Clenbuterol diminishes aerobic performance in horses

CHARLES F. KEARNS and KENNETH H. MCKEEVER

Equine Science Center, Department of Animal Science, Rutgers the State University of New Jersey, New Brunswick, NJ

ABSTRACT

KEARNS, C. F., and K. H. MCKEEVER. Clenbuterol diminishes aerobic performance in horses. *Med. Sci. Sports Exerc.*, Vol. 34, No. 12, pp. 1976–1985, 2002. **Purpose:** The purpose of this 8-wk study was to examine the effect of therapeutic levels of clenbuterol on aerobic performance and hemodynamics associated with exercise. **Methods:** Twenty-three unfit Standardbred mares were divided into four experimental groups, clenbuterol (2.4 $\mu g \cdot kg^{-1}$ body weight twice daily) plus exercise (20 min at 50% $\dot{V}O_{2max}$; CLENEX; $N = 6$), clenbuterol only (CLEN; $N = 6$), exercise only (EX; $N = 5$), and control (CON; $N = 6$). All horses performed an incremental exercise test (GXT) to measure maximal oxygen consumption ($\dot{V}O_{2max}$), blood lactate concentration, total plasma protein concentration, and hematocrit. Plasma volume, heart rate, right ventricular pressure (RVP), and pulmonary artery pressure (PAP) were measured before and after the treatment/training. Each horse also performed an exercise capacity test (ECT) in which they ran at their pretreatment $\dot{V}O_{2max}$ speed until exhausted. **Results:** There were no significant changes in blood lactate, total protein, or hematocrit for any group during either the GXT or ECT. CLENEX decreased ($P < 0.05$) $\dot{V}O_{2max}$ (−6.2%) and velocity to $\dot{V}O_{2max}$ (−10.0%), whereas both CLENEX and CLEN decreased ($P < 0.05$) in time to exhaustion (−20.5 ± 4.7 and −20.9 ± 5.6%). EX alone increased ($P < 0.05$) $\dot{V}O_{2max}$ (+6.5%), velocity to $\dot{V}O_{2max}$ (+10.0%), velocity to produces lactate concentration of 4 mmol (+13.5%), and time to exhaustion (+32.3 ± 15.0%). Plasma volume was altered ($P < 0.05$) in CLENEX (−10%) and EX (+27%) but not in CLEN. Posttest recovery HR was higher ($P < 0.05$) at 2 min post-GXT in the CLENEX, CLEN, and CON compared with their pretest values; RVP remained elevated at 2 min of recovery in the CLEN and CON groups; however, in the EX, recovery HR and RVP had returned to pre-GXT levels by 2 min of recovery. **Conclusions:** These data suggest that the combined effect of therapeutic levels of clenbuterol and training decrease aerobic performance and that the resultant reduction in plasma volume may affect improvements in cardiovascular function during recovery normally seen with exercise training. **Key Words:** EQUINE, EXERCISE TESTING, HEMODYNAMICS, BETA-AGONIST

The β2-sympathomimetic agent clenbuterol was originally used as an agent to increase muscle mass in a variety of research and livestock species (5,11,30,41). Although it effectively caused a repartitioning of nutrients and altered body composition, its use was discontinued due to a variety of side effects associated with the consumption of clenbuterol-tainted meat (26,49). Typical symptoms associated with the ingestion of clenbuterol-tainted meat included skeletal muscle tremors, tachycardia, cephalagia, myalgia, nervousness, dizziness, and nausea (17,26).

More recently, clenbuterol has been used as a bronchodilator to alleviate bronchospasms in the horse (44), in much the same way as albuterol and salbutamol have been in humans. Also, approximately 75% of horses examined after a high-intensity race experience exercise-induced pulmonary hemorrhage (EIPH) (18). Part of the mechanism thought to be associated with EIPH is the large increase in pulmonary artery pressure that occurs in all horses during exercise coupled with changes in airway resistance (18). This has led some to suggest that clenbuterol could potentially be used to treat EIPH by altering airway resistance and attenuating pulmonary artery "hypertension" (42,44). However, clenbuterol's action as a vasoconstrictor may actually increase rather than decrease pulmonary artery blood pressure. Due to the widespread use of inhalers to combat bronchospasm, concern has been raised as to the potential ergogenic properties of these β2-agonists, even when prescribed at therapeutic doses (1). Data regarding the ergogenic effect of short-term use of β2-agonist in humans have been equivocal (1,4,7,35,36,46) whereas data in the healthy horses have failed to show any significant increase in performance (20,23,24,42,43,48). However, there are no data concerning the relationship between exercise performance and long-term administration of therapeutic levels of clenbuterol. In fact, many studies have only examined the effects of a single treatment (1,27,42,46). Given the fact that many equine and human athletes use both legal and illegal (12,40) β2-agonist for extended periods of time, it is not unreasonable to assume that long-term usage with these drugs may have an effect that is compounded over time. Training has been shown to prevent clenbuterol-induced effects in rats (14,19). Studies to examine the combined effects of long-term systematically administered β2-agonist and exercise

Address for correspondence: Kenneth H. McKeever, Ph.D., FACSM, Equine Exercise Physiology Laboratory, Department of Animal Sciences, 84 Lipman Drive, New Brunswick, NJ 08901-8525; E-mail: McKeever@aesop.rutgers.edu.
Submitted for publication July 2001.
Accepted for publication July 2002.

0195-9131/02/3412-1976/$3.00/0
MEDICINE & SCIENCE IN SPORTS & EXERCISE®

DOI: 10.1249/01.MSS.0000038973.96796.1E

training are difficult to do in humans. For many reasons, the horse is an ideal comparative model for examining this problem in that it trains for athletic competitions, both sprinting and endurance events, similar to human athletes, while also using a prescribed β2-agonist therapy. Therefore, it was the purpose of this study to examine the role of long-term clenbuterol administration on aerobic performance in the Standardbred mare. Specifically, the first objective of the study was to test the hypothesis that clenbuterol and exercise training would alter markers of aerobic performance in horses. Additionally, because all horses experience a large increase in pulmonary artery blood pressure during exercise that may be linked to EIPH, and because clenbuterol has the potential to alter the hemodynamic response to exercise, a secondary hypothesis was to test whether clenbuterol would have any effect on heart rate, right ventricular pressure, and pulmonary artery blood pressure during and after exercise.

