Glucose Homeostasis in Type 1 Diabetic Rats after Acute Physical Activity

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ABSTRACT

Moura LP, Bertolini NO, Ghezzi AC, Bertucci DR, Bonfim MR, Serafim THS, Pereira AS, Garuffi M, Mello MAR, Luciano E. Glucose Homeostasis in Type 1 Diabetic Rats after Acute Physical Activity. JEPonline. 2011;14(6):8-19. Physical activity is considered an extremely effective therapy in cases of type 1 diabetes (DM-1), as it promotes glucose uptake independent of insulin action. However, there are few studies on the effect of a single session of exercise on glucose uptake in DM-1 (i.e., in the absence of insulin). Therefore, the purpose of this study was to assess the effect of a single exercise session on glucose homeostasis in DM-1 rats. For this purpose, 30 male rats were divided into three groups: sedentary control (SC), sedentary diabetic (SD), and exercise diabetic (ED). DM was induced by administration of alloxan and identified by the value of fasting glucose. The physical activity consisted of a single swimming session at the anaerobic threshold intensity for diabetic rats (3.5% body weight overload) for 30 min. The oral glucose tolerance test (OGTT) was performed immediately after the physical activity. The animals were sacrificed 48 hr after the OGTT, and samples were taken from the blood, liver, gastrocnemius, and mesenteric and subcutaneous adipose tissue. We observed that DM caused significant reduction in body weight. A single session of physical activity did not modify the response to the OGTT or glucose. However, it resulted in increased HDL cholesterol and hepatic glycogen content. These results suggest that, despite not having an effect on glucose homeostasis, acute physical activity performed at anaerobic threshold intensity leads to beneficial changes in the context of type 1 diabetes.

Key words: Diabetes, Glucose Tolerance, Acute Exercise
INTRODUCTION

Diabetes mellitus (DM) is a global public health problem. It is estimated that there are more than 220 million diabetics worldwide. The World Health Organization estimates that the number of people with diabetes in the world will reach 300 million by 2025 (9). In general, diabetes is characterized by increased concentrations of blood glucose resulting from reduced secretion or action of the hormone insulin. It can be classified into diabetes mellitus type 1 (DM-1) or type 2 diabetes (13).

Diabetes mellitus type 1 is considered an autoimmune disease that destroys the pancreatic beta cells in the Islets of Langerhans. It primarily affects young people, and it is sometimes referred to as juvenile diabetes. As DM-1 patients do not produce enough insulin, they require exogenous administration of the hormone. Thus, they are considered insulin-dependent. In contrast, type 2 diabetes usually affects people over 40 yrs of age. It is known as non-insulin-dependent diabetes that tends to have a slower progression and a later onset (13). Type 2 diabetes is characterized by reduced sensitivity to insulin action. The primary contributing factors are poor diet and sedentary lifestyle (22).

According to recent WHO estimates, about 3 million deaths worldwide can be attributed to DM-1 every year (31). In addition, the complications of diabetes are serious and equally worrisome, as they decrease the quality and life expectancy of patients (34). Type 1 diabetes (DM-1) is considered a major cause of blindness. It is also associated with the occurrence of kidney disease, macrovascular diseases, arteriosclerotic vascular disease, liver changes, inflammatory processes, a variety of debilitating neuropathies (14), and tissue damage (3,8,42).

Tissue damage resulting from hyperglycemia can be caused by four mechanisms: activation of the polyol pathway, activation of the hexosamine pathway, activation of protein kinase C, and formation of advanced glycation end-products (3,8,42). In addition, there is a reduction in both muscle and liver glycogen stores due to the decrease in insulin concentrations and consequent reduction in peripheral glucose uptake. Hypoinsulinemia increases the concentrations of circulating fatty acids, aggravating the disorders mentioned above, and it may also adversely affect individual physical performance (41). In view of these deleterious consequences, it is important to conduct studies aimed at elucidating the mechanisms of glucose homeostasis in DM-1 and identify new and effective therapeutic measures.

