

## Acute caffeine intake lowers glycemia before and after acute physical exercise in diabetic rats<sup>1</sup>

### *Ingestão aguda de cafeína reduz a glicemia sanguínea antes e após o exercício físico agudo em ratos diabéticos*

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#### ABSTRACT

##### Objective

The present study investigated the effects of caffeine supplementation combined with acute physical exercise on the glycemic response of diabetic rats.

##### Methods

Thirty-two 60-day-old rats with a mean weight of 238±3 g were divided into four groups: control, control caffeine, diabetes, and diabetes/caffeine. Diabetes was induced by 60 mg/kg of streptozotocin intraperitoneally. The control groups received an acute dose of caffeine (6 mg) or saline 60 minutes before exercise. The animals were then forced to swim for 60 minutes carrying a ballast weighing 6% of their body weight, producing lactacidemia compatible with the maximum lactate production during the steady state (5.5 mmol/L). After the acute exercise session, the animals were sacrificed and their blood collected for glucose analysis. The cardiovascular responses were measured before and after supplementation by tail cuff plethysmography. The one-way Analysis

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of Variance (Anova) was realized with *post hoc* of Student-Newman-Keuls to analyse the statistical differences between the supplementations, considering  $p < 0.05$ .

### Results

Caffeine at a dose of 6 mg/kg did not change the cardiovascular responses. However, compared with the control groups, caffeine reduced the blood glucose (42%,  $p < 0.05$ ) of diabetic rats after 60 minutes of exercise.

### Conclusion

Acute caffeine ingestion together with exercise can increase glucose uptake without changing cardiovascular responses in animal models.

**Indexing terms:** Caffeine. Diabetes Mellitus. Exercise. Glycemia.

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## RESUMO

### Objetivo

O presente estudo verificou os efeitos da suplementação com cafeína associada ao exercício físico agudo sobre a resposta glicêmica em ratos diabéticos.

### Métodos

Foram utilizados 32 animais, com 60 dias de idade, e  $238 \pm 3$  g de peso, divididos em quatro grupos: controle, controle cafeína, diabetes e diabetes/cafeína. O modelo de diabetes foi induzido pela administração intraperitoneal de 60 mg/kg de estreptozotocina. De forma aguda, os animais receberam 6 mg de cafeína ou salina para os grupos-controles 60 minutos antes do exercício físico. Os animais realizaram um protocolo de natação de 60 minutos de exercício físico, com sobrecarga de 6% do peso corporal com lactacidemia compatível com a máxima produção de lactato em estado estável (5,5 mmol/L). Após o exercício físico agudo, foi realizada a eutanásia dos animais para coleta de sangue e análises glicêmicas. Antes e após a prescrição das suplementações, ocorreu a aferição das respostas cardiovasculares por meio de um pletismógrafo de cauda. Foi realizado o teste estatístico Análise de Variância one way com *post hoc* de Student-Newman-Keuls para analisar as diferenças estatísticas entre as suplementações, sendo considerado  $p < 0,05$ .

### Resultados

A prescrição de cafeína na dose de 6 mg/kg não alterou respostas cardiovasculares. No entanto, a cafeína promoveu uma significativa redução na glicemia sanguínea (42%;  $p < 0,05$ ) após 60 minutos do protocolo de exercício nos ratos diabéticos em relação aos grupos-controles.

### Conclusão

A ingestão aguda de cafeína associada ao exercício físico agudo pode aumentar a captação de glicose sem alterar as respostas cardiovasculares em modelo animal.

**Termos de indexação:** Cafeína. Diabetes Mellitus. Exercício. Glicemia.

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## INTRODUCTION

Diabetes Mellitus (DM) is a metabolic disorder characterized by a change in the homeostasis of energy substrates, causing hyperglycemia secondary to low pancreatic secretion of the hormone insulin or low response of peripheral insulin receptors to the hormone<sup>1</sup>. Its prevalence is increasing exponentially, acquiring pandemic characteristics in many countries. In Brazil the mean occurrence of adult diabetes (individuals aged 18 years or more) is 5.2%,

representing 6,399,187 people who confirmed having the disease. The prevalence increases with age: DM affects 18.6% of the population aged more than 65 years<sup>2</sup>.