MATERIALS AND METHODS

Animals and drug administration. Twenty-three healthy, untrained Standardbred mares (age: 10 ± 3 yr) were evaluated. The mares were unfit, but accustomed to the lab and running on the treadmill before the start of the experiment. During the trial, the horses were housed as a group on a pasture. Each mare was fed approximately 6 $kg \cdot d^{-1}$ of alfalfa and grass hay and approximately 3 $kg \cdot d^{-1}$ of a commercially available grain ration (split into two feedings). Water and pasture grazing were provided *ad libitum*. The Rutgers University Institutional Animal Care Review Board approved all methods and procedures used in this experiment, and the study adhered to ACSM animal care standards.

Horses were divided into four experimental groups: clenbuterol and exercise (CLENEX; $N = 6$) and clenbuterol only (CLEN; $N = 6$) were both orally administered 2.4 $\mu g \cdot kg^{-1}$ body weight of clenbuterol (Boehringer Inglelheim, UK) twice daily as a syrup (for an average volume of 20 mL) on a schedule of 5 d on and 2 d off for the duration of the study. CLENEX also aerobically trained for 3 $d \cdot wk^{-1}$. Exercise (EX; $N = 5$) were used as the training group and aerobically trained for 3 $d \cdot wk^{-1}$. Control (CON; $N = 6$) was used as the control group. Both EX and CON were administered similar volumes of molasses twice daily on a 5 d on and 2 d off schedule.

Before receiving any drug treatment, all animals completed a series of baseline testing. Baseline testing was comprised of an incremental graded exercise test (GXT) to measure maximal aerobic capacity ($\dot{V}O_{2max}$), an index of performance. An exercise capacity test (ECT) was administered to determine the ability to endure high-intensity exercise. Horses were then trained for 8 wk, and at the end of the 8-wk training period, all baseline tests were repeated.

Training program. The exercise program consisted of continuous treadmill running 3 $d \cdot wk^{-1}$ for 8 wk. The horses ran initially for 15 $min \cdot d^{-1}$ at a work rate set at an intensity of 50% $\dot{V}O_{2max}$. After 1 wk, the duration of run time was increased to 20 $min \cdot d^{-1}$ and was held at this duration for the remainder of the study. During the exercise training, the treadmill was set at a fixed 6% grade.

Graded exercise test (GXT). This test was designed to measure maximal oxygen uptake and several indices of exercise performance. Before the test, the horses were weighed, and catheters (Angiocath, 14 gauge, Becton Dickinson, Inc., Parsippany, NJ; and a 7 French catheter introducer, Argon Medical, Athens, TX) were inserted percutaneously into the left and right jugular veins, respectively, by using sterile techniques and local lidocaine anesthesia. The horses were then walked onto the treadmill where a pressure-sensing catheter (Millar Instruments, Houston, TX and Electrocatheter, Rahway, NJ) was inserted and positioned for the measurement of pulmonary artery pressure, right ventricular pressure, and heart rate. Verification of the position of these pressure-sensing catheters was performed before and after exercise by using the representative blood pressure waveforms recorded on the physiological recording system (Biopac, Santa Barbara, CA). The horses then stood quietly for approximately 10–15 min equilibration period. Fifteen minutes of hemodynamic data, standing calorimetry data, and a baseline blood sample (10 mL) were obtained after this equilibration period.

During the incremental exercise tests the animals ran on a high speed horse treadmill (Sato I, Equine Dynamics, Inc., Lexington, KY) at a fixed 6% grade and wore the indirect open-flow calorimeter apparatus (Oxymax-XL, Columbus Instruments, Inc. Columbus, OH) used to measure oxygen uptake. The tests started at an initial speed of 4 $m \cdot s^{-1}$ for 1 min. Speed was then increased to 6 $m \cdot s^{-1}$ followed by incremental 1-$m \cdot s^{-1}$ increases every 60 s until the horses reached fatigue. Fatigue was defined as the point where the horse could not keep up with the treadmill despite humane encouragement. After this, the treadmill was stopped, and 10 min of postexercise calorimetry and hemodynamic data were collected. Oxygen uptake was measured continuously during the test and recorded at 10-s intervals by using the open-flow calorimetry system. Analog hemodynamic data were recorded continuously and digitized for later analysis (Biopac Physiological Recording System).

Blood chemistry. Blood samples (20 mL) were obtained during the tests at rest, during the last 10 s of each increment of the test, and at 5 min, and 10 min postexercise to measure hematocrit, total protein, and blood lactate concentration. Blood samples were placed into prechilled tubes containing EDTA (Vacutainer, Becton Dickinson, Inc., Franklin Lakes, NJ) and were immediately placed on ice.

Hemodynamics. Pulmonary artery pressure (PAP) and right ventricular pressure (RVP) were measured continuously in real time using a micromanometer catheter transducer (Millar Instruments, Houston, TX). The transducer was calibrated before and after each horse performed a GXT against a mercury manometer and position was verified before and after the GXT using representative waveform. All data was recorded on a physiological recording system (Biopac Systems Inc., MP 100, Goleta, CA) and stored on computer. Analog pressure data was measured and digitized

on computer (Acknowledge; Biopac Systems Inc., Santa Barbara, CA). Heart rate (HR) was calculated by counting the number of pressure waves in the final 10 s of each stage of the exercise test.

Plasma volume. Resting plasma volume was measured using a modified Evans blue dye dilution method (32). All measures were made while the horses were standing quietly (at rest) in their respective stalls 2 d before the GXT. Calculations of total blood volume and red cell volume were made using previously published methods (32,38).

Exercise capacity test. Horses warmed up by running for 2 min at 3 $m \cdot s^{-1}$. Then the horses ran at the speed calculated to produce $\dot{V}O_{2max}$ in their pretreatment GXT. They ran until they could no longer keep up with the treadmill despite humane encouragement. Time to exhaustion was measured as the time in seconds when the test was terminated. Before the test, the horses were weighed, and a catheter was placed into the left jugular vein as per the $\dot{V}O_{2max}$ test. This test was repeated during the posttreatment testing phase of the experiment using the pretest $\dot{V}O_{2max}$ speed.

Blood samples (30 mL) were obtained at rest, at the termination of the ECT, and at 5 and 10 min postexercise to measure hematocrit, total protein, and blood lactate concentration. Samples were kept on ice and run immediately after collection. Blood lactate concentrations was measured in triplicate using a lactate analyzer (Sport 1500, YSI, Inc., Yellow Springs, OH). Hematocrit and plasma protein were measured in duplicate using the microhematocrit technique and refractometry (32).

Statistical analysis. Results are expressed as means ± standard error of the mean (SEM). For comparison by group and time a two-way ANOVA for repeated measures was used with the *a priori* level of statistical significance set at $P < 0.05$. *Post hoc* differences were determined using the Tukey test, and correlation coefficients were derived using the Pearson product moment (Sigma Stat 2.0; SPSS Inc., Chicago, IL).

