

Effect of Acute Administration of Clenbuterol on Athletic Performance in Horses

Guilherme C. Ferraz, PhD,^a Antonio R. Teixeira-Neto, PhD,^a Flora H.F. D'Angelis, PhD,^a José C. Lacerda-Neto, PhD,^b and Antonio Queiroz-Neto^a

ABSTRACT

The aim of the study was to determine the effect of clenbuterol on the anaerobic threshold of horses on a treadmill with increasing physical stress, measuring heart rate (HR) and blood levels of lactate, glucose, and insulin. Twelve Arabian horses were submitted to two physical tests separated by a 10-day interval. Clenbuterol (CL) at 0.8 µg/kg or saline (control—C) was administered intravenously 30 minutes before the test. The treadmill exercise test consisted of an initial warmup followed by a gradually increasing effort. There was no statistical difference in either V_2 or V_4 (velocity at which plasma lactate concentration reached 4 and 2 mmol/L, respectively) between the two experimental groups. For the CL group, V_{200} , V_{180} , V_{160} , and V_{140} (velocity at which the rate heart is 140, 160, 180, and 200 beats/minute, respectively) decreased significantly. At rest as well as times 4, 6, and 10 minutes, insulin levels were higher in the group that received clenbuterol ($P < .05$). Contrary to what was expected, apparently, there was no improvement in aerobic metabolism in animals when given a therapeutic dose of the bronchodilator. The elevated heart rate observed could have been attributable to the stimulation of cardiac β_1 adrenoceptors and the increased insulin levels to the stimulation of pancreatic β_2 receptors.

Keywords: Horse; Exercise; Clenbuterol; Performance; Insulin

INTRODUCTION

Clenbuterol hydrochloride is a selective β_2 sympathomimetic, largely used as a bronchodilator in horses suffering from recurrent airway obstruction (RAV). This drug is available commercially in gel, granular or injectable forms.¹ According to the guidelines of the Association of

Racing Commissioners International (ARCI) for the classification of exogenous substances, clenbuterol is categorized as a class 3 agent; its identification in biologic samples after a race results in disqualification of the competitor.²

During the reflex of “fight or flight,” nerve fibers of the sympathetic nervous system and the adrenal medulla release catecholamines that activate adrenoceptors, producing such effects as increase in cardiac output and heart rate (HR), increased blood flow to the muscles and reduced blood flow to the splanchnic organs, glycogenolysis, and lipolysis.

In addition to clenbuterol, other drugs that make up the group of selective β_2 agonists are salbutamol, pirbuterol, and fenoterol. The selectivity of these drugs for β_2 agonist activity is determined by the ratio β_2/β_1 , which is 4 for clenbuterol, indicating moderate selectivity. Therefore, at low doses this drug acts preferentially on β_2 receptors. At higher doses, it may interact with β_1 adrenoceptors,² resulting in increased HR.

Although studies in Thoroughbred horses showed that oral^{1,3} as well as intravenous⁴ administration of clenbuterol at 0.8 µg/kg did not alter cardiorespiratory variables, this drug continues to be used illegally for the purpose of improving athletic capacity in healthy horses.

Studies to date on the effect of clenbuterol on athletic performance of horses have used Thoroughbreds as the experimental model, rather than breeds whose energy metabolism is predominantly aerobic. Therefore, the aim of the current investigation was to evaluate the behavior of some physiologic variables in Arabian horses submitted to exercise of increasing intensity.

MATERIALS AND METHODS

Animals and Handling

Twelve trained male and female Arabian Horses were used in the study,⁵ all from the Horse Breeding Sector of the College of Agricultural and Veterinary Sciences—UNESP, Jaboticabal, Brazil, with a mean body weight (\pm SE) of 390 ± 25.4 kg and mean age of 8.6 ± 3.3 years. The horses, which appeared healthy, were submitted to a complete clinical examination, then divided into the two experimental groups. The study followed the Ethical Principles in Animal Experimentation adopted by the Brazilian College of

From the Department of Animal Morphology and Physiology, Faculty of Agricultural and Veterinary Sciences (FCAV), São Paulo State University (UNESP), Jaboticabal Campus, Jaboticabal, SP, Brazil^a; and the Department of Animal Surgery and Clinical Sciences, FCAV, UNESP^b.