In diabetes Mellitus, the clinical picture resultant from the metabolic disorder characteristic of the syndrome could be controlled by non-pharmacological treatments, such as physical exercise or dietary substances that can improve the metabolic status and its consequences. Clinical studies have shown that caffeine intake before

exercise increases fat oxidation and carbohydrate uptake by activated muscle cells<sup>3</sup>, possibly improving the metabolic status of diabetic patients. Verguawen *et al.*<sup>4</sup> have shown that caffeine can increase skeletal muscle glucose uptake in rats by increasing the intracellular concentration of calcium ions (Ca<sup>2+</sup>) and the expression of Adenosine Monophosphate-Activated Protein Kinase (AMPK), which may be an interesting strategy to reduce insulin resistance in skeletal muscle and control glycemia in diabetics.

Caffeine is an alkaloid from the methylxanthine group (1,3,7-trimethylxanthine) that is rapidly absorbed by the gastrointestinal tract and metabolized by the liver. Its biological half-life varies from 2.5 to 4.5 hours<sup>5</sup>. Caffeine's main mechanisms of action include the intracellular mobilization of Ca<sup>2+</sup>, catecholamine increase, and adenosine receptor antagonism<sup>6</sup>.

Caffeine stimulates insulin secretion by pancreatic  $\beta$ -cells by increasing intracellular Ca<sup>2+</sup><sup>7</sup>. In skeletal muscle caffeine can increase the expression of Glucose Transporter type 4 (GLUT4) by increasing intracellular Ca<sup>2+</sup> concentration and AMPK expression<sup>7,8</sup>. Caffeine also antagonizes the adenosine receptors involved in glycogenolysis and gluconeogenesis found in the cell membranes of hepatocytes<sup>9</sup>.

Physical exercise promotes important changes in glucose homeostasis and possibly a rapid blood glucose decrease in diabetics. This action can be observed by monitoring glycemia during planned physical activity<sup>3,10</sup>. The ingestion of energy substrates or substances that promote glycemic control is a good strategy for preventing a sharp increase in blood pressure or rebound hypoglycemia during and after physical activity<sup>11</sup>.

Preclinical (animal model) investigations of the mechanism by which caffeine benefits diabetics or how the benefits are optimized are still scarce. Some data have suggested that caffeine or aerobic exercise may increase cellular glucose uptake and thereby reduce insulin resistance. However, the effect of associating both strategies for metabolically controlling diabetes

is unknown. Hence, a study showing the potential effects of caffeine on lipid metabolism, glycemic and insulin control, and insulin response, further enhanced by physical activity, could encourage new diabetes treatment strategies.

Thus, the objective of the present study is to verify the effects of caffeine and acute physical exercise on the glycemic and cardiovascular response of diabetic rats.

## METHODS

Thirty-two 60-day-old Wistar rats with a mean weight of 238 $\pm$ 3 g were kept at a room temperature of 23 $\pm$ 2 °C, controlled moisture of 55% $\pm$ 10%, and an inverse 12-hour light/dark cycle.

The study was approved by the local Animal Research Ethics Committee under Protocol number 026/2011.

### Experimental design

The animals were divided into four groups: (1) Control, (2) Control Caffeine, (3) Diabetes, and (4) Diabetes/Caffeine. The diabetic groups were given intraperitoneally 60 mg/kg of Streptozotocin (STZ) (Sigma, St. Louis, EUA) dissolved in a 0.01M citrate buffer (pH 4.5) after a 12-hour fast. The diabetic groups consisted of animals with a fasting blood glucose level >250 mg/dL, as classified by a previous study<sup>12</sup>.

After an 8-hour fast, the rats received by gavage either 6 mg/kg of caffeine (same dosage used by Graham *et al.*<sup>3</sup>) diluted in water or a placebo (0.9% NaCl solution). These supplements were administered 60 minutes before the exercise protocol because of caffeine's biological half-life<sup>6</sup>.

