Training exercise at the aerobic/anaerobic metabolic transition prevents glucose intolerance in neonatal alloxan treated rats

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Abstract

Background: Type 2 diabetes is a non-insulin-dependent disease that occurs in a slowly and delaying manner, representing about 90% of all the diabetic patients. Physical inactivity appears to be one of the main causes of type 2 diabetes. The present study aimed to analyze the effects of aerobic physical exercise on the glucose homeostasis of adult rats subjected to neonatal administration of alloxan, an experimental model on type 2 diabetes.

Methods: Female newborn Wistar rats (6 days old) received Alloxan (250mg/Kg body weight), intraperitoneally (alloxan group); or vehicle citrate buffer (0,01M) (control group). After weaning (28 days), the animals were subdivided in to 4 groups: control (C); trained control (CT); Alloxan (A) and trained Alloxan (AT). Trained animals were submitted to swimming, 1 h/day, 5 days/week, supporting an overload equivalent to the aerobic/anaerobic metabolic transition. Before the training, all the animals were subjected to an effort test for identification of the aerobic/anaerobic metabolic transition using the maximal lactate steady state procedure. At 28, 60, 90 and 120 days old, the animals were subjected to glucose tolerance test (GTTo) , analyzed by the total area under the serum glucose curve and had peripheral insulin sensivity evaluated by HOMA insulin sensivity index.

Results: The area under serum glucose curve during GTTo was always higher in the A group in relation to C group. At the 90th and 120th days, there was a decrease in this area in the AT group when compared to the A group. At 90 days, TC group presented lower values for HOMA index than C group.
**Conclusion:** The neonatal administration of alloxan induced a persistent glucose intolerance and physical training at the aerobic/anaerobic metabolic transition counteracted this intolerance.

**Background**

Diabetes Mellitus results from the reduced secretion and/or the diminished action of insulin. Diabetic individuals are classified into two distinct groups, taking into account if diabetes is caused by the lack of insulin (insulin-dependent diabetes mellitus - IDDM) or by the resistance to its action (non-insulin-dependent diabetes mellitus - NIDDM).

The NIDDM is a disease that occurs in a slowly and delaying manner, representing about 90% of all the diabetic patients. Obesity, high caloric diet and physical inactivity appear to be among the main causes of type 2 diabetes [1].

Insulin resistance impairs glucose uptake by the muscle and adipose tissue and increases hepatic glucose production. Both derangements contribute to hyperglycemia. There are evidences that insulin resistance is the abnormality more precociously detected in a diabetic picture, preceding the beginning of the hyperglycemia [2].

In 1989, Portha *et al.* developed an experimental model of insulin resistance by streptozotocin neonatal administration to rats [3]. Later, Kodama *et al.* (1993) developed another experimental model of insulin resistance in rats through the substitution of the estreptozotocin for the alloxan [4]. In this study, the alloxan was administered in Wistar rats of 2, 4 or 6 days of age. At 60 days old, the rats which received alloxan at the 2nd day of life presented slightly
increased serum glucose, in the fed state, whereas those which received the drug at the 4th and 6th days had shown significantly higher serum glucose than the controls.

In previous studies we (Oliveira et al. 2004) analyzed the model developed by Kodama et al. (1993)[4] and verified that male Wistar rats which received alloxan (200 mg/Kg body weight) at 2 days of age recovered themselves from the glucose intolerance at the 90th day [5]. Other metabolic disturbances such as high circulating FFA concentration persisted through the whole experimental period.

Physical exercise programs proved to be efficient in the glucose homeostasis control of diabetic patients, improving insulin sensitivity and glucose tolerance [6,7,8]. In general, aerobic exercises are recommended for type 2 diabetes [6,7,9]. However, strength exercises also showed beneficial effects in the glycemic control of these patients [10,11,12,13]. Since there are limitations in the research with human beings, animal models become useful in the study of this question.

Thus, the aim of the present study was to analyze the effect of the physical exercise performed at the intensity corresponding to the aerobic/anaerobic metabolic transition on glucose homeostasis of adult Wistar rats submitted to the neonatal administration of alloxan.

Methods
Animals and its treatment

The studies were carried out on new born female Wistar rats, maintained at 25 ± 1º C on a 12-h light-dark cycle, with free access to the water and food
[food consisted in simplified rodent diet, prepared in our laboratory in accordance to the AIN-93G [14]) . Body weight as well as food and water intake of all animals were recorded once a week. All experiments with the animals were performed in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Council of Europe nº 123, Strasbourg, 1985).