RESULTS

Side effects. Horses receiving chronic clenbuterol administration demonstrated several side effects that persisted for 10 d. These effects included extreme sweating (the horses given clenbuterol were dripping copious amounts sweat while standing in their stalls vs no visible sweating in the nondrug horses) and severe agitation. Horses administered molasses failed to demonstrate any side effects.

Graded exercise test (GXT). The EX group demonstrated an increase in $\dot{V}O_{2max}$ ($P < 0.05$; +6.5%), whereas the CLENEX group demonstrated a decrease (−6.2% Fig. 1A). There were no changes in $\dot{V}O_{2max}$ in either the CON or CLEN groups. The velocity to reach $\dot{V}O_{2max}$ increased ($P < 0.05$; +10%) in the EX group but decreased ($P < 0.05$; −10%) in the CLENEX group (Fig. 1B). There were no changes ($P > 0.05$) in the velocity to reach $\dot{V}O_{2max}$ in either the CON or CLEN groups.

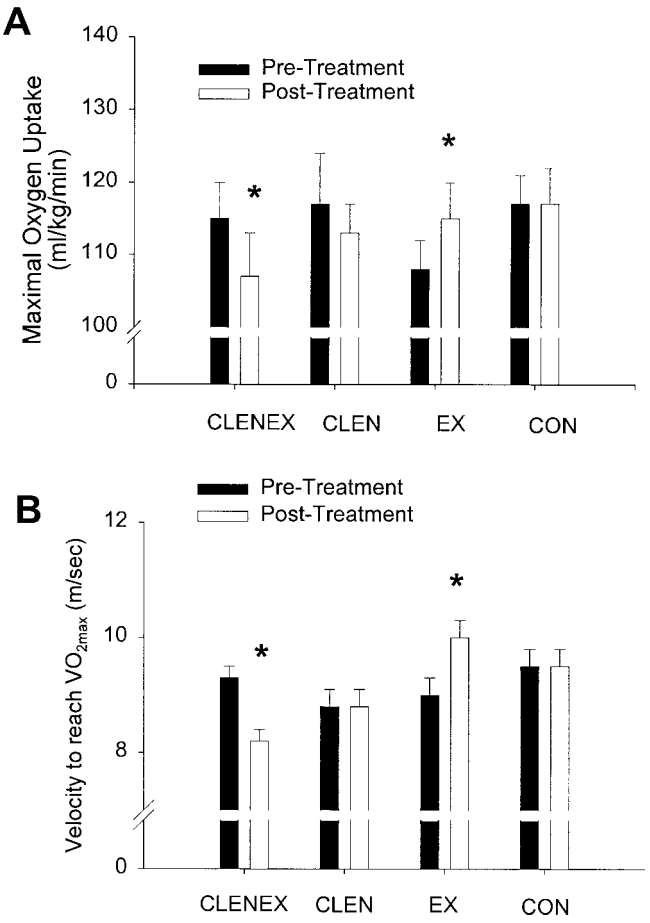

FIGURE 1—A, Maximal oxygen consumption ($\dot{V}O_{2max}$); and B, velocity to reach $\dot{V}O_{2max}$ in clenbuterol plus exercise-treated (CLENEX), clenbuterol-treated (CLEN), exercise (EX), and control (CON). Values are means ± SE; * significantly different from pretreatment values for each group.

Blood chemistry. Blood lactate concentration, total protein, and hematocrit all increased with increasing exercise intensity (data not shown). There were no changes ($P > 0.05$) in blood lactate concentration (Fig. 2A), total protein or hematocrit due to either clenbuterol and/or exercise treatment. However, the velocity to reach 4 mmol lactate (VLa4) increased ($P < 0.05$; +13.5%) in the EX group (Fig. 2B). There were no changes ($P > 0.05$) in VLa4 for the other three groups.

Exercise capacity test (ECT). The time to fatigue during the ECT was increased ($P < 0.05$) by 32.3 ± 1.5% in the EX group, and decreased ($P < 0.05$) in the CLENEX (−20.5 ± 4.7%) and CLEN groups (−20.9 ± 5.6%) (Fig, 3, A and B). There were no changes ($P > 0.05$) in ECT in the CON group. There were no significant treatment effects for hematocrit, total protein, or lactate at any sampling period for the ECT.

Hemodynamics. Hemodynamic data are summarized in Table 1. There were no differences ($P > 0.05$) in resting HR, RVP, or PAP. Similarly, there were no differences ($P > 0.05$) between groups for maximal HR or the RVP and PAP measured at the speed eliciting maximal oxygen uptake. Recovery HR was higher ($P < 0.05$) than pretreatment

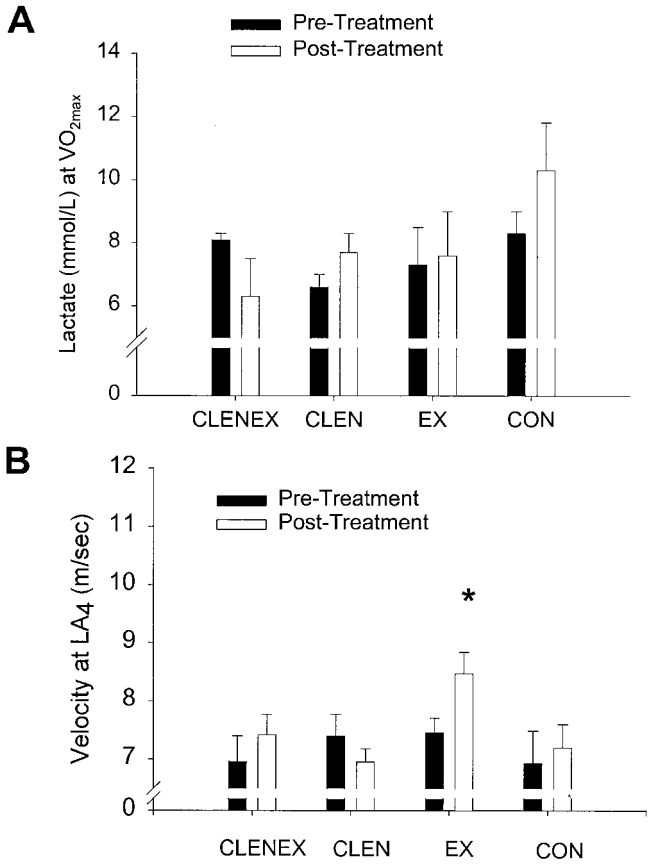

FIGURE 2—A: Lactate at $\dot{V}O_{2max}$ and B: velocity producing 4 mmol lacate (V_{LA4}) in clenbuterol plus exercise-treated (CLENEX); clenbuterol-treated (CLEN); exercise (EX); and control (CON). Values are means ± SE; * significantly different from pretreatment values for each group.