### Exercise protocol

All animals were adapted to water by allowing them to swim 10 minutes a day for seven days before the experimental protocol began. On the test day, after receiving the supplements, all

animals performed a 60-minute, predominantly aerobic swimming exercise carrying a ballast weighing 6% of their bodyweight, which induced lactacidemia compatible with the maximum lactate production during the steady state (5.5 mmol/L). This protocol, proposed by Gobatto *et al.*<sup>13</sup>, uses a 40 cm-deep tank with water heated to 30 °C ± 1 °C.

### Determination of blood pressure and heart rate

Heart Rate (HR), Systolic Blood Pressure (SBP), and Diastolic Blood Pressure (DBP), were determined by tail cuff plethysmography (Insight®, *Ribeirão Preto*, Brazil). All animals were familiarized with the device by undergoing three measurements a day for five days before the experimental protocol. On the test day, before and after the supplements and before the exercise session, the HR and blood pressure of each animal were measured at least three times. Heart rate, SBP, and DBP were recorded by the device's software after each measurement.

### Glycemic analyses

Blood glucose was measured before and after the gavage by tail vein puncture. Approximately 25 µL of blood were collected and analyzed by the glucose meter ACCU-CHEK® Active (Roche, Switzerland).

The rats were sacrificed immediately after the exercise protocol, and their blood were collected and stored in tubes containing fluoride for enzymatic analysis of blood glucose. The samples were centrifuged at 1500 rpm for ten minutes to separate the serum and analyzed by a semiautomatic biochemical analyzer Diaglobe CA-2006® (New York, United States of America) using the BioSystems kit (Barcelona, Spain).

### Statistical analysis

All results were expressed as mean ± Standard Error of the Mean (SEM). The statistical analyses relied on one-way Analysis of Variance (Anova). The significance level was set at 5% ( $p < 0.05$ ). When appropriate, the differences between the groups were determined by the *post hoc* Student-Newman-Keuls test.

## RESULTS

Caffeine intake did not change the cardiovascular variables significantly (Table 1).

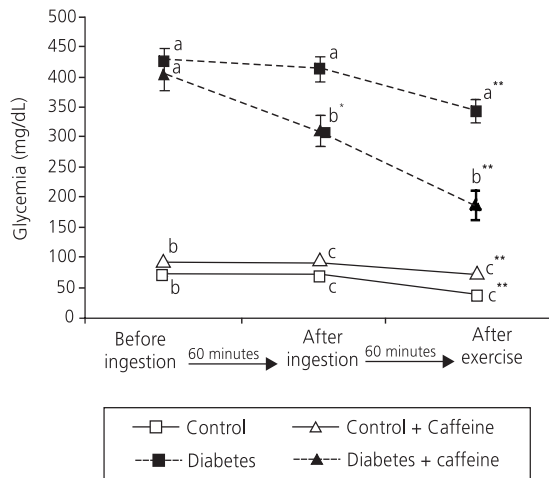
The blood glucose of the Diabetes/Caffeine group decreased by 25% (from 403 mg/dL to 311 mg/dL;  $p < 0.05$ ) 60 minutes after the ingestion of 6 mg/kg of caffeine (without exercise) compared with the fasting group. The blood glucose of the Diabetes group was 42% higher than that of the Diabetes/Caffeine group (Diabetes=387 mg/dL and Diabetes/Caffeine=187 mg/dL;  $p < 0.05$ ) after the physical activity protocol (Figure 1).

**Table 1.** Cardiovascular responses before and after the respective supplementations before exercise (n=32).

Groups	HR (bpm)				SBP (mm/Hg)				DBP (mm/Hg)			
	Before ingestion		After ingestion		Before ingestion		After ingestion		Before ingestion		After ingestion	
	M	SE	M	SE	M	SE	M	SE	M	SE	M	SE
Control	375 ± 5		336 ± 6		124 ± 2		126 ± 3		79 ± 4		80 ± 4	
Control caffeine	450 ± 24		359 ± 28		125 ± 1		126 ± 2		83 ± 3		81 ± 3	
Diabetes	434 ± 17		433 ± 17		126 ± 1		120 ± 5		82 ± 2		81 ± 2	
Diabetes/Caffeine	436 ± 19		433 ± 25		123 ± 2		123 ± 1		81 ± 3		82 ± 3	

Note: The data are expressed as Mean ± Standard Error of the Mean (SEM). The intra- and intergroup comparisons were done by Student-Newman-Keuls *post hoc* one-way Analysis of Variance.