Regular physical activity has been found to be of great importance for both type 1 and 2 diabetics (2), because such an intervention improves glycemic homeostasis in these individuals. Chronic physical activity increases the gene expression of glucose transporters in skeletal muscle and the intermediaries of the uptake signaling pathway, resulting in long-term reduction of blood glucose levels (16). Similarly, acute exercise can increase the translocation of muscle glucose transporter (GLU-4), mediated by muscle contraction. Due to muscle cell depolarization resulting from contraction, which leads to the Ca\(^{2+}\) channel opening, the calcium-calmodulin complex is activated and the concentration of adenosine monophosphate (AMP) increases. In turn, AMP functions as a second messenger that transmits a signal setting off GLUT-4 translocation to the periphery of the membrane (11,17,29,32,33).

Physical activity, therefore, may interfere with glucose uptake independent of insulin. It may also act as a transducer of several signaling pathways common to muscle insulin signaling. For this reason, physical activity can be considered a tool for understanding the mechanism of glucose homeostasis in diabetes mellitus, allowing new therapeutic targets to be identified, especially for
DM-1 (22). However, there are few studies aimed at identifying the effects of a single session of physical activity on glucose uptake in DM-1. Therefore, the goal of this study was to analyze the effect of a single session of moderate physical activity on glucose homeostasis, independent of insulin, in alloxan-induced diabetic rats.

METHODS
Ethics approval
This work is in accordance with the codes of practice in UK legislation, and is part of a larger study, entitled "Protocols of aerobic, interval, and swimming periodized training in rats," which was reviewed and approved by the research ethics committee of the Faculdade de Ciências da Universidade Estadual Paulista, UNESP, Rio Claro/SP Campus, Brazil, Process No. 1501/48/01/08.

Sample
Thirty adult male Wistar strain rats were from the Central Animal Breeding House of the Universidade Estadual Paulista (UNESP), Botucatu/SP, Brazil. The rats were approximately 70 days old at the beginning of the experiment. The experimental animals were housed in collective cages made of polyethylene measuring 37 x 31 x 16 cm (five rats per cage), kept at a room temperature of 25°C under 12:12-h light-dark (LD 12:12) photoperiod cycles and fed balanced, standard Purina® and water ad libitum. The rats were divided into three groups with 10 animals/group, as follows: sedentary control (SC), consisting of eutrophic rats that did not exercise; sedentary diabetic (SD), consisting of alloxan-induced diabetic rats that did not exercise; and exercise diabetic (ED), consisting of alloxan-induced diabetic rats submitted to a single session of physical activity.

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, injected into the penile vein (20). After this procedure, the rats were relocated in cages, where they received a glucose and water solution (15%) and food ad libitum (19) during the first 24 hr after the administration of alloxan. Five days after drug administration, a blood glucose test was taken to confirm the diabetic state; animals with a blood glucose level equal to or above 150 mg/dL were identified as diabetic. The control group was injected with citrate buffer and thus, submitted to the same stress as the animals that were administered alloxan.

Adaptation to water immersion
All animals, both those subjected to swimming and those that were sedentary, were subjected to water adaptation in order to reduce the stress of the animal at the time of the experiment (36). The water adaptation process consisted of exposing the animal to shallow water at a temperature of 30 ± 1°C for 60 min for 5 days. The animals in the ED group underwent adaptation in the 5 days preceding the physical activity session, while the animals in groups SC and SD remained in shallow water to mimic the stress endured by the ED group.

Single physical activity session
One week after the confirmation of diabetes onset, and following the period of water adaptation, the animals in the ED group underwent one swimming session for 30 min. A load equivalent to 3.5% of body weight was attached to the animal's chest, as required to reach the intensity equivalent to the anaerobic threshold for DM-1 rats (26).
Glucose tolerance test – OGTT
The OGTT was performed when all the animals fasted for 12 hr and immediately after the physical activity session carried out by the ED group. The first blood sample was obtained by a small cut on the tip of the tail before the start of physical activity. Immediately after physical activity, another blood test was performed, followed by administration of an 80% glucose solution through a gastric polyethylene probe (20% of body weight). Blood samples were collected after 30, 60 and 120 min with heparinized capillary tubes calibrated to 25 µL in order to determine the serum glucose concentrations. A single cut at the tail end of the animal was sufficient to carry out all collections. The glucose concentrations were determined by the glucose oxidase method using commercial kits (Laborlab®). The results were analyzed by calculating the area under the curve (AUC) for serum glucose during the test by the trapezoidal method (23), using ORIGIN 6.0 software (2000).