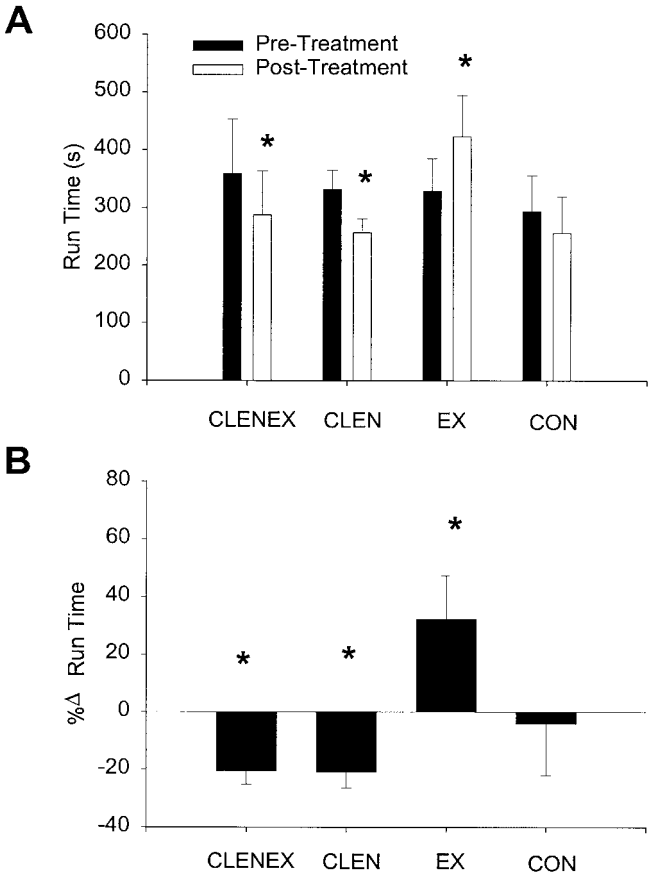

FIGURE 3—A, Exercise capacity test; and B, percent change in run time in clenbuterol plus exercise-treated (CLENEX), clenbuterol-treated (CLEN), exercise (EX), and control (CON). Values are means ± SE; * significantly different from pretreatment values for each group.

values for CLENEX, CLEN, or CON but was reduced ($P < 0.05$) for EX at 2 min of recovery (Fig. 4, A and B). There was a significant interaction ($P = 0.035$) between group and period for recovery HR. RVP was not different ($P > 0.05$) at $\dot{V}O_{2max}$ (Table 1) for any group. RVP was higher ($P < 0.05$) at 2 min post-GXT in the CLEN and the CON groups compared with pretest values; however, in the EX group, recovery RVP had returned to pre-GXT levels by 2 min of recovery (Fig. 5). There was a significant interaction ($P = 0.001$) between group and period for recovery RVP. There were no differences ($P > 0.05$) in PAP for any intensity for any group (Table 1).

Plasma volume. Plasma volume significantly increased (+27%) in the EX group but significantly decreased (−10%) in the CLENEX group (Fig. 6, A and B). There were no significant changes in either the CON or CLEN. Percent changes in plasma volume were correlated ($P = 0.01$; $R = 0.984$) to percent changes in $\dot{V}O_{2max}$ in the four treatment groups (Fig. 7). Changes in plasma volume were correlated ($P = 0.03$; $R = -0.469$) to changes in recovery HR at 2 min (data not shown).

DISCUSSION

The major finding of this paper was that even minimal therapeutic concentrations of the β2-sympathomimetic drug, clenbuterol, adversely affect aerobic performance, high-intensity exercise capacity and the animal's ability to recover from exercise. Animals treated with clenbuterol and exercise demonstrated a proportional reduction in $\dot{V}O_{2max}$ (−10%) and plasma volume (−10%). And although not significant ($P = 0.09$), there was a modest, and proportional, decrease in the measured $\dot{V}O_{2max}$ (−3.5%) and plasma volumes (−3.0%) of the clenbuterol-only horses. Functionally, the reduced plasma volume may explain in part the longer postexertion cardiovascular recovery times seen in the trained clenbuterol-treated horses when they are compared with the trained horses that did not get the drug. In fact, changes in plasma volume were correlated ($P = 0.01$; $R = 0.984$) to changes in $\dot{V}O_{2max}$ in the four treatment groups. Supporting these conclusions were the elevated postexertion recovery heart rate (HR) and the elevated right ventricular pressure (RVP). These data are also supported by echocardiographic data collected from these same horses in a concurrent study (47) that showed elevations in ventricle and atrial chamber dimensions and cardiac output, stroke volume, and HR immediately after a maximal exercise test

TABLE 1. Hemodynamic data for heart rate (HR), right ventricular pressure (RVP), and pulmonary artery pressure (PAP); all data were collected during the graded exercise test for each horse in the clenbuterol (CLEN), clenbuterol and exercise (CLENEX), exercise (EX), and control (CON) groups before and after treatment; all results are means ± SEM.

	CLENEX	CLEN	EX	CON
HR resting ($b \cdot min^{-1}$)				
Pre	53.0 ± 4.8	70.7 ± 11.9	69.6 ± 8.6	56.0 ± 7.2
Post	52.0 ± 4.0	69.0 ± 3.4	52.0 ± 4.8	61.0 ± 7.2
HR max ($b \cdot min^{-1}$)				
Pre	219.0 ± 4.3	222.0 ± 7.8	216.0 ± 6.8	224.0 ± 5.2
Post	217.0 ± 9.6	225.0 ± 6.1	212.0 ± 8.0	219.0 ± 4.0
RVP resting (mm Hg)				
Pre	50.2 ± 3.9	56.8 ± 9.3	55.2 ± 4.8	48.8 ± 2.9
Post	52.2 ± 2.6	63.1 ± 2.8	47.0 ± 4.9	60.0 ± 3.5
RVP max (mm Hg)				
Pre	130.4 ± 6.5	128.9 ± 7.3	132.3 ± 4.1	122.3 ± 5.1
Post	127.9 ± 4.2	127.3 ± 3.7	122.4 ± 6.4	129.1 ± 4.1
PAP resting (mm Hg)				
Pre	37.5 ± 3.9	47.3 ± 7.2	46.2 ± 3.5	42.1 ± 4.1
Post	34.8 ± 2.5	43.6 ± 2.4	45.4 ± 4.5	42.9 ± 4.3
PAP max (mm Hg)				
Pre	117.0 ± 11.0	111.8 ± 9.50	109.6 ± 5.10	109.4 ± 4.4
Post	106.1 ± 4.2	103.0 ± 3.7	105.7 ± 0.3	103.8 ± 0.3
PAP 2 min post (mm Hg)				
Pre	54.1 ± 5.0	50.6 ± 4.80	61.3 ± 6.8	53.4 ± 8.9
Post	53.1 ± 6.4	51.3 ± 3.2	52.3 ± 6.6	60.3 ± 9.7

