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# Central nitric oxide inhibition modifies metabolic adjustments induced by exercise in rats

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**Abstract**

The influence of the central nervous system on metabolic function is of interest in situations deviating from basal states, such as during exercise. Our previous study in rats demonstrated that central nitric oxide (NO) blockade increases metabolic rate, reducing mechanical efficiency during exercise. To assess the role of brain nitric oxide in the plasma glucose, lactate and free fatty acids (FFAs) concentrations of rats submitted to an incremental exercise protocol on a treadmill until fatigue, 1.43 μmol (2 μl) of $N^{\omega}$-nitro-L-arginine methyl ester (L-NAME, $n = 6$), a NO synthase inhibitor, or 2 μl of 0.15 M NaCl (SAL, $n = 6$) was injected into the lateral cerebral ventricle (icv) of male Wistar rats immediately before exercise (starting at 10 m/min, with increments of 1 m/min every 3 min until fatigue, 10% inclination). Blood samples were collected through a chronic jugular catheter at rest and during exercise until fatigue. During exercise, the L-NAME-treated animals had the following metabolic response compared to controls: (1) an increased hyperglycemic response during the first 60% of time to fatigue; (2) higher plasma lactate levels; and (3) a significant transitory increase in plasma free fatty acids during the dynamic phase of exercise that returned to basal levels earlier than controls during the steady state phase of exercise. In addition L-NAME-treated rats fatigued earlier than controls. The data indicate that the inhibition of the brain nitrergic system induced by icv L-NAME treatment disrupted the accuracy of the neural mechanism that regulates plasma glucose and free fatty acids mobilization during exercise in rats.


*Keywords:* Exercise; Glucose; Lactate; Free fatty acids; Fatigue

The central regulation of hepatic glucose production, catecholamine outflow from the adrenal medulla, and the mobilization of free fatty acids (FFAs) from the adipose tissue during exercise have been shown to be mediated by direct sympathetic innervation [7,14]. In such cases, during exercise, the sympathetic drive acts redundantly together with some hormones to increase FFAs mobilization, to stimulate liver glyconeogenesis and glycogenolysis, as well as to inhibit peripheral glucose uptake, thus conserving blood glucose, and inducing FFAs utilization as energetic fuel [4].

In our previous study, intracerebroventricular (icv) infusion of $N^{\omega}$-nitro-L-arginine methyl este [L-NAME—a nitric oxide synthase (NOS) blocker] induced a significant increase in metabolic rate and a decrease in mechanical efficiency, reducing running performance in rats [12]. However, we do not know whether fuel sources were also altered with the higher metabolic rate as a result of L-NAME-treated rats. Matsumura et al. [16] demonstrated that intracerebroventricular infusion of L-NAME increases plasma epinephrine and norepinephrine concentrations at rest, inducing hyperglycemia. They attributed these findings mainly to the enhanced sympathetic outflow. However, it is not known whether this central inhibitory effect on sympathetic outflow by brain NO would affect plasma glucose, lactate, and FFAs levels during exercise in the same extension as it did in metabolic rate [12]. Therefore, the influence of the central nervous system on metabolic adjustments during exercise is of particular interest. Exercise represents a physiological state in which metabolic and hormonal adaptations are required to increase the supply of glucose and FFAs levels to the working muscle and to maintain an adequate flow of glucose to the

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brain [6]. It has been well established that the sympathoadrenal system is activated during exercise, resulting in increased plasma concentrations of epinephrine and norepinephrine [7]. However, there is no evidence about the relative role of central nitric oxide (NO) on metabolic adjustments during exercise. So, we hypothesized that brain NO inhibition might be induce a much more increase on sympathetic outflow to the liver, adipose tissue, and adrenal medulla during exercise. As a result, we could observe a hyperglycemic and lactic response and an augmented free fatty acids mobilization, despite of the metabolic demands of the exercising muscles.

The aim of this study was to assess the effects of the central administration of the NOS inhibitor L-NAME on plasma glucose, lactate and FFAs concentrations of untrained rats submitted to progressive exercise until fatigue.

Male Wistar rats (200–280 g) were individually housed under 14/10 h, light–dark cycles and had free access to water and rat chow. Following anesthesia achieved using 2,2,2-tribromoethanol (300 mg/kg body weight i.p.), the rats were fixed to a stereotaxic apparatus (David Kopf Instruments, M-900, Tujunga, CA, USA) and a guide cannula (22 G) was implanted into the right lateral cerebral ventricle using a previously described technique [15,20]. A chronic jugular vein catheter was also implanted into these animals as described previously [14]. All animals were allowed to recover for at least 1 week before being submitted to the experiments. The rats were familiarized with exercise on the motor-driven treadmill by running at 10% inclination for 5 min per day for the 5 consecutive days prior to the experiments. In the first and second day of familiarization, they exercised at a speed of 10 m $min^{-1}$, while in the subsequent days of familiarization they exercised at a speed of 11, 13 and 15 m $min^{-1}$ consecutively. The purpose of this preliminary exercise was to show the animals in which direction to run. All experiments were approved by the Ethics Committee for the Care and Use of Laboratory Animals at the Federal University of Minas Gerais and were carried out in accordance with the regulations described in the Committee's Guiding Principles Manual (protocol 012/05).

