Response of Creatine Kinase (CK) Levels in Diabetics Wistar Rats After Acute Physical Exercise

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ABSTRACT

Junior MC, Spagnol AR, Pereira AS, Novais IP, de Castro MR, Anaruma CP, Valgas CP, Luciano E. Response of Creatine Kinase (CK) Levels in Diabetics Wistar Rats After Acute Physical Exercise. JEPonline 2013;16(2):99-111. This study examined the effects of acute exercise on hyperglycemia and kinetic behavior of creatine kinase (CK) in diabetic rats. Wistar rats were distributed into two groups: Exercise Control (C) and Exercise Diabetic (D). Diabetes mellitus was induced by alloxan monohydrate Sigma® (32 mg·kg⁻¹ of body weight). After 7 days, a glucose test was carried out. Animals with values equal to or higher than 200 mg·dL⁻¹ were considered diabetic. After 15 days, both groups were subjected to a single session of 30 min of swimming with a load of 4.5% body weight attached to the dorsal region. Biochemical analyses were performed moments before, immediately after, and at 18 hrs, 24 hrs, 48 hrs, and 96 hrs after aerobic physical exercise through the collection of blood using heparinized capillary. The serum was withdrawn and used for determination of creatine kinase (CK). The CK concentrations were higher at the time immediately after exercise in diabetics (P<0.05). In the control group, there was a tendency of decreased levels of CK after exercise, and at 24 hrs CK levels were lower than at pre exercise. There were significant differences between groups, thus characterizing a higher state of injury in diabetic animals throughout the experiment. Although moderate aerobic exercise caused a reduction of glucose levels in the diabetic rats, there was an increase in the levels of CK that reflects a greater muscular injury.

Key Words: Aerobic Exercise, Creatine Kinase, Diabetes Mellitus
INTRODUCTION

Currently, diabetes mellitus (DM) is considered a global public health problem. It is estimated that there are more than 220 million diabetics worldwide (46). There is the expectation that DM is going to increase, mainly due to population growth, obesity, aging, and the prevalence of a sedentary lifestyle (47).

Diabetes mellitus is considered to be a metabolic disorder characterized by a reduction or failure in the secretion or action of insulin, thus resulting in the pathological state of hyperglycemia. The pathology can be classified as two types: type 1 diabetes mellitus (DM1) and type 2 (DM2). The DM1 influences mainly the young individuals. It promotes the destruction of pancreatic β cells in the islets of Langerhans, usually by an autoimmune process that leads to a reduction in the quality of life and longevity (43). By comparison, DM2 is characterized by reduced sensitivity to insulin action. It is considered a disease that is caused by a sedentary lifestyle and inadequate nutrition (31).

The chronic hyperglycemia that is characterized in untreated DM1 is often related to cardiovascular diseases with microvascular complications and neuropathies that result in blindness, hypertension, and a decrease in the sensation of the extremities. There is also renal dysfunction, inflammatory processes, fatigue, and muscular injury (9,14,21,44). The deleterious effects of hyperglycemia are related to increased production of reactive oxygen species (ROs), which in high amounts promote increased cell damage (45). The result may be structural changes in proteins and the DNA of cells that causes damages in functionality.

According to the American Diabetes Association (1), it has been found that regular physical exercise is beneficial to patients with both types of diabetes. In particular, a training program for low, moderate or high intensity (16,28) is related to an increase in the expression of the major glucose transporter protein in the skeletal muscle (GLUT-4) and the increase insulin sensitivity by its cellular receptor. However, it is also recognized that acute exercise causes a decrease in blood glucose because muscle contraction results in an increase in translocation of GLUT-4 through various mechanisms, including calcium influx, the calcium-calmodulin complex formation, and the activation of AMP-activated protein kinase (AMPK). These mechanisms increase the uptake of glucose and improves glycemic homeostasis (16,33,36,40).