**Neonatal alloxan administration**

At 6 days old, the female pups received alloxan monohydrate injection (Sigma-Aldrich Inc., St Louis, MO, USA) dissolved in citrate buffer 0.01 M, pH 4.5 (250 mg/Kg body weight), intraperitoneally, after a 16-h fast. As controls, we used same-age, vehicle-injected (citrate buffer) rats.

**Experimental groups**

From weaning (28 days) to the adult age (120 days), the animals were randomly divided into four groups and treated as follows:

- **Controls (C) n=8**: citrate buffer-injected rats;
- **Trained Control (CT) n=8**: citrate buffer-injected rats, trained by swimming.
- **Alloxan (A) n= 8**: alloxan injected rats;
- **Trained Alloxan (AT) n=8**: alloxan injected rats, trained by swimming.
Oral glucose tolerance test (GTTo)

The rats were fasted for 16 h, at 28, 60, 90 and 120 days old. Glucose (200 g/L) was administered into the stomach through a gastric catheter at the final dose of 2.0 g/Kg body weight. Blood samples for determination of serum glucose (25 µL) concentrations were obtained from a cult at the tip of the tail at 0, 30, 60 and 120 min. Blood samples (75 µL) for insulin concentration determination were also obtained at 0 min, in order to calculate the HOMA insulin sensitivity index. Blood glucose level was determined by the glucose-oxidase method, and insulin, by radioimmunoassay [15]. The blood glucose responses during the GTTo were evaluated by the total area under the serum glucose (mMx120 min).

Insulin Sensitivity

Peripheral insulin sensitivity was evaluated in the rats at 28, 60, 90 and 120 days old through the calculation of the HOMA (Homeostasis Model Assessment) insulin sensitivity index, using the following equation: serum insulin (pmol/L) X serum Glucose(mmol/L)/22.5. According to this method, a high HOMA index denotes low sensitivity to the insulin [16].

Effort test

At 28 days old, all the animals were submitted to an effort test for identification of the exercise intensity corresponding to the anaerobic/aerobic metabolic transition using the Maximal Lactate Stead State protocol. The MLSS is equivalent to the highest blood lactate concentration at which lactate entrance
into the blood is compensated by the its removal during exercises with constant loads [17] and its determination has shown useful for exercise prescription and for evaluation of the aerobic capacity. Recently, our research group described a protocol for the determination of the MLSS for rats during swimming exercise [18], which was used in the present study. Briefly, the test protocol for the determination of the MLSS consisted of sessions of swimming exercise supporting constant and increasing overloads in relation to the body weight, until the blood lactate concentration does not stabilize during the exercise session. Each test consisted of 25 minutes of continuous swimming with a load followed by blood collection through a cut at the tip of the tail, each 5 minutes, for lactate concentration measurement. There was a 48 hours interval between the sessions. The highest exercise intensity in which the increase in the blood lactate concentration did not exceed 1 mmol/L, from the 10th to the 25th, was considered as the maximal lactate steady state [18,19].

Training Protocol

The physical training protocol consisted in swimming in individual tanks, with an overload corresponding to the individual aerobic/anaerobic metabolic transition identified by the maximal lactate steady state. The animals were trained 1 h/day, 5 days/week, during 16 weeks. Each 30 days, the animals were subjected to new MLSS tests for adjustment of the overload.
Statistics

The results are shown as mean ± standard deviation and were analysed statistically by T-student test, two-way ANOVA followed by Newman-Keuls test or Friedman test, where appropriated. The significance level taken was 5%.

Results

General evaluations

The results referring to body weight, food intake and water intake are shown in table 1. Group C presented higher area under the curve of body weight than the other groups (CT, A, AT). AT group presented higher food intake when compared with the sedentary groups (C and A). There was no difference in water intake between groups.

TABLE 1

Evaluations previous to training

In the GTTo performed at 28 days (figure 3), the blood glucose level of the rats that received alloxan neonatally (A) was higher than the control rats (C), as seen by the increased area under the serum glucose curve in this group. There was no difference in the Homa insulin sensivity Index between groups (C and A) when they were evaluated at 28 days. At 28 days of age, all the animals were subjected to a test for identification of the individual maximal lactate steady state (MLSS), and were subdivided in to the 4 groups that composed the
experiment. The test was carried out with loads varying from 5.0% to 6.5% of the body weight. The animals did not present MLSS with the same load of exercise, within the same group. As an example, figure 1 represents the lactate concentration kinetics during the effort test of an animal of each group. In the control group, 25% of the animals presented MLSS with 5% body weight load and the mean lactate concentration of 7.3 ± 2.4 mmol/L; 75% of the animals presented MLSS with 6.5% body weight load and mean of lactate concentration of 6.0 ± 2.6 mmol/L. In the alloxan group, 50% of the animals presented MLSS with 5.5% body weight load and the mean lactate concentration of 3.8 ± 0.7 mmol/L, 33.3% of the animals presented MLSS with 6.0% body weight load and mean of lactate concentration of 6.5 ± 0.6 mmol/L and 16.7% of the animals presented MLSS with 6.5% of body weight load and mean of lactate concentration of 10.4 ± 0.6 mmol/L.