Animal euthanasia and biological material collection
At the end of the experiment and 48 hr after the OGTT, the animals were anesthetized with CO₂ and exsanguinated. The collected blood was used for serum separation and subsequent determination of free fatty acids (FFA), triglycerides (TG), total cholesterol (TC), high (HDL) and low (LDL) density lipoproteins and glucose using commercial kits (Laborlab®). Liver and gastrocnemius muscle aliquots were weighed. One sample was used to determine the concentration of glycogen while the other was used for the analysis of tissue triglycerides (24). The mesenteric and subcutaneous adipose tissue was completely removed after the analysis.

Statistical analysis
The experimental results are presented as mean ± standard deviation. Differences between groups were identified by analysis of variance (one-way ANOVA), followed by a Bonferroni post-test. The Kruskal-Wallis test was adopted whenever the homogeneity of variance (Levine’s test) presented P values = 0.05. The paired t-test was used to identify significant differences between the initial and final stages of the study. We used the statistical package SPSS 13.0 for Windows, with P < 0.05 considered significant for all tests.

RESULTS
During the experimental protocol, we observed that the diabetes-induced animals showed a reduction in body weight while control animals showed an increase in body weight. Moreover, at the end of the experiment, the control group weight was higher than that of the other groups (Figure 1). One week after the confirmation of diabetes onset, the animals were subjected to acute physical activity, followed by the oral glucose tolerance test (OGTT). Figure 2A shows the glycemic profile 30, 60 and 120 min before and after the administration of glucose. With regard to the glycemic profile pattern during the test, the animals from the sedentary diabetic and sedentary control groups showed increased blood glucose levels until 60 min after administration of glucose, followed by a decline at 120 min. This did not occur with the ED group, which continued to have elevated blood glucose at the final moment of the test. Also, a significant difference was noticed in the blood glucose levels of the diabetic groups compared to the control group throughout the test. There was no difference between the two groups of diabetic animals. This pattern was also observed for the glucose area under a curve values (Figure 2B).
Figure 1. Values of body weight (g) ± standard error at the beginning and end of the experimental protocol. SD = Sedentary Diabetic; ED = Exercise Diabetic. *Difference between initial and final result; a Difference with SC; b Difference with SD; c Difference with ED.

Figure 2. A- Glucose and B - Area under the glucose curve values during the glucose tolerance test. Results are expressed as mean ± standard deviation. SC = Control; SD = Sedentary Diabetic; ED = Exercise Diabetic. a Difference with the control.
The serum biochemical parameters measured at the moment the animals were euthanized are shown in Table 1. We observed that blood glucose levels remained significantly elevated in the diabetic groups compared to the control group. Concerning the lipid profile, only the HDL cholesterol of the ED group was significantly high compared to other groups. There were no differences in the other parameters between the two diabetic groups.

**Table 1. Serum biochemical parameters at the moment of euthanasia.**

<table>
<thead>
<tr>
<th></th>
<th>SD (n = 5)</th>
<th>SD (n = 9)</th>
<th>ED (n = 8)</th>
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</thead>
<tbody>
<tr>
<td>Glucose (mg/Dl)</td>
<td>84.2±15.6</td>
<td>328.1±69.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>329.0±96.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>AGL (µEq/L)</td>
<td>0.47±0.27</td>
<td>0.39±014</td>
<td>0.46±0.22</td>
</tr>
<tr>
<td>Total cholesterol (mg/Dl)</td>
<td>87.7±13.5</td>
<td>91.0±26.5</td>
<td>98.6±29.1</td>
</tr>
<tr>
<td>HDL cholesterol (mg/Dl)</td>
<td>9.6±0.7</td>
<td>9.5±1.3</td>
<td>12.2±1.7&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>LDL cholesterol (mg/Dl)</td>
<td>71.9±13.0</td>
<td>77.7±26.8</td>
<td>79.9±26.6</td>
</tr>
<tr>
<td>Triglycerides (mg/Dl)</td>
<td>183.3±43.9</td>
<td>222.5±165.3</td>
<td>356.9±213.9</td>
</tr>
</tbody>
</table>

Results expressed as mean ± standard deviation. SC = Sedentary Control; SD = Sedentary Diabetic; ED = Exercise Diabetic. <sup>a</sup>Control difference; <sup>b</sup>SD difference; <sup>c</sup>ED difference.