Evidences show that the chronic hyperglycemia may result in damage to the cell membrane of almost all tissues due to the reaction of excess glucose with lipids and proteins of the cellular membrane, thus reducing its stability (27). Krause and colleagues (25) have indicated that diabetic subjects experience disorders of skeletal muscle tissue (e.g., fiber atrophy, Z-line disruption, mitochondrial abnormalities, and loss of muscle fibers especially type I, even before displaying neuropathy).

During physical exercise it is known that hyperglycemia (37), the increased production of ROs (11), and the decreased levels of glycogen due to hypoinsulinemia (21) may make the diabetic individual more susceptible to fatigue and muscular injuries. Although physical activity has beneficial effects in terms of reducing blood glucose, little is known about its influence on levels of creatine kinase (CK) in diabetic rats (25,38). Yet, CK is found almost exclusively in skeletal and cardiac muscular tissue (4,34,35). Since people with diabetes are likely to be more susceptible to muscular injury, the purpose of this study was to analyze the effects of acute exercise on hyperglycemia and kinetic behavior of CK in diabetic rats.
METHODS

Animals

Twenty recently weaned male Wistar rats with 45 days old (at the beginning of experiment) were selected from the Central Biotery Facility of the São Paulo State University (UNESP), Campus Botucatu. The animals were housed in polyethylene cages measuring 37 x 31 x 16 (5 rats per cage), under controlled temperature conditions 21º C and photoperiod (12 hrs light/dark), fed with a standard rat chow (Purina) and water ad libitum for the experimental groups (in the Biotery of Biodynamic Laboratory of the Department of Physical Department - Institute of Bioscience – UNESP – Rio Claro). All experimental procedures were in accordance with the standards of the Brazilian College of Animal Experimentation (COBEA) and were approved by the Ethics Committee on Animal Use (CEUA) of Institute of Bioscience at UNESP – Rio Claro Campus.

Induction of Diabetes Mellitus and Experimental Design

The rats were moderately anesthetized with diethyl ether and then received alloxan monohydrate Sigma® (32 mg·kg⁻¹ of body weight) dissolved in 0.01 M citrate buffer, pH 4.5. After induction of DM by alloxan, the animals were relocated in cages and received a glucose and water solution (15%) during the first day, and food (29). Seven days after drug administration, a blood glucose test was carried out (commercial kit- Laborlab® enzymatic colorimetric method of glucose oxidase/peroxidase) to confirm the diabetic state. The rats considered diabetics were those that presented glucose level equal or above 200 mg·dL⁻¹. After these procedures, body weight was recorded and the distribution of the animals to the control and diabetic groups according to blood glucose levels.

Acute Physical Exercise Session

Two weeks after the confirmation of the DM, the animals of the exercise control group and the exercise diabetic group were subjected to a single session of swimming for a period of 30 min. A load equivalent to 4.5% of body weight was attached to the dorsal region of animals. The load was considered aerobic as verified by Araújo et al. (12) and by Oliveira et al. (13). The rats were then divided into two groups:

- Exercise Control (C): Rats that were subjected to the exercise protocol (n = 10);
- Exercise Diabetic (D): Diabetic rats that were subjected to the exercise protocol (n = 8).

Serum Evaluations

Biochemical analyses were performed in the moments before, immediately after, as well as 18 hrs, 24 hrs, 48 hrs and 96 hrs after aerobic physical exercise by collecting blood through two heparinized capillaries. The collected blood was centrifuged at 3000 rev·min⁻¹ for 15 min and the hematocrit was measured to check the level of hydration of the animals. Subsequently, the supernatant (serum) was removed and used to determine creatine kinase (CK) (measured at 37°C) by kinetic enzymatic method using commercial kits Laborlab®. For glucose analysis, a capillary with 25 µL of blood was collected from the animals’ tails in the moments before, immediately after, as well as 18 hrs, 24 hrs, 48 hrs, and 96 hrs after aerobic exercise. The blood glucose concentrations were determined through the enzymatic colorimetric method of glucose oxidase/peroxidase, using commercial kits Laborlab®.