FIGURE 1

Evaluations during the training period

There was no difference in the Homa Index between groups when they were evaluated at 28 and 60 days old. At 90 days old, the animals from control trained group (CT) were more sensitive to insulin than the other groups (C, CT, A). At 120 days old, there was no statistical difference between groups. When comparing HOMA index values at 28, 60, 90 and 120 days for each group, no difference was observed (figure 2).

FIGURE 2

At 60 days old, alloxan (A) and trained alloxan (AT) animals kept high serum glucose concentrations during the GTTo when compared to the control
animals (C). On the other hand, at 90 and 120 days old the area under the serum glucose curve of the trained alloxan (AT) group was decreased when compared to the sedentary alloxan animals group (A) (figure 3).

FIGURE 3

Discussion

The aim of this study was to analyze the effects of the physical exercise performed at an intensity equivalent to the metabolic aerobic/anaerobic metabolic transition on glucose homeostasis of diabetic female Wistar rats, from weaning (28 days) until adult age (120 days).

Our results show that the sedentary control group (A) showed higher body weight than the other groups while the trained alloxan group (AT) presented the highest food intake without transforming this energy in body weight gain.

Obesity is one of the main causes of type 2 diabetes. In the present study, exercise protocol prevented body weight gain. Data from literature shows that regular physical activity and the reduction in the body weight gain reduce the incidence of type 2 diabetes in patients with glucose intolerance [20]. Eriksson et al. (1999) showed a reduction in the body weight gain associated with a decrease in glucose intolerance, after an intervening physical activity program in glucose intolerant patients, during one year period [21]. In short-term period (period of 8 weeks), Boulé et al. (2001) reported as main benefit of the physical activity the reduction of Hemoglobin HbA_1c (A1C) [22].
The GTTo carried out at 28 days showed that the animals had a satisfactory response to the neonatal alloxan administration, presenting a higher area under the serum glucose curve than the controls. In the subsequent evaluations at the 60\textsuperscript{th}, 90\textsuperscript{th} and 120\textsuperscript{th} days of experiment, the sedentary alloxan group remained glucose intolerant.

In another experiment carried out in our laboratories, Oliveira et al (2005) observed that rats which received alloxan (200 mg/Kg of body weight) at 2 days old presented spontaneous regression of the glucose intolerance [5]. In the present study, the maintenance of the higher blood glucose concentrations by the alloxan treated sedentary animals was probably due to an increase in the age at which the animals received the alloxan (6 days) and in the dose (250mg/Kg). According to Kodama et al. (1993) when alloxan is administered to neonatal rats, the earlier the drug administration the less severe is the blood glucose derangement observed later in life [4].

According to American Diabetes Association, patients who present blood glucose intolerance and/or altered fasting blood glucose are potential candidates to interventions in order to monitor and control the type-2 diabetes outbreak [23]. This same association still defends that the use of the oral glucose tolerance test and the evaluation of the fasting blood glucose are the best tests for tracking the development of type-2 diabetes.

The United Kingdom Prospective Diabetes Study (1995) demonstrated that the dysfunction of β cells is initiated about 10 to 12 years before type-2 diabetes being installed, offering, therefore, a large range interval for prophylactic procedures [24]. The sedentary alloxan injected rats of the present study kept this pre diabetic condition during all the experimental period,
indicating that such model could be useful for future studies aiming the development of procedures for type 2 diabetes prevention, since there are limitations in the research with human beings.

The maximal lactate steady state identification has been employed successfully for the aerobic capacity evaluation of experimental animals [18]. In addition, this protocol allows the identification of the individual aerobic capacity of the animals. In the present study, the procedures used for glucose intolerance induction led to a reduction of blood lactate concentrations in most of the AT animals at the MLSS intensity when compared to C animals. On the other hand, the overloads at the MSLL were similar for both groups.

Randle proposed a mechanism, in studies with heart and diaphragm of rats, known as glucose-Fatty Acid cycle. It was observed that increase of FFA concentration in plasma decreased glucose uptake and oxidation by these tissues [25]. In general, type 2 diabetes leads to an increase in circulating FFA, which are used by peripheral tissue, including skeletal muscles, saving glycogen [26, 27]. This may have led to a reduced glycolitic activity resulting in lower lactate production in alloxan treated rats.