Reprint requests: Antonio Queiroz-Neto, Via de Acesso Prof. Paulo Donato Castellane, 14884-900, Jaboticabal, SP, Brasil

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Animal Experimentation and was approved by the University's Institutional Animal Care and Use Committee.

Incremental Exercise Test

The horses were adapted to exercise on a high-performance treadmill (Galopper treadmill, Sahinco LTDA, Palmital, SP, Brazil) and then submitted to the exercise test (ET) for a duration of 30 minutes. The warm-up exercise was first carried out for 4 minutes at a speed of 4.0 m/second, which was then increased at 1-minute intervals to 5.0, 6.0, 7.0, 8.0, 9.0, and 10 m/second. At this stage of maximum stress, the exercise proceeded with deceleration, returning to a velocity of 3.0 m/second for 20 minutes, which corresponded to the active cooling-down period. The entire phase of physical stress, with increase in velocity, was performed with the treadmill at 10% inclination.

Groups

The horses were divided into two experimental groups, control (C, $n = 12$) and clenbuterol (CL, $n = 12$), which were submitted to two physical tests separated by a 10-day interval, where a cross-over type design was used. Thirty minutes before the first incremental exercise test (IET), six animals of group C received saline intravenously, and six animals of group CL received clenbuterol (Ventipulmin, injectable solution, Boehringer Ingelheim do Brasil Quim. and Farm. Ltda, São Paulo, SP, Brazil) intravenously at a dosage of 0.8 $\mu\text{g}/\text{kg}$. In the second IET, the procedure was the same but reversed for the two groups.

Blood Sampling

A standard operating procedure (SOP) for blood sampling was created to establish proper procedures for collection, processing, and storage. Blood was drawn 15 seconds before the end of each velocity step of the test exercise. Before the exercise, the area close to the left jugular vein was shaved and aseptically prepared for venous catheterization. An extension tube was connected to a 12-gauge catheter to facilitate drawing blood with the animal in motion. After each collection, the entire assembly was rinsed with 2.5% heparin.

Heart Rate

Heart rate (HR) (beats/minute) was determined with a digital heart rate monitor (S610 Polar, Port Washington, NY), where three measurements were taken at each stress step. The means obtained at each stress step were plotted and analyzed by linear regression to derive the V_{140} , V_{160} , V_{180} , and V_{200} (velocities at which HR was 140, 160, 180, and 200 beats/minute, respectively).

Lactate

Lactate concentration was determined in 0.5 ml of blood, which was separated and processed in Eppendorf tubes containing 1.0 ml 1% sodium fluoride. The hypotonic solution caused hemolysis and inhibition of glycolysis, thereby preventing coagulation and lactate production by

erythrocytes. Lactate was determined by using an electrochemical lactate analyzer (YSI 1500 Sport L-Lactate Analyzer, YSI Incorporated, Yellow Springs, OH) in which the samples were assayed in duplicate. Average values were plotted against velocity, and exponential regression analysis was used to determine V_2 and V_4 (velocities at which blood lactate concentration was 2 and 4 mmol/l, respectively).

Glucose

Blood samples (3.0 ml) reserved for glucose assay were processed with anticoagulant containing the glycolysis inhibitor potassium fluoride, and later analyzed by spectrophotometry (Bio 2000, Bioplus, Barueri, SP, Brazil).

Insulin

Ten milliliters blood was obtained in vacutainer tubes containing sodium heparin and immediately centrifuged under refrigeration (ALC, Multispeed refrigerated centrifuge PK121R, NJ), and the plasma was stored frozen at -20°C . Plasma insulin was determined by solid phase radioimmunoassay using a commercial kit (DPC [Coat-a-count], Diagnostic Products Corp., Los Angeles, CA), as described previously.⁶ All 144 plasma analyses were performed in duplicate. Intra-assay and interassay coefficients of variation were $6.6 \pm 1.3\%$ and $10.2 \pm 2.1\%$, respectively.

Statistical Analysis

The effect of exercise on the physiologic variables was studied using analysis of variance (ANOVA) for repeated measures, with the aim of determining significant differences within each stress step, followed by Tukey's test when necessary. Student's *t*-test for paired samples was also performed to compare differences among groups. A *P* value $\leq .05$ was considered statistically significant.