Statistical Analysis

The data obtained in each group are described as mean ± standard deviation. They were statistically analyzed using analysis of variance (ANOVA) for repeated measures and the post-hoc Tukey for glucose kinetic variables, CK, and hematocrit. The area under the curve (AUC) was calculated by the
trapezoidal method. The comparisons of area under the curve of CK, body weight, food intake, and hydric intake were compared by the Student t test. The level of significance was predetermined at 5%, using the Statistica 7 software.

RESULTS

Diabetes mellitus induction was demonstrated by the glycemia serum test (Figure 3), and also by observation of the disease classic symptoms such as polyphagia (Figure 1A), polydipsia (Figure 1B), and polyuria. There was also a significant decrease in body weight of the animals in the diabetic group compared to the animals of the control group (C=416.14 ± 32.37; D=347.40 ± 32.41). Two weeks after inducing DM, the animals were subjected to acute physical exercise and blood collections were performed.

Figure 1. (A) Graph of Food Intake in g·100 g⁻¹ of Body Weight. (B) Graph of Hydric Intake in mL·100 g⁻¹ of Body Weight. *Indicates significant difference between groups (P<0.05).

Figure 2 shows the kinetics of hematocrit during the moments before, immediately after, and at 18 hrs, 24 hrs, 48 hrs, and 96 hrs after physical exercise. In this chart, it is possible to notice a change in the behavior of the hematocrit after exercise, characterizing a reduction in concentrations of water in the animals. This scenario of water loss in the animals of the control group returned to normality after 24 hrs, while the diabetic group persisted for 96 hrs.
Figure 2. Comparison of Kinetic Hematocrit at Pre, Post, 18 hrs, 24 hrs, 48 hrs, and 96 hrs After Exercise Between Groups.  

- Significant difference in relation to group diabetic pre.  
- Significant difference in relation to group control pre.  
- Significant difference in relation to control at the same time of analysis. Significance level, P<0.05.

During glycemic kinetics in Figure 3, the high concentrations of glucose in the diabetic group can be observed due to a disorder caused in the pancreatic beta cells resulting in the reduction or even the absence of insulin secretion. The same did not occur in the control group, confirming the installation of DM1 due to the infusion of alloxan. Regarding the effect of acute physical exercise, it promoted a decrease in the concentration of blood glucose in the diabetic animals and not in the control animals, returning to normality after 18 hrs.

Figure 3. Comparison of Glycemia Kinetic at Pre, Post, 18 hrs, 24 hrs, 48 hrs, and 96 hrs After Exercise Between Groups.  

- Significant difference in relation to group diabetic pre.  
- Significant difference in relation to group control pre.  
- Significant difference in relation to control at the same time of analysis. Significance level, P<0.05.
As shown in Figure 4A, the CK plasma concentrations were higher after exercise (Pos vs. Pre) for the diabetic animals, but returned to normal in all other subsequent moments (P<0.05). In the control group, there was a tendency for decreased plasma concentrations of CK immediately after exercise. At 24 hrs, CK levels were found lower when compared to the pre-exercise value (followed by a return to normal after 48 hrs).

Figure 4B represents an analysis of the area under the plasma concentration curve of CK in the moments before, immediately after, and at 18 hrs, 24 hrs, 48 hrs, and 96 hrs after exercise. A significant difference (P<0.05) exists between the two groups, thus characterizing the occurrence of muscle injury in diabetic animals during the period of 96 hrs.

**Figure 4.** (A) Comparison of Creatine Kinase (CK) Kinetic at Pre, Post, 18 hrs, 24 hrs, 48 hrs, and 96 hrs After Exercise Between Groups. (B) Comparison of Area Under Curve (AUC) of CK Between Groups. *a*Significant difference in relation to the diabetic group pre. *b*Significant difference in relation to the control group pre. *c*Significant difference in relation to control at the same time of analysis. *d*Significant difference in AUC in relation to control. Significance level, P<0.05.

