic acid plus 40% ethanol solution. The absorbance was read at 550 nm and the cationic vesicles content was expressed as percentage of control[15].

Hydrogen peroxide production

Hydrogen peroxide production by peritoneal macrophage or blood neutrophil was measured as described by Pizatto et al.[17]. This assay is based on the horseradish peroxidase (HRPO)-dependent conversion of phenol red into a colored compound by H_2O_2. After the 60 min of adhesion procedure and non-adherent cells washing macrophages or neutrophils (final volume 0.1 mL) were incubated in the presence of glucose (5 mM), phenol red solution (0.56 mM), and HRPO (8.5 U/mL) in the dark for 1 h at 20°C. After this period, absorbance was measured at 620 nm on a plate reader. The concentration of H_2O_2 was determined from a standard curve prepared in parallel. H_2O_2 production is expressed as percentage of control[17].

Superoxide anion production

Superoxide anion production was estimated by the reduction of nitroblue tetrazolium (NBT) assay. Peritoneal macrophage or blood neutrophil suspensions (0.1 mL) were added to the wells of a 96-well flat-bottomed tissue culture plate (10^5 cells/well) and left to adhere for 60 min. The non-adherent cells were washed twice with PBS. Peritoneal macrophages or blood neutrophils (0.45 ml) suspended in PBS was incubated for 1 h at 37°C in the presence of 0.1% NBT. The reaction was stopped by adding 0.45 ml of acetic acid. Then the mixture was centrifuged for 30 s at 2.500 *g*. Reduction of NBT results in the formation of blue formazan which was detected spectrophotometrically at 560 nm[17]. The results are expressed as percentage of control.

Statistical analysis

The Shapiro-Wilk test was used to verify the data normality per group, and the Levene test was used to analyze the homogeneities between group variances. No parametric statistic was employed for biochemical and immune parameters. Changes in body mass during two experimental weeks (pre- *vs.* post-training) were analyzed using Mann-Whitney test. Statistical analysis was performed by Kruskal-Wallis test. Tukey Post hoc test was applied for multiple comparisons and the data were analyzed using the Graph Pad Prism *software* version 6.0. The results were presented as mean ± SEM. A value for $p<0.05$ was taken to indicate statistical significance.

RESULTS

Total body mass

Body mass (Table 1) from SE group increased significantly after 2 weeks (35.4 %, $p<0.001$). On the other hand, DI and DIT groups did not modify significantly the body mass when compared to pre-to post-training ($p>0.05$). In the post-training the diabetic state and exercise caused attenuation of the gain of body mass from DI and DIT group when compared to SE group ($p<0.05$) (Table 1).

Blood biochemical parameters

The diabetic group increased the fasting glycaemia by 84%, decreased the serum high density lipoprotein (HDL-C) cholesterol levels by 64,5% and increased the triacylglycerol (TAG) by 13.8% when compared to SE group, respectively, ($p<0.05$) (Table 2). Diabetic condition did not modify low density lipoprotein cholesterol (LDL-C), very low density lipoprotein (VLDL-C) and total cholesterol (TC) when compared to sedentary rats ($p>0.05$) (Table 2). The interval training protocol did not modify the blood biochemical parameters in the diabetic rats submitted to interval training (DIT) when compared to DI group ($p<0.05$) (Table 2).

Parameters of polymorphonuclear cells and peritoneal macrophages

Phagocytic capacity, cationic vesicles content, superoxide anion and hydrogen peroxide production by neutrophils were assayed in all experimental groups (Table 3). Diabetic condition increased the phagocytic capacity by 61.3% when compared with SE (161.3 ± 9.4% and

100.0 ± 5.2%, respectively, p<0.01) (Table 3). After 2 weeks of interval training the phagocytic capacity of DIT group increased 2.7-fold when compared to SE group (p<0.0001). Interestingly, in the diabetic rats submitted to the interval training (DIT), the phagocytic capacity was even higher (1.3-fold) when compared to the DI group (p<0.01) (Table 3).

The diabetes induced a 1.9-fold increment in the cationic vesicles content compared to the SE group (100.0 ± 2.5% and 294.7 ± 14.3%, respectively, p<0.01) (Table 3). The interval training protocol increased the cationic vesicles content by 3.5-fold in the diabetic rats when compared with the sedentary rats (p<0.0001). Furthermore, the cationic vesicles content from DIT group was 53.7% higher when compared with DI group (p<0.01) (Table 3).

Diabetes increased by 69.8% the superoxide anion production of DI compared with SE group (169.8 ± 8.7% and 100.0 ± 3.9%, respectively, p<0.01). Interval training increased 69.4% the superoxide anion production of DIT compared with SE group (p<0.01). Between DI and DIT groups there was no difference in the production of this reactive oxygen species (p>0.05) (Table 3).