RESULTS

Administration of clenbuterol did not alter V_2 and V_4 (Fig. 1), but did cause a decrease, as seen in Fig. 2, in the values of V_{200} , V_{180} , V_{160} , and V_{140} , with corresponding *P* values of .03, .05, .04, and .02, respectively.

Glucose levels were altered with exercise, as demonstrated in Fig. 3A. Plasma glucose concentration decreased initially and then increased at the 6 m/second step ($P \leq .05$), which remained elevated in the subsequent steps of the test exercise. A comparison of glucose levels between the experimental groups showed no statistical difference.

Effects of exercise on plasma insulin concentration are presented in Fig. 3B. There was a reduction ($P < .05$) in insulin levels during exercise in the two experimental groups, which showed a tendency toward reversal at the end of the exercise phase. At rest, as well as at times 4, 6, and 10 minutes, plasma insulin was higher in the group that received clenbuterol, in which the *P* values were 0.01, 0.02, 0.04, and 0.05, respectively. This trend was

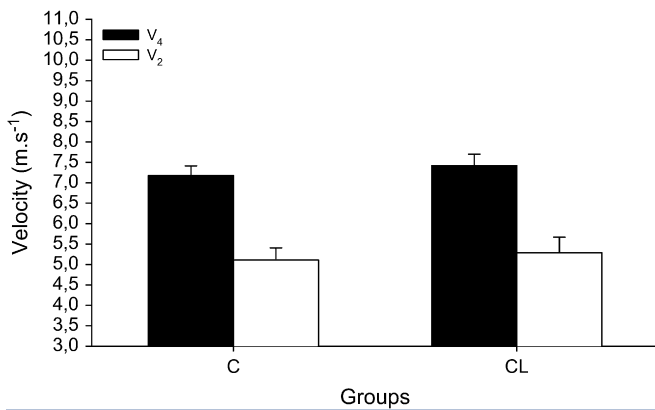


Figure 1. Alterations of the velocity at which plasma lactate concentration reached 4 (V_4) and 2 mmol/l (V_2), after intravenous administration of 0.8 $\mu\text{g}/\text{kg}$ clenbuterol in Arabian horses during incremental exercise on a treadmill. $N = 12$ for C (control) and CL (clenbuterol).

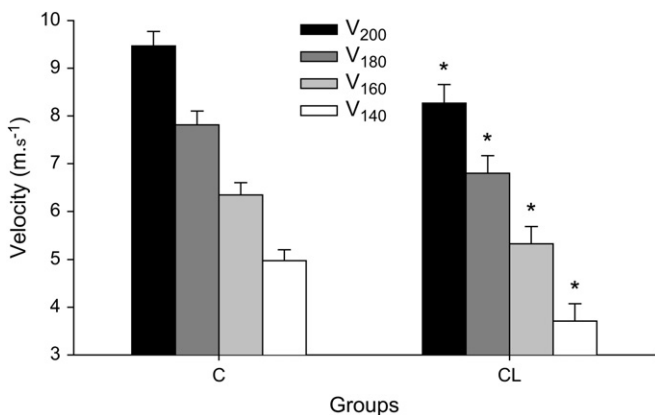


Figure 2. Alterations of the velocity at which the heart rate reached 140, 160, 180, and 200 beats/min after intravenous administration of 0.8 $\mu\text{g}/\text{kg}$ of clenbuterol in Arabian horses during incremental exercise on a treadmill. $N = 12$ for C (control) and CL (clenbuterol).
* Significant decrease in relation to control group ($P \leq .05$).

apparent at other steps of the exercise test but did not show a statistical difference.

DISCUSSION

Because it is a bronchodilator, clenbuterol has been used in healthy horses in an attempt to improve aerobic capacity during exercise. However, the data obtained in this study do not point to this effect. Studies with Thoroughbred horses submitted to intravenous³ or oral¹ administration of clenbuterol did not find any significant change in the onset of blood lactate accumulation. Similarly, the current experiment reveals no significant difference in V_2 and V_4 between the experimental groups. One explanation for