All the proceedings were carried out between 13:00 and 17:00 h at a room temperature of $21 \pm 2\,^{\circ}C$. On the day of the experiment, the animals were allowed to rest for 1 h in the rodent treadmill chamber before being submitted to the test. A needle (30 G) protruding 0.3 mm from the tip of the guide cannula was introduced into the right lateral cerebral ventricle by connecting it to a Hamilton syringe. Immediately prior to exercise, 2.0 μL of 0.15 M NaCl ($n = 6$) or 2.0 μL of L-NAME (1.43 μmol, $n = 6$) was injected into the right lateral ventricle. The dose of brain L-NAME was based on the results of our previous experiments that showed that the response of reduction in workload was clearly L-NAME dose-dependent [11]. Rats were randomly assigned to groups receiving either saline or L-NAME solution. Immediately after the injections, the animals were submitted to progressive running exercise until reaching fatigue.

Progressive work was performed on a motor-driven treadmill (Columbus Instruments, OH, USA, Modular Treadmill) at a constant slope of 10°. The rats started running at 10 m $min^{-1}$ and treadmill speed was increased by 1 m $min^{-1}$ every 3 min until fatigue. Fatigue was defined as the point at which the animals were no longer able to keep pace with the treadmill [25,26]. Time to fatigue (minutes) and workload (kgm) were considered indexes of running performance.

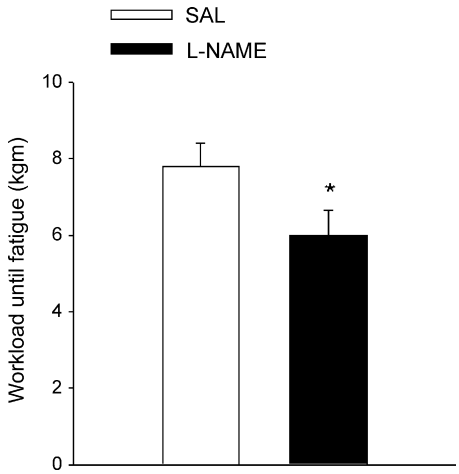


Fig. 1. Effect of icv injection of L-NAME (1.43 μmol/2 μL) or 0.15 M NaCl (2 μL, SAL) on workload until fatigue. $n = 6$ in each group. *Significantly different from the control group ($p < 0.05$).

During the experimental situation, blood samples (0.3 mL) were collected at rest, using a jugular vein catheter [8], before L-NAME or SAL intracerebroventricular infusion, at 3, 6, 9, 12, 15, and 21 min following initiation of exercise and at fatigue point. The blood volume collected in each sample was replaced by normal donated blood to avoid reduction in the blood volume of the animal. The blood samples were centrifuged and the plasma separated. The plasma samples were then frozen ($-20\,^{\circ}C$) until biochemical analyses were carried out using the enzymatic method of glucose and lactate oxidase (Glucose Analyser YSI 300 Plus), and the spectrophotometric method of FFAs (WACO kit).

Workload (W; kgm) was calculated as $W$ = body weight (kg) × TTF × treadmill speed (m $min^{-1}$) × $\sin\theta$ (treadmill inclination) [1,2,15], where TTF is time to fatigue (minutes). The data are reported as mean + S.E.M. Two experimental groups and six sequential times on treadmill defined a split plot design with six replications. Target response mean between groups were compared at each treadmill speed. Also, within each group, mean at each treadmill speed was compared to rest levels solely. All comparisons were made using Student's $t$-test ($\alpha \leq 0.05$).

Intracerebroventricular injection of L-NAME in untrained normal rats resulted in a marked decrease in workload (L-NAME: $6.0 \pm 0.6$ kgm versus SAL: $7.8 \pm 0.6$ kgm, $p < 0.01$) (Fig. 1) and time to fatigue (L-NAME: $19.6 \pm 2.5$ min versus SAL: $23.3 \pm 1.9$ min, $p < 0.01$) compared to SAL-treated rats.

As seen in Fig. 2A and D, exercise induced a rise in plasma glucose level in control animals, which was only evident as early as 12 m/min (9 min of exercise) (2A) and 80% of maximal work (2D), and continued to rise until fatigue. L-NAME-treated rats exhibited a rise in plasma glucose concentration throughout the experiment, however showing an increased hyperglycemic response compared to control animals during the first 60% of maximal work.