Table 2 shows the results regarding the glycogen and triglycerides concentration in the liver and gastrocnemius muscle at the end of the experiment.

**Table 2. Tissue parameters (mg/100mg) after the experimental protocol, expressed as mean ± SD.**

<table>
<thead>
<tr>
<th></th>
<th>SC (n = 5)</th>
<th>SD (n = 9)</th>
<th>ED (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver glycogen</td>
<td>3.20±1.35</td>
<td>2.14±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.40±0.44</td>
</tr>
<tr>
<td>Muscle glycogen</td>
<td>0.07±0.20</td>
<td>0.11±0.05</td>
<td>0.16±0.10</td>
</tr>
<tr>
<td>Liver triglycerides</td>
<td>10.7±1.4</td>
<td>10.3±2.4</td>
<td>10.7±2.9</td>
</tr>
<tr>
<td>Muscle triglycerides</td>
<td>0.74±0.02</td>
<td>1.68±0.60</td>
<td>2.58±1.55</td>
</tr>
</tbody>
</table>

Results expressed as mean ± standard deviation. SC = Sedentary Control; SD = Sedentary Diabetic; ED = Exercise Diabetic. <sup>a</sup>Control difference; <sup>b</sup>SD difference; <sup>c</sup>ED difference.

**Table 3. Mass, in grams, of the mesenteric adipose and subcutaneous tissues at the end of the experiment.**

<table>
<thead>
<tr>
<th></th>
<th>SC (n = 5)</th>
<th>SD (n = 9)</th>
<th>ED (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesenteric fat</td>
<td>1008.1±1026.5</td>
<td>402.7±133.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>694.3±482.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Subcutaneous fat</td>
<td>1475.6±749.3</td>
<td>208.9±207.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>344.3±350.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Results expressed as mean ± standard deviation. SC = Sedentary Control; SD = Sedentary Diabetic; ED = Exercise Diabetic. <sup>a</sup>Control difference control; <sup>b</sup>SD difference; <sup>c</sup>ED difference.
The liver glycogen values of the SD group were significantly higher than those of the control group. The weight of both the mesenteric adipose tissue and the subcutaneous tissue were significantly higher in the control group than the diabetic groups.

DISCUSSION

The purpose of this study was to identify the effects of a single session of moderate physical activity on the glucose homeostasis of DM-1 rats without exogenous administration of insulin. The DM-1 experimental model can be obtained through the use of chemicals that destroy the pancreatic beta cells. Alloxan and streptozotocin (STZ) are the most widely used drugs for this purpose. Although STZ is more commonly used for the induction of DM in rats, it has an oncogenic effect and, in the long term, there is a need for insulin use in animals induced in adulthood (25). In contrast, alloxan acts directly and selectively in pancreatic beta cells, destroying them irreversibly with low oncogenic effect (19,34). Therefore, we chose to use alloxan rather than STZ for the induction of the diabetes model in this study.

In fact, blood glucose levels presented by the animals in groups SD and ED show that alloxan was effective in inducing DM and that this effect was maintained throughout the experiment. Two weeks after the induction of DM-1, a significant reduction in the body weight of the DM-1 animals was detected, both sedentary and exercised. The DM-1 animals had lower final body weight compared to the control group and less total mesenteric and subcutaneous fat at the end of the experiment.

This reduction in body weight has also been found in other studies. Leme (18) found that changes in body weight in alloxan animals began on the first day after drug administration and that weight continued to decline throughout the experiment. Gomes (10) observed that alloxan animals lost weight during the 6 weeks after drug administration. However, Gomes (10) also found that the diabetic animals that exercised regularly showed no significant difference in body weight compared with sedentary and exercised controls. This finding showed that physical activity is effective in maintaining body weight and lean mass in these animals.