HR: Heart Rate; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure.



**Figure 1.** Glycemia variation according to supplementation. The values are expressed as means ± standard error of the mean (n=32).

Note: \*Indicate intragroup statistical differences before and after supplementation ( $p < 0.05$ ); \*\*Indicate intragroup statistical differences before and after exercise ( $p < 0.05$ ); Different letters (a, b, c, d) indicate statistical differences between the groups ( $p < 0.05$ ; Student-Newman-Keuls test after one-way analysis of variance).

## DISCUSSION

Glycemic control promoted by diet and exercise greatly helps to prevent the frequent bouts of hyperglycemia and hypoglycemia experienced by diabetics before and after exercise. Moreover, glycemic control promotes a positive long-term impact on the clinical status of diabetics, delaying the tissue damage associated with this metabolic disorder. Caffeine intake significantly decreased STZ-induced hyperglycemia in the study rats before and after exercise.

Although the study STZ-induced diabetes model kills 10% to 30% of pancreatic  $\beta$ -cells<sup>14</sup>, caffeine intake associated with acute physical activity reduced hyperglycemia by 19%, lowering blood glucose from 436 mg/dL to 343 mg/dL. Therefore, caffeine may stimulate insulin secretion by pancreatic  $\beta$ -cells as has been demonstrated by other studies: its activity cascade and amplification pathways promote insulin secretion by blocking Adenosine Triphosphate (ATP)-sensitive potassium channels in the pancreas, increasing calcium

concentration<sup>15,16</sup>. Additionally, caffeine has also promoted the expression of GLUT4 in the skeletal muscle of animals not exposed to acute physical activity even in dosages (7.6 mg/dL)<sup>7</sup> similar to the present dosage (6 mg/dL). Caffeine may act in two fronts: first, it increases the number of glucose transporters, promoting lower insulin resistance; and second, it stimulates insulin secretion, which, together with the predominantly aerobic exercise and low lactate threshold, results in better skeletal muscle glucose signaling and uptake efficiency, which in turn attenuates the hyperglycemic status in the study model of diabetes.

It is well established that physical exercise may increase insulin sensitivity, GLUT4 expression, and glycogen synthase activity in the muscle cells of type-2 diabetics, and this effect may last as much as 48 hours<sup>17,18</sup>.

Caffeine also increases incretins, such as Glucagon-Like Peptide 1 and Glucose-Dependent Insulinotropic Polypeptide, so in theory, it may increase insulin secretion<sup>19</sup>. The intake of 180mg of caffeine by type-2 diabetics increases blood glucose uptake during the Oral Glucose Tolerance Test, contrary to the group that consumed a decaffeinated beverage<sup>20</sup>.

Noordzij *et al.*<sup>21</sup> showed that caffeine's stimulation of the cardiovascular system is directly proportional to the amount of caffeine consumed. The effect of caffeine on cardiovascular responses arises from the classic effect of caffeine of increasing catecholamine release, directly affecting the sympathetic nervous system and consequently increasing blood pressure<sup>6,22</sup>. However, significant differences between the groups did not occur in the present study, possibly because of the caffeine dosage used (6 mg/kg).

## CONCLUSION

Caffeine decreased glycemia significantly without promoting cardiovascular changes. Future studies should try to better clarify caffeine's dose-

response effect before and after physical activity, which may indicate that caffeine intake is an interesting strategy to control glycemia.

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#### CONTRIBUTORS

LA SILVA collected data, searched the literature, performed the statistical analyses, created and interpreted the graphs, wrote the manuscript, and discussed the results. RA PEREIRA collected data and wrote the manuscript. JA TÚRMINA collected data and searched the literature. II KERPPERS wrote the manuscript and discussed the results. LR ALTIMARI wrote the manuscript and discussed the results. CRM MALFATTI performed the statistical analyses, created and interpreted the graphs, wrote the manuscript, and discussed the results.

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