(47). Both the CLENEX and EX groups should have experienced similar beneficial training effects such as a higher maximal aerobic capacity, increases in plasma volume, and because of the greater potential for maintaining venous return, shorter cardiovascular recovery times. However, those improvements in cardiovascular function that were seen in the EX horses with training were not apparent in the CLENEX horses. Thus, one could speculate that the combination of a reduced aerobic capacity and longer postexertion recovery times in the CLENEX horses compared with the EX horses may reflect both a cardiovascular and thermoregulatory instability that be related to the lower plasma volumes. These findings may have important implications both for equine (3,15,23,24,44) and human athletes involved in similar training regimes that use prescription β2-agonist drugs (1,34,45) to control respiratory ailments such as exercise-induced asthma (22).

There are several other new findings in this study. This is the first study to directly measure oxygen uptake and then assess changes in $\dot{V}O_{2max}$ after treatment with clenbuterol. Studies using rodent measured run time to volitional fatigue as a measure of fitness (8,12). Run time volitional fatigue is a derivative measure that correlates well with maximal oxygen uptake in rodents, but it is not a direct measure. Second, this is also the first study to examine long-term clenbuterol administration in nongrowing and weight-stable animals. Rodents are constantly growing, as is evident by the necessity to report growth curves. Growth changes over the course of the study can be a confounding variable. The study by Duncan et al. (14) did not compare the results of the control-untreated, and therefore we cannot make any argument regarding these data. Third, no other study has measured the interaction of clenbuterol, training, and plasma volume. It is unknown whether training and exercise would reduce plasma volume in rodents, an animal that does not sweat, or whether this is indeed a sweating-mediated mechanism. Because humans do sweat during exercise, the findings from the current study do represent novel and important data. Fourth, there are no data regarding clenbuterol treatment and cardiac function during exercise. We are the first to measure the pressure of the right ventricle and the pulmonary artery during each step during the GXT.

Clenbuterol's effects on aerobic performance. The reductions in aerobic performance seen in the present study are consistent with findings from previous studies in other species that used much higher doses (~$mg \cdot kg^{-1}$ body weight) of clenbuterol (13,14,19,29,51,52). Previous studies of the horse (20,23,24,42,43,48) have not demonstrated any alterations in performance; however, those studies used either acute (1 d) or short-term (<5 d) therapeutic doses (0.8–3.2 $\mu g \cdot kg^{-1}$ body weight) of clenbuterol. This is the first study in the horse to demonstrate any adverse effects on performance by using doses in a prescribed therapeutic range recommended by the manufacturer.

Clenbuterol has been shown to adversely affect aerobic performance in mice (19) and rats (14,52). Ingalls and coworkers (19) investigated the effects of eight weeks of clenbuterol administration (1.6 $mg \cdot kg^{-1}$, 4 $d \cdot wk^{-1}$) on exercise capacity in mice. In that study, clenbuterol-treated mice decreased total work by 25% whereas the exercise-trained mice increased total work by 58% in a run-to-exhaustion treadmill test. Thus, it could be suggested that clenbuterol treatment eliminated the exercise-induced increase in work in the clenbuterol plus exercise mice (19). Alternatively, it could be inferred that exercise eliminated the clenbuterol-induced decrease in total work or that exercise acted "protectively" against the deleterious effects of clenbuterol. These data were extended by a study by Duncan and colleagues (14), who looked at the effects of clenbuterol (2 $mg \cdot kg^{-1} \cdot d^{-1}$) on sprint and swim performance in rats. The clenbuterol-treated rats of that study ran 57% less total distance than untreated animals. Furthermore, many clenbuterol rats were unable to complete the swim and sprint testing protocols. In order for the clenbuterol-treated rats of that study to finish their exercise protocol, their running speed was reduced by 43%, and the tail weights were

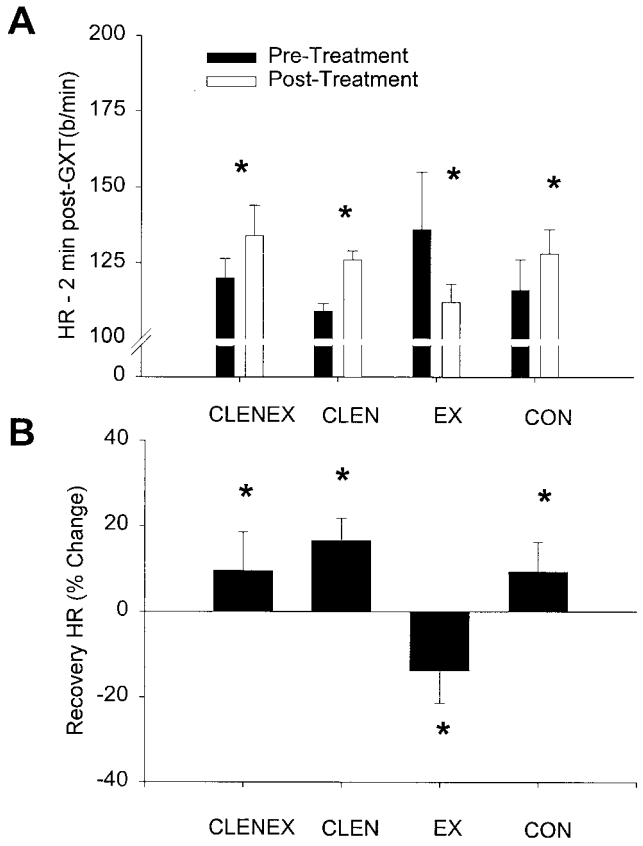

FIGURE 4—Heart rate (HR): A, 2 min post-GXT during recovery; and B, percent recovery HR in clenbuterol plus exercise-treated (CLENEX), clenbuterol-treated (CLEN), exercise (EX), and control (CON). Values are means ± SE; * significantly different from pretreatment values for each group.