The reduction in body weight at the beginning of DM-1 induction is well described in the literature. It is associated with the role of insulin in the body. In general, this hormone acts by triggering storage of excess carbohydrates and converting them into fat, promoting amino acid uptake and inhibition of protein catabolism (21). Lack of insulin causes hyperglycemia by decreasing peripheral glucose uptake. This change is accompanied by increased lipolysis in the adipose tissue and elevated serum concentrations of free fatty acids and triglycerides, as well as intense catabolism and inhibition of protein synthesis. This leads to use of the body's energy reserves and, consequently, to weight reduction (24).

In this study, the physical activity protocol used was acute and, as expected, unable to promote change in body weight. However, an acute protocol can potentially help identify the mechanisms of glucose homeostasis (22). Therefore, an analysis of the effects of acute exercise was conducted using an oral glucose tolerance test. During testing, the glucose levels of the SD and ED groups were higher than the control group, which is a characteristic feature of glucose tolerance in diabetic animals and validates the protocol used to induce DM (25).
Although the differences were not statistically significant, the ED animals had lower blood glucose levels at baseline and up to 60 min of testing compared to the SD animals, which suggests that part of the blood glucose uptake is due to exercise-induced translocation of glucose transporters (11,17,29,32,33) and the effect of local release of bradykinin (41). Nonetheless, it was observed that, unlike the SC and SD groups, the ED group had increased glucose levels in the last OGTT analysis.

According to Rogatto et al. (30), glycemic profiles during and after acute physical activity have been quite varied due to differences in the activities employed. This may be related to increased secretion of certain hormones during exercise, such as catecholamines, glucagon, and growth hormone, which act by stimulating glycolysis and lipolysis and, therefore, favor the use of lipids as an energy source instead of glucose (5,7). Moreover, for rats, physical activity in the water is part of a flight instinct, given that it is a stressful activity. One can assume that the secretion of corticotropin and cortisol will increase, further contributing to lipolysis (6,37).

Forty-eight hours after the OGTT, the animals were euthanized and the collected blood was used to check the serum biochemical parameters. Differences were found in the blood glucose levels of the diabetic animals in both groups compared to the controls, thus confirming diabetes mellitus (2).

When the circulating lipid profile (FFA, total cholesterol, LDL cholesterol, and triglycerides) was analyzed, we found that just one week of following the confirmed inducement of experimental diabetes is not sufficient to promote clinical symptoms of dyslipidemia, even with degradation of the adipose tissue. This may be because the peripheral muscle tissue has good permeability to fatty acids (7). As diabetic animals were not able to use glucose as an energy substrate, they used fat as an energy source for muscles, minimizing, for this short period, the onset of dyslipidemia.

On the other hand, when the effect of an exercise session is isolated, it becomes evident that the exercised diabetic animals have higher HDL cholesterol synthesis, which is in concordance with the findings of Grandjean et al. (12). These authors reported that normocholesterolemic and hypercholesterolemic men experienced an increase in HDL cholesterol after just one session of aerobic physical activity. Although our results indicate that a single physical activity session is not enough to reduce the blood glucose levels of diabetic rats, the increase in HDL cholesterol is very relevant because this lipid fraction is well known for its antiatherogenic role, being inversely associated with coronary heart disease and other vascular disorders (9).

With regard to the tissue glycogen content, the sedentary diabetic animals had lower hepatic glycogen content than the control group and the exercise diabetic group. This finding confirms the results of research studies by Luciano et al. (20) and Gomes et al. (10). These results indicate that a single session of exercise is able to promote an increase in hepatic glycogen content, which may be related to increased activity of the glycogen synthase enzyme (32), without changes in the muscle content.

**CONCLUSION**

In summary, the results of this study suggest that acute moderate physical activity is unable to change the glycemic profile from a glucose tolerance test in type 1 diabetic rats without insulin
use or to reduce the blood glucose values after 48 hr. However, this acute activity can effectively increase HDL and hepatic glycogen content that demonstrates, despite little action on glucose homeostasis, even one session of physical activity can yield health benefits for type 1 diabetes.

CONFLICT OF INTEREST
The authors declare that there are no conflicts of interest regarding the present study.

AUTHOR CONTRIBUTIONS
All of the authors contributed to the study, not only with regard to sample collections but also with regard to the preparation of this manuscript. All of the authors have read and approved of the final version of this manuscript.

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