Diabetic condition did not alter the hydrogen peroxide production compared with SE group (p>0.01). The interval training did not modify the hydrogen peroxide production in the DIT group compared with SE (p>0.05). There was no difference also between DI and DIT groups (p>0.05) (Table 3).

Phagocytic capacity, cationic vesicles content, superoxide anion, hydrogen peroxide production by peritoneal macrophages are shown in Table 4. There was no difference in the phagocytosis from DI group when compared to SE group (p>0.05). After 2 weeks of interval training, three times a week, the phagocytosis by peritoneal macrophages increased by 84.4% in the DIT group when compared with sedentary rats (SE) (p<0.05). Furthermore, the phagocytosis by peritoneal macrophages from DIT group was 44% higher when compared to DI group (p<0.05) (Table 4).

Diabetic condition (DI) increased by 65% the cationic vesicles when compared with SE group (p<0.05). When diabetic rats accomplished the interval training (DIT) the cationic vesicles content increased by 1.1-fold when compared to SE group (p<0.05). The cationic vesicles content from diabetic rats submitted to interval training (DIT) was 41% higher when compared to DI group (p<0.05) (Table 4)

The diabetes (DI) decreased the superoxide anion production by 35.1% when compared with SE group (p<0.001). When diabetic rats accomplished the interval training (DIT) the superoxide anion production was decreased by 37.4% when compared to SE group (p<0.001) and there was no difference between DI and DIT groups (p<0.05) (Table 4).

Hydrogen peroxide production increased by 6.9% when compared with SE group (p<0.0002) after 4 weeks of interval training (IT). The diabetes (DI) increased hydrogen peroxide production by 6.9% when compared with SE group (p<0.0002). The interval training did not alter the hydrogen peroxide production of DIT compared with DI group (p>0.05) (Table 4).

DISCUSSION

Our main finding was that IT protocol applied in this study, 30-s exercise with 30-s rest, three times a week, during two weeks, increased the functionality of blood neutrophils and peritoneal macrophages parameters. Nevertheless, the exercise neither did attenuate resting hyperglycemic state nor decrease blood lipids in the group of diabetic rats submitted to interval training.

Our results in this STZ-induced diabetes model showed body mass loss, and the training protocol did not alter body mass reduction caused by the diabetic state (Table 1). It has been demonstrated that rats treated with STZ to induce type 1 DM possess a compromised ability for normal skeletal muscle growth and impaired body weight gain[11,18]. Corroborating such information, our group have previoulsy showed that after six or eight weeks of STZ-induced diabetes (60 mg/kg) resulted in significant less body mass compared with controls in rats[11]. Similar findingns were also achieved by others, where eight weeks of STZ-induced diabetes (120 mg/kg) resulted in significantly less body mass, less absolute muscle mass, and less epididymal fat mass compared with control rats[19].

The IT accomplished during two weeks, did not modify the hyperglycemia in the diabetic rats submitted to interval training compared with the DI group (Table 2). Few studies have been reported alterations of hyperglycemia with the interval training in type 1 diabetcs. Our group carried out a study with diabetic rats that accomplished a program of Interval Training (30-s exercise with 30-s rest, for 30 minutes, during 6 weeks) and there was a decreased of hyperglycemia in the interval training diabetic group[11]. In other study with diabetic rats that accomplished treadmill running high--intensity exercise protocol, during six weeks, there was also a decrease of hyperglycemia in the trained diabetic group after six weeks of exercise[20]. We infer that the divergence between our results and the previous reported studies maybe be mainly related to volume of training (two weeks, total of six sessions) applied in the present study. According to Gibala et al.[9] high-intensity interval training is infinitely variable with the specific physiological adaptations induced by this form of training determined by

Table 1. Body mass (g) pre- and post- training moments from sedentary (SE), diabetic (DI) and interval training diabetic (DIT) groups during two experimental weeks.

	SE	DI	DIT
Pre-training body mass (g)	277.4 ± 4.9	287.5 ± 13.5	289.9 ± 23.3
Post-training body mass (g)	375.6 ± 12.5*	300.3 ± 13.3[a]	274.3 ± 22.8[a]

Data are expressed as mean ± SEM. *P<0.001 compared with the pre-training. [a]P<0.05 compared with post-training SE group.

Table 2. Serum glucose, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), triacylglycerol (TAG) and total cholesterol (TC) concentrations from sedentary (SE), sedentary diabetic (DI) and interval training diabetic (DIT) groups at the end of two weeks of training.

	SE	DI	DIT
Glucose (mg.dL^{-1})	87.2± 4.9	151.6 ± 14.7[a]	184.4 ± 14.6[a]
HDL-C (mg.dL^{-1})	27.1 ± 5.4	17.5 ± 1.5[a]	17.7 ± 1.5
LDL-C (mg.dL^{-1})	35.7 ±1.6	42.9 ± 5.1	45.5 ± 7.3
VLDL-C (mg.dL^{-1})	27.7 ±1.8	26.5 ± 3.2	26.5 ± 3.2
TAG (mg.dL^{-1})	116.5 ± 8.4	132.6 ± 6.0[a]	129.5 ± 4.0
TC (mg.dL^{-1})	87.4 ± 3.5	87.7 ± 5.3	84.5 ± 7.0

Data are expressed as mean ± SEM. [a]P<0.05 compared with SE group.