**DISCUSSION**

Animal models have been widely used in research that is designed to understand the mechanisms of diabetes and DM1 induction by alloxan. Clearly, it is a very effective way to develop this particular pathology (26). Using this technique the present study examined the induction of diabetes through the
test of glycemia and by the observation of the classic symptoms of the disease such as polyuria, polyphagia, polydipsia, and weight reduction.

There were no significant differences in hematocrit and, in agreement with Oliveira et al. (13), the hypothesis of dehydration has been discarded as a susceptibility to this disease. However, after acute physical exercise, diabetic animals had a state of reduced water levels, viewed by increased concentrations of hematocrit, caused in higher propensity to a dehydrated state, which lasted until the end of the experiment. This deficiency in the fluid replacement may have occurred due to the state of polyuria, which may have been caused by an excessive increase in the concentrations of glucose (hyperglycemia). Hyperglycemia promotes an increase in filtered and excreted loads of glucose by the kidneys, causing glycosuria and osmotic diuresis. This osmotic effect results in decreasing the reabsorption of liquid that may cause the appearance of polyuria resulting in dehydration and, consequently, polydipsia (33,39). In the present study, the increase in hematocrit was restored after 24 hrs in the control group, emphasizing the effect of polyuria in the dehydration of diabetic animals on post-exercise.

Regarding glycemia, when subjected to the exercise protocol, rats of the control group showed no significant difference between the pre and immediately after physical exercise (Figure 3). Already in the diabetic group, there was a significant reduction in glycemic values in the period immediately after acute physical exercise. Such finding in relation to the control group is expected for a healthy population and occurs due to increased concentrations of catabolic hormones, such as adrenaline and glucagon that promote the synthesis of glucose from the liver and muscle glycogen. However, in diabetic individuals this pathway is altered (19). This finding corroborates the results.

The reduction of glycemia caused by physical exercise was independent of insulin, which was found drastically reduced or even absent in the diabetic group. The mechanisms involved in this glycemic reduction through glucose uptake may have been: (a) increased activation of protein kinase activated by adenosine monophosphate (AMPK) (19,36,40); (b) increased concentration of calcium ions within the cell (48); (c) the activity of nitric oxide synthase (NOS) and the also synthesis of nitric oxide (NO) (5); and (d) increase in bradykinin concentration and/or even hypoxia (3,36).

The elevation of the AMPK, seen as the major cause of the glucose influx stimulated in the physical exercise by mechanisms not yet completely understood, cause the translocation of vesicles containing GLUT-4 to the cell membrane (16,28). According to Hardie et al. (20), this activation is caused by the decrease in cellular energy status, that is, a situation in which the AMP/ATP ratio is increased, and conformational changes in the molecule, leaving it susceptible to phosphorylation and activation by AMPK, thus increasing the translocation of glucose transporters (36).

Although the acute physical exercise contributes to the glycemic decrease in diabetic individuals, this does not reach normal levels. However, it is known that when exercise is performed chronically, it improves glycemic control in diabetic animals because it eases even further the peripheral glucose uptake and metabolism of glycogen and proteins (30,33).

As pointed out by Mann et al. (31), disorders of carbohydrate metabolism may indirectly injure the muscles, as in the case of DM1. In this regard, the present study focused on measuring the levels of creatine kinase (CK) to check the level of muscle injury in diabetic rats exposed to acute exercise. According to Brancaccio and colleagues (8), CK is used as a marker of muscle injury levels, allowing for the identification of the state of the muscle. Thus, high levels of circulating CK represent muscle injury in healthy people, which may be correlated with the severity and/or duration of the physical
activity (3). Foschini et al. (15) showed signs of injury in the muscles of individuals who did not have disease, but nonetheless had high concentrations of CK.