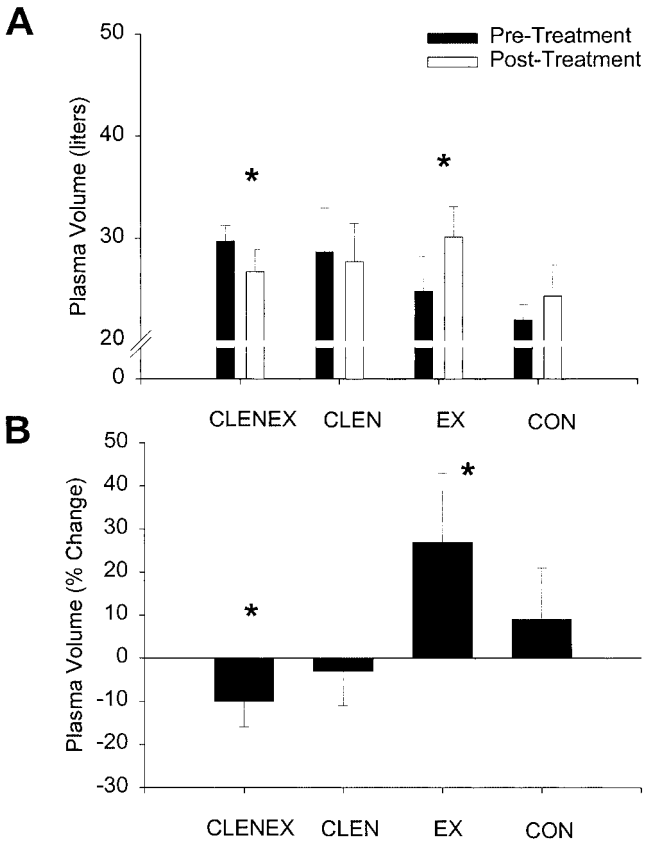

FIGURE 6—Plasma volume: A: pretreatment; and B, percent change in plasma volume from pretreatment values in clenbuterol plus exercise-treated (CLENEX), clenbuterol-treated (CLEN), exercise (EX), and control (CON). Values are means ± SE; * significantly different from pretreatment values for each group.

removed from their tails (14). Clenbuterol also prevented the aerobic training-induced improvement in insulin-stimulated glucose uptake in rats (52). These data (14,19,52) demonstrated that exercise, in combination with clenbuterol, can eliminate the deleterious effects of clenbuterol alone, a situation that we did not see in horses of the present study.

A similar reduction in work capacity was seen in horses of the present study during both the ECT and GXT. Horses in both clenbuterol-treated groups were unable to sustain a high intensity of work output. The exercise intolerance was most evident in the CLENEX horses, which not only had a

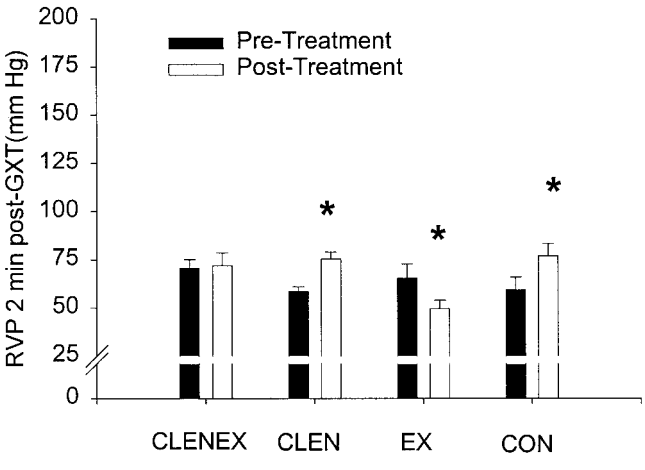

FIGURE 5—Right ventricular pressure (RVP) 2 min post-GXT during recovery and clenbuterol plus exercise-treated (CLENEX), clenbuterol-treated (CLEN), exercise (EX), and control (CON). Values are means ± SE; * significantly different from pretreatment values for each group.

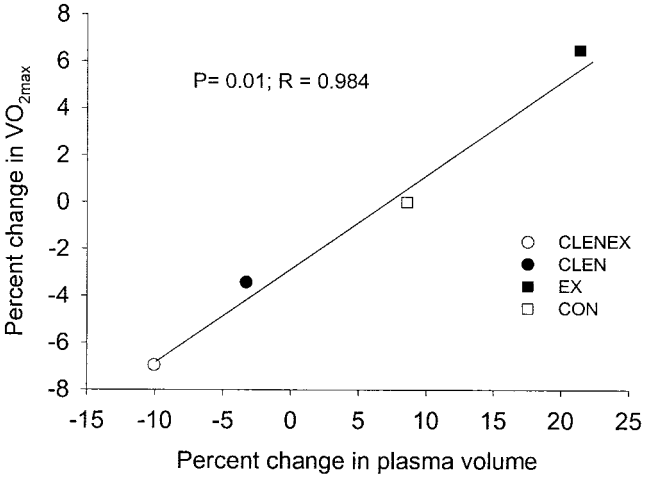

FIGURE 7—Scatter diagrams and linear regression lines relating percent change in $\dot{V}O_{2max}$ to percent change in plasma volume for clenbuterol plus exercise-treated (CLENEX, *open circle*); clenbuterol-treated (CLEN, *closed circle*); exercise (EX, *closed square*); and control (CON, *open square*). Values are means.

diminished maximal aerobic capacity but also a reduction in the speed that elicited $\dot{V}O_{2max}$. And although not significant at the $P < 0.05$ level, the CLEN group did show a tendency for a reduction in $\dot{V}O_{2max}$ (−3.5%). Heart rate at $\dot{V}O_{2max}$ was not significantly different posttreatment in either clenbuterol-treated group. Taking into account their lower $\dot{V}O_{2max}$, this would indicate an increase in work of the heart at any given submaximal workload as evidenced by a higher heart rate at these workloads (data not shown). Together, this suggests that there was a leftward shift in the heart rate to work curve in the CLENEX and CLEN horses. This is in contrast to the rightward shift in the heart rate to work curve in the EX group. Thus, it appears that clenbuterol administration altered this training-induced adaptation that benefits aerobic performance. Interestingly, there were no changes in blood lactate kinetics in any of the treatment groups, except for an improvement in V_{LA4} in the EX group. The later is a marker of endurance capacity that one would expect to be improved with aerobic conditioning. The fact that V_{LA4} was not improved in the CLENEX horses further adds to the suggestion that clenbuterol administration somehow alters the normal improvement in aerobic performance seen as an adaptation to training. Finally, horses in the CLEN and CLENEX groups had a 20% reduction in time to fatigue during their high intensity ECT, whereas the horses in the EX group demonstrated a substantial improvement in the time to fatigue. The ability to perform the high-intensity ECT relies heavily on both the aerobic capacity of the animal and its ability to tolerate anaerobic metabolism. We did not collect data to differentiate the exact mechanism behind the differences in time to fatigue; however, it may be linked to alterations in both aerobic and anaerobic capacity. Together the observed data support the hypothesis that clenbuterol deleteriously effects the ability to tolerate both aerobic and high-intensity work.