Our results show that when the animals were 90 days old i.e 60 days after beginning of the physical training, the exercise protocol used in the present study was efficient in reducing the area under the serum glucose curve during the GTTo in AT rats, evidencing a significant improvement in the glucose tolerance (figure 3). A similar improvement in the glucose tolerance in AT rats was observed at 120 days.

Knowing at which moment and how physical activity can contribute in counteracting or preventing type-2 diabetes may be a useful information to
encourage diabetics patients in keeping the regular practice of the physical activity. Page et al. (1992) showed that 6 months of regular physical activity practice was not enough to incorporate this habit to the lifestyle of the patients [28].

Taking into account the comparisons carried out along the experiment (28, 60, 90 and 120 days), an improvement in glucose tolerance was observed for the trained control group, which at the 90th and 120th days presented lower area under the serum glucose curve than at the 60 days. These data are further strengthened by the HOMA index data, where the same group presented an increase in insulin sensitivity at 90 and 120 days of experiment. In a similar study with human beings predisposed to develop type-2 diabetes, Knowler et al. (2002) demonstrated that the practice of 150 minutes of physical activity per week was more efficient in preventing the outbreak of type-2 diabetes than metformin treatment (850 mg) twice a day [29].

Conclusion

The neonatal administration of alloxan induced a persistent glucose intolerance and physical training at the aerobic/anaerobic metabolic transition counteracted this intolerance.
Abbreviations


Competing Interests

The author(s) declare that they have no competing interests.

Authors' contributions

CSAM, conceived of the study, developed the study protocol, reviewed the references, abstracted data, analyzed the data, and wrote the paper. CR, GGA, MBA, FBM-G, CAMO, FAV and EL, participated in the design of the study, reviewed the manuscript, and advised on revisions to the manuscript. MARM. conceived of the study, gave very important financial support and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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References


FIGURE LEGENDS

Figure 1: Effort test. Blood Lactate (mmol/L) during effort test for evaluation of the maximal lactate steady state, after weaning (28 days) of an animal of each group, as an example. For the control rat, the maximal lactate steady state was reached with a load equivalent to 6.5% of the body weight and the mean blood lactate concentration was 4.4 ± 0.4. For the alloxan group, the maximal steady state lactate was reached with a load equivalent to 5.5% of the body weight and the mean blood lactate concentration was 4.5 ± 0.2 mmol/L.

Figure 2: HOMA. HOMA insulin sensitivity Index* (homeostasis model assessment) of the animals at weaning (28 days - C and A) and at 60, 90 and 120 days old. Values showed as mean ± standard deviation, n=8 animals per group
C=Control; TC= Treinad control; A= Alloxan e TA= Treinad alloxan
a = C vs TC; e = TC vs TA

Figure 3: Area under serum glucose curves during GTTo. Areas under serum glucose curves during GTTo (mmol/L x 120 min) of the animals at weaning (28 days - C and A) and at 60, 90 and 120 days old. Values showed as mean ± standard deviation, n=8 animals per group
C=Control; TC= Treinad control; A= Alloxan e TA= Treinad alloxan
b = C vs A; c = C vs TA; d = A vs TA; e = TC vs TA

Table 1: Areas under the curves of body weight (g x 16 weeks), food intake (g of chow/100g of body weight x 16 weeks) and water intake (ml of water/100g of body weight x 16 weeks) of the animals.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body Weight</th>
<th>Food intake</th>
<th>Water intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>3648.7 ± 212.4</td>
<td>103.0 ± 11.0</td>
<td>127.9 ± 21.2</td>
</tr>
<tr>
<td>TC</td>
<td>3281.2 ± 212.0 ( ^a )</td>
<td>131.3 ± 8.7</td>
<td>148.6 ± 22.6</td>
</tr>
<tr>
<td>A</td>
<td>3258.3 ± 202.2 ( ^b )</td>
<td>106.9 ± 6.5</td>
<td>124.4 ± 5.2</td>
</tr>
<tr>
<td>TA</td>
<td>3093.2 ± 291.2 ( ^c )</td>
<td>137.6 ± 19.6 ( ^{c,d} )</td>
<td>156.9 ± 4.1</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation, n=8 animals per group.
C=Control; TC= Treinad control; A= Alloxan e TA= Treinad alloxan
a = C vs TC; b = C vs A; c = C vs TA; d = A vs TA
EFFORT TEST

Control

Alloxan

Figure 1
Figure 2
Figure 3

AREA UNDER GLUCOSE CURVE DURING GTT₀

mmol/L x 120 min

Groups

C 28 60 90 120
TC 60 90 120
A 28 60 90 120
TA 60 90 120

Days

- 28
- 60
- 90
- 120

Figure 3