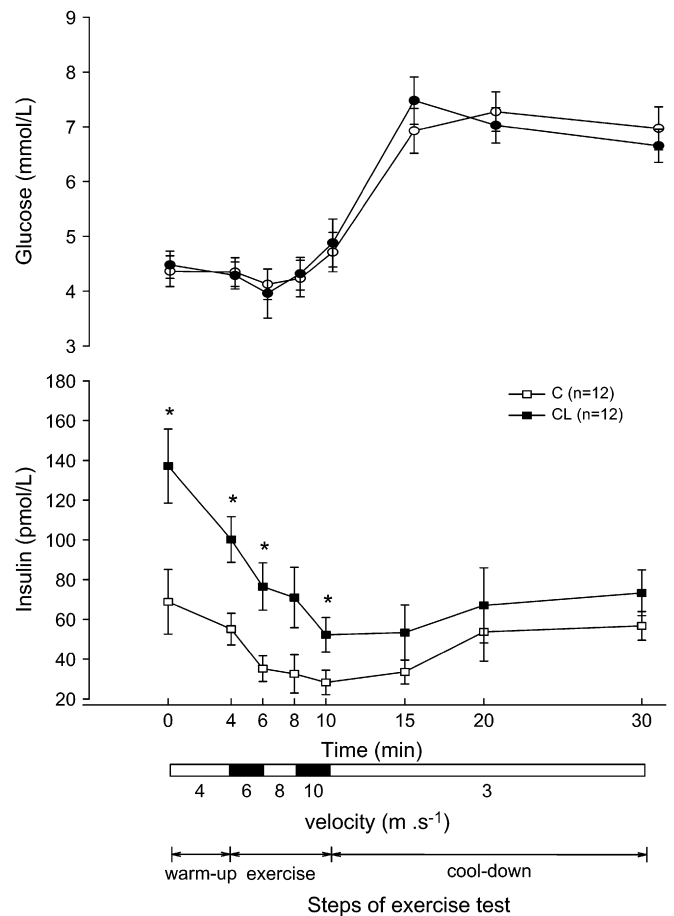


Figure 3. Changes in blood glucose (A) and serum insulin (B) values of horses submitted to an incremental exercise on a treadmill after intravenous administration of 0.8 $\mu\text{g}/\text{kg}$ clenbuterol (CL) or physiologic solution (C).
* Significant difference between CL and C ($P \leq .05$).

this finding is that clenbuterol administration was not capable of affecting both partial pressure of carbon dioxide and arterial pressure.

For velocities related to heart rate, V_{140} , V_{160} , V_{180} , and V_{200} , which are commonly used for the evaluation of athletic performance, the results indicate that group velocities were lower in CL than group C. This finding can be explained by the interaction of clenbuterol with cardiac β_1 adrenoreceptors, which, on stimulation, induces a positive chronotropic and inotropic effect.⁷ This observed effect demonstrates that the utilization of clenbuterol for ergogenic purposes is senseless because it overloads the heart during physical stress.⁸ Some authors^{9,10} cite necrosis of the myocardium as a toxic effect, secondary to relative hypoxia, resulting from the increase in oxygen demand by the heart during tachycardia.

There was a decrease in plasma insulin concentrations in both experimental groups, with an increase in stress intensity. According to one study,¹¹ in incremental exercise there is an increase in blood levels of catecholamines that

interact with pancreatic α_2 receptors, which in turn are responsible for the reduction in insulin secretion. However, when compared with the control group, the increase in insulin levels was greater in the group that received clenbuterol, indicating an interaction of this drug with pancreatic β_2 -adrenoceptors, which, when sensitized, increase insulin production.¹⁰

Increased plasma insulin concentrations in CL horses did not result in differences in glucose levels between the two groups. Plasma glucose levels varied only with increases in the intensity of stress. Initially, there was a reduction in plasma glucose, which is explained by its mobilization, at least in part to type I muscle fibers, in the initial phases of physical stress.¹² Sequentially, starting at the 6-m/second step, there was a tendency toward elevated glucose levels, which could be explained as an effect of the release of catecholamines,^{6,13} which occurs during intense exercise and induces an increase in plasma glucose because of increased glycogenolysis and neoglycogenesis.^{13,14} Therefore, the adrenergic effect can be overlying the β_2 -hyperinsulin response induced by clenbuterol, because it did not produce lower glucose levels. The fact that glucose levels at rest were not affected by insulin concentration needs further evaluation.

In conclusion, administration of clenbuterol in horses did not improve aerobic capacity. Moreover, clenbuterol can harm cardiac response and exacerbate elevated insulin levels in the equine.

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