The reductions in aerobic performance seen with clenbuterol administrations may have been due to peripheral changes in muscle, or they may be reflect of central limitations, or both. The former is supported by the observation that clenbuterol produces many muscle-directed alterations, such as a slow to fast migration in myosin heavy chain profile (2,53) and a reduction in oxidative and glycolytic enzyme activities (13,25,52) in rats and mice (19). In the present study, clenbuterol treatment induced a migration from slow to fast myosin heavy chain (2) but did not alter the maximal activity of any glycolytic or aerobic enzymes measured (unpublished data) in these horses. The changes in the muscle fiber profile could potentially increase explosive work ability but would severely limit any aerobic or endurance capacities in these horses. Alternatively, the reduction in work capacity may also be related to or exacerbated by the observed substantial reductions in plasma volume and subsequent detrimental effects on cardiovascular capacity and stability.

Changes in plasma volume, cardiovascular function, and recovery. Blood volume and plasma volume are highly correlated to cardiovascular function and aerobic capacity in humans (8) and horses (38). Plasma volume increases with exercise training in humans (8–10), dogs (34), and horses (28,33). The increase in plasma volume with exercise training is called a training-induced hypervolemia (8). The mechanism behind this increase in plasma volume in the horse involves renal sympathetic nerve stimulation of the renin-angiotensin-aldosterone cascade and a net retention of sodium and water by the kidneys (31,33). This extra total body water and plasma volume provides fluid for cardiovascular (venous return, filling volume, and cardiac output) stability and thermoregulatory (sweating and skin blood flow) stability (8).

Exercise training in the present study resulted in a significant elevation in plasma volumes for all the horses in the EX group. The magnitude of this increase (+27%) was very similar to that previously reported in horses (33). This increase in plasma volume, combined with the increases in $\dot{V}O_{2max}$, and the more rapid recovery HR and RVP suggest that the extra plasma volume enhances cardiovascular stability in response to an acute bout of exercise, a conclusion supported by work in humans (8). Plasma volume decreased in the CLENEX group and trended downward in the CLEN group. Interestingly, the magnitude of the decrease seen in the CLENEX (−10%) versus CLEN (−3%) was proportional to the decrease in maximal aerobic capacity seen in both groups. Thus, a major finding of the present study is the appearance that clenbuterol counteracts or blocks the normal hypervolemic response to exercise training and even results in decreases in plasma volume in the CLENEX horses. Those horses should have experienced an increase in vascular fluid volume similar to the EX horses, an adaptive response that is beneficial to cardiovascular function because venous return can be better maintained in the face of an exercise challenge. This extra plasma volume results in shorter postexertion cardiovascular recovery times enhancing apparent cardiovascular stability. One could possibly define cardiovascular "stability" as the ability to rapidly return to baseline values after an exercise challenge. Thus, in a functional sense, elevated postexertion HR in the CLENEX, CLEN, and CON; and RVP in the CLEN and CON groups may reflect a degree of both a cardiovascular and thermoregulatory instability when compared to the rapid recovery response demonstrated by the EX animals.

Human studies have suggested that greater recovery responses to exercise maybe indicative of greater cardiac effort and, if induced by disease or drugs, may be predictive of risk for sudden death (6,16,37). In those cases, pathological data have indicated that the alteration in the recovery response may be related to changes in cardiac morphology (16) or sympathetic vagal tone (6). Clenbuterol has also been shown to cause cardiac hypertrophy (50) and collagen infiltrations of the left ventricle (29). These changes would be predictive to further increase cardiac effort by reducing cardiac compliance. Furthermore, in a concurrent study in these horses (47), clenbuterol induced changes in the structural dimensions of the heart, as assessed by echocardiography, that were consistent with cardiac remodeling and reduced cardiac compliance.

A major difference between this study and the previous literature regarding clenbuterol is that exercise training was not protective against the deleterious effects of clenbuterol. In fact, the CLENEX group suffered the largest reductions in aerobic performance whereas the CLEN group demonstrated only minor changes. In previous work in other species, exercise training has been shown to be protective of the detrimental effects of clenbuterol, ameliorating the reductions in work capacity (19,29,51,52). Mice given clenbuterol plus exercise training demonstrated similar run-to-exhaustion times when compared with control mice (19), and exercise training lessened the clenbuterol-induced infiltration of collagen in the ventricles of clenbuterol-treated rats (14). In the present study, horses receiving a combination of clenbuterol and exercise training demonstrated a larger decline in $\dot{V}O_{2max}$ and plasma volume than horses receiving clenbuterol alone.

An explanation for why exercise training actually amplified the clenbuterol-induced reductions in aerobic capacity and did not act protectively was unclear. The lack of a protective response to training may be reflective of the mild intensity of the exercise training protocol used in the current study, which was set at the lower threshold for significant aerobic improvement (39). It may have also been related to clenbuterol-induced alterations in fluid homeostasis. Although exercise training alone significantly increased $\dot{V}O_{2max}$ and ECT, the effect of training in the CLENEX group may not have been intense enough to counter clenbuterol's deleterious effects. However, despite the mild exercise intensity, the clenbuterol-treated horses of the present study still demonstrated great difficulty in their ability to maintain the study's prescribed exercise protocol. We believed that it was better to maintain both exercise-training groups at similar work intensities rather than to increase the work intensity of the EX group.

An alternative explanation as to why exercise training further reduced exercise capacity in clenbuterol-treated horses of the present study, and was not protective as seen in studies of rats (14) and mice (19), may be related to the fact that neither rats nor mice sweat to thermoregulate. Horses, on the other hand, like humans, sweat profusely to thermoregulate. It is possible, that the disruption of the mechanisms associated with fluid homeostasis plays an important part in the action of clenbuterol in the horse. As stated above, it appears as though clenbuterol alters the normal hypervolemic response to exercise training. We did not quantify sweat rate at any time; however, the observed side effects (data not shown) of clenbuterol administration included extreme sweating for hours postdrug administration (copious amounts dripping off the clenbuterol-treated horse vs no visible sweating in the control horses), increased heart rate, and agitation while the horses stood in their stalls. These effects persisted for 2 wk in both clenbuterol-treated horses. The CLENEX group was lathered more than the EX group immediately after all exercise training bouts. The CLENEX horses continued to sweat long into their recovery, in some cases for nearly an hour after exercise. It is possible that the CLENEX group was unable to replenish the volume of water that was lost as a normal consequence of exercise as well as the extra fluid lost while sweating in their stalls and, therefore, showed a much greater reduction in plasma volume than the CLEN group (−10% vs −3.5%). The observed loss in plasma volume would certainly reduce the horse's ability to thermoregulate after a bout of exercise.