After 24 hrs of aerobic exercise (Figure 4A), the CK values reached levels similar to the pre exercise period, though, after 48 hrs these values increased again, showing the tendency for CK to increase in diabetic animals. These findings are in agreement with earlier reports (8,11,15) of CK values peaking at 24 to 48 hrs after exercise. However, the mechanisms involved in the decrease followed by an increase are not entirely clear and may involve the lack of the hormones insulin, growth hormone (GH), and growth factors similar to the insulin (IGF-1) that contributes to the recovery of damage caused by exercise (25). Hence, an analysis of the concentrations of CK during all time periods was performed in order to check the levels of muscle injury and recovery throughout the experiment. In Figure 4B it can be seen that the diabetic animals maintained higher levels of damage during the entire period of experiment.

One possible mechanism related to the increased injury in diabetic animals may be due to increased production of reactive oxygen species (ROS), which can cause cellular damage and injury compared to exercise (22,23). In basal levels, due to hyperglycemia, the production of ROS is increased in these animals (6). Then, too, exercise adds to the production (11,45), which may cause a redox imbalance leading to serious cellular damage, including apoptosis, damage to cellular DNA, and cell rupture (6,11,45) that increases the values of serum CK.

The increase in ROS probably contributes to a higher incidence of muscle injury in diabetic animals by promoting up-regulation in the genes related to atrophy (such as atrogin-1 and MuRF-1), concomitant with a down-regulation in the genes associated with muscle growth (JunD, MyoD, myogenin, MCK, MHCIIB, MLC1-3) in diabetic rats (2,32). Furthermore, Russell and colleagues (42) demonstrated that an increase in the blood glucose concentrations may promote protein degradation and suppress the synthesis in myotubes in vitro. According to the researchers, the protein degradation was associated with an activation of the p38 and caspase-3/-8 pathway, leading to activation of the ubiquitin-proteasome pathway, and simultaneously promoting the suppression of phosphorylation-PKR-dependent and IF2c (stimulatory protein of protein synthesis).

Regarding the higher level of muscle injury in diabetic animals, a reduction in the levels of anabolic hormones and elevation of catabolic hormones corroborate this fact. For instance, cortisol, a hormone that promotes protein degradation, is found in high concentrations above control values in patients with DM1, especially if they are not in the ideal glycemic control (10,41). The inhibitor of plasminogen activator (PAI-1) is another hormone implicated in complications of DM1 pathology (7). Interestingly, increased PAI-1 was also found to be a potent inhibitor of skeletal muscle regeneration following a condition of microtrauma (24) and, therefore, may be a factor that contributes to an increase in CK after physical exercise.

As to the anabolic hormones, aside from individuals with DM1 not having sufficient concentrations of insulin (anabolic hormone), they have reduced concentrations of growth hormone (GH) and growth factor similar to insulin (IGF-1) when compared to the control group (18). Besides IGF-1 acting mainly in the increase and signaling of protein synthesis, it also acts directly in the suppression of protein degradation pathways (such as atrogin-1, ligase E2, and ubiquitin-proteosome system). According to Gomes et al. (17) and Gomes et al. (18), IGF-1 and GH levels are low, which might be implicated in the increased concentrations of CK serum.

Another factor that may affect the level of injury is the intensity of physical exercise. Judging by the difference in the response of CK, the data suggest that exercise for the diabetic group would have to
be of greater intensity (considering that this pathology promotes reduction in the levels of physical performance) (13,25). However, the intensity used in this study was based on previous studies from our laboratory that have shown to be suitable for the diabetes model in question (13).

CONCLUSION

Based on the results of this study, it can be concluded that acute moderate aerobic physical exercise decreases the concentration of serum glucose in type 1 diabetic rats without the use of drugs. However, this acute physical activity may cause an increase in CK levels for diabetic animals that suggests a higher level of muscle injury caused by the pathology.

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