Table 3. Blood neutrophils phagocytic capacity, cationic vesicles content, superoxide anion and hydrogen peroxide production in sedentary rats (SE), sedentary diabetic (DI), interval training diabetic (DIT) rats.

	SE	DI	DIT
Phagocytic capacity (%)	100.0 ± 5.2	161.3 ± 9.4[a]	372.2 ± 12.1[ab]
Cationic vesicles content (%)	100.0 ± 2.5	294.7 ± 14.3[a]	453.1 ± 10.9[ab]
Anion superoxide production (%)	100.0 ± 3.9	169.8 ± 8.7[a]	169.4 ± 14.6[a]
Hydrogen peroxide production (%)	100.0 ± 1.3	98.6 ± 4.4	102.9 ± 3.3

Data are expressed as mean ± SEM. [a]P<0.01 compared with SE group. [b]P<0.0001 compared with DI group.

Table 4. Peritoneal macrophages phagocytic capacity, cationic vesicles content, superoxide anion, hydrogen peroxide production and adhesion obtained from sedentary rats (SE), diabetic (DI) and interval training diabetic rats (DIT).

	SE	DI	DIT
Phagocytic capacity (%)	100.0 ± 2.5	127.9 ± 1.4	184.4 ± 2.4[ab]
Cationic vesicles content (%)	100.0 ± 3.2	154.4 ± 1.5[a]	218.5 ± 3.8[ab]
Anion superoxide production (%)	100.0 ± 5.6	64.9 ± 11.0[a]	62.6 ± 7.1[a]
Hydrogen peroxide production (%)	100.0 ± 1.6	106.9 ± 1.2[a]	105.5 ± 1.2

Data are expressed as mean ± SEM. [a]P<0.01 compared with SE group. [b]P<0.05 compared with SE group.

a myriad of factors including the precise nature of the exercise stimulus (i.e. the intensity, duration and number of intervals performed, as well as the duration and activity patterns during recovery).

Some studies have shown that two weeks of interval training might improve skeletal muscle fatty acid oxidation, what could increase the use of the blood lipids as energy substrate for the muscles[21,22]. However, the IT protocol applied in this study, 30-s exercise with 30-s rest, three times a week for two weeks, did not alter blood lipids from DIT group compared to DI (Table 2). According to Taskinen[23], the hyperglycemia caused by insulin deficiency affects key enzymes and pathways in lipid metabolism. Therefore, the lack of change in blood lipids might be related to the absence of effect caused by the IT protocol in the fasting glycaemia. Other possibility is that the very low volume of training applied by us (two weeks, only six sessions of exercise) is not enough to cause any systemic alteration in the parameters assayed by us.

Poor glycemic control has been shown to be an important risk factor for infections in type 1 diabetics[24,25]. Several studies have shown impairment of neutrophil function, a disorder that contributes to the high incidence of infections in diabetes[26]. Interestingly, in our study, the functionality of neutrophils and peritoneal macrophages from the DI group increased significantly (Table 3). Among many possible factors responsible for the stimulation of functionality of neutrophils and peritoneal macrophages in diabetic rats, hyperglycemia seems to be the most important. It seems that the increased plasma glucose level may activate innate immune cells by increasing plasma advanced glycation end products (AGEs)[27] and by influencing intracellular carbohydrate metabolism[11].

Exercise can have both positive and negative effects on immune system. While moderate activity may enhance immune function over sedentary levels, excessive, prolonged and high-intensity exercise may impair immune function[28]. Some studies have shown that relatively short periods of intense training (1 to 3 weeks), may reduce the function of innate immune[29,30]. In our study, two weeks of high intensity exercise increased the phagocytic capacity and cationic vesicles content from neutrophils and macrophages in the training diabetics rats (Table 3-4)). These parameters showed that lysosomal capacity of immunotoxicity increased from neutrophils and macrophages, which in turn may reduce the risk of infections in the diabetic rats. Our group evaluated the effects of six weeks of interval training on functionality neutrophils and peritoneal macrophages of a STZ-induced diabetes model and also showed increased of functionality of immune cells in the trained diabetics rats[11]. Other important result of our study was that high intensity exercise, carried out for two weeks, did not negatively alter the function of innate immune cells.

CONCLUSION

In summary, our data show that STZ-induced diabetes model resulted in an increased functionality of blood neutrophils and peritoneal macrophages The low-volume high-intensity physical exercise further increased the innate immune cells' functionality. Nevertheless, two weeks of interval training was not sufficient to modify the biochemical disturbances caused by STZ-induced diabetes.

All authors have declared there is not any potential conflct of interests concerning this article.

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