Another factor associated with the hypervolemic response is an increase in the total content of protein in the vascular space. This increase in plasma protein content leads to the retention of water via oncotic mechanisms. It has been shown in rats that clenbuterol exacerbates the symptoms associated with protein deficiency (46). In that study, plasma albumin concentrations, as well as total liver proteins were significantly reduced despite an increase in gastrocnemius muscle protein in the clenbuterol-treated rats (45). It was hypothesized that clenbuterol preferentially increases the pool of muscle protein at the expense of the body's other protein stores. Both clenbuterol-treated horses of the present study significantly increased their fat-free mass (21). Although there were no changes in plasma protein concentration in any group of the present study, there must have been an increase in the content of plasma protein in the EX group so as to maintain the concentration in the face of an increase in plasma water. Because plasma volume decreased in the CLENEX group without a decrease in plasma protein concentration, it seems reasonable to conclude that there was a reduction in the total content of protein within the vascular space. This alteration in the availability of plasma proteins could have had potentially detrimental effects on the horse's ability to retain water. Therefore, the net negative loss in the vascular fluid volumes seen in the present study may partially explain the differences in the exercise performance between the two clenbuterol-treated groups. The mechanisms regulating the decrease in total content of protein and the subsequent reduction of plasma volume have yet to be elucidated. The phenomenon may be related to an alteration in renal tubular function and/or a disruption in the neuroendocrine control of renal fluid homeostasis. This is the first study to date, in any species, that has reported alterations in fluid homeostasis due to clenbuterol treatment, and clearly more work needs to be done in this area. What is clearer, however, is that the significant loss of plasma volume in the CLENEX horses might explain why exercise was not protective of clenbuterol's deleterious effects as it has been shown to be in rats (14,29,51,52) or mice (19).

Summary and conclusion. The training-induced increase in plasma volume in the EX group may explain the faster recovery for HR and RVP. Data from the present study suggest, however, that the combined effect of clenbuterol and training may result in a reduction in plasma volume that appears to affect aerobic capacity and cardiovascular function. These data have strong implication for human athletes using β2-agonists. Obviously, any decrease in performance is undesirable, but it is the potential health risks that are the most important. The impairment of postexertion recovery could put an athlete at greater risk for cardiac injury. Furthermore, the reduction in plasma volume greatly increases the potential for thermal injury, especially

when exercising in a hot and humid environment. This last point has particular relevance to the equine athlete, as horses must produce large amounts of sweat to thermoregulate, even in a cool and temperate environment.

The authors would like to thank Ms. Micki Mooney for her assistance with the running of the assays and analysis of the data.

This project was supported by the New Jersey State Initiative on Equine and N. J. Agriculture experiment Station Project no. 99501.

REFERENCES

1. Bedi, J. F., H. Gong, and S. M. Horvath. Enhancement of exercise performance with inhaled albuterol. *Can. J. Physiol. Pharmacol.* 13:144–148, 1988.
2. Beekley, M. D., K. H. Mckeever, J. M. Ideus, J. B. Brown, and C. F. Kearns. Chronic clenbuterol administration alters myosin heavy chain composition in Standardbred mares (Abstract). *Med. Sci. Sports Exerc.* 33:S1904, 2001.
3. Belloli, C., G. Re, F. Arioli, et al. Differences between longitudinal and circular smooth muscle in β-adrenergic control of motility of isolated equine ileum. *Am. J. Vet. Res.* 58:1422–1426, 1997.
4. Caruso, J. F., J. F. Signorile, A. C. Perry, et al. The effects of albuterol and isokinetic exercise on the quadriceps muscle groups. *Med. Sci. Sports Exerc.* 27:1471–1476, 1995.
5. Claeys, M. C., D. R. Mulvaney, F. D. Mccarthy, M. T. Gore, D. N. Marple, and J. L. Sartin. Skeletal muscle protein synthesis and growth hormone secretion in young lambs treated with clenbuterol. *J. Anim. Sci.* 67:2245–2254, 1989.
6. Cole, C. R., E. H. Blackstone, F. J. Pashkow, C. E. Snader, and M. S. Lauer. Heart-rate recovery immediately after exercise as a predictor of mortality. *N. Engl. J. Med.* 342:662–663, 2000.
7. Collomp, K., R. Candau, F. Lasne, Z. Labsy, C. Prefaut, and J. De Ceaurriz. Effects of short-term oral salbutamol administration on exercise endurance and metabolism. *J. Appl. Physiol.* 89:430–436, 2000.
8. Convertino, V. A. Blood volume: its adaptation to endurance training. *Med. Sci. Sports Exerc.* 23:1338–1348, 1991.
9. Convertino, V. A., L. C. Keil, E. M. Bernauer, and J. E. Greenleaf. Plasma volume, osmolality, vasopressin, and renin activity during graded exercise in man. *J. Appl. Physiol.* 50:123–128, 1981.
10. Convertino, V. A., L. C. Keil, and J. E. Greenleaf. Plasma volume, renin, and vasopressin responses to graded exercise after training. *J. Appl. Physiol.* 54:508–514, 1983.
11. Dalrymple, R. H., P. K. Baker, P. K. Gengher, D. L. Ingle, J. M. Pensack, and C. A. Ricks. A repartitioning agent to improve performance and carcass composition in broilers. *Poult. Sci.* 63: 2376–2383, 1984.
12. Delbeke, F. T., N. Desmet, and M. Debackere. The abuse of doping agents in competing bodybuilders in Flanders. *Int. J. Sports Med.* 16:66–70, 1995.
13. Dodd, S. L., S. K. Powers, I. S. Vrabis, D. Criswell, S. Stetson, and R. Hussain. Effects of clenbuterol on contractile and biochemical properties of skeletal muscle. *Med. Sci. Sports Exerc.* 28:669–676, 1996.
14. Duncan, N. D., D. A. Williams, and G. S. Lynch. Deleterious effects of chronic clenbuterol treatment on endurance and sprint exercise performance in rats. *Clin. Sci.* 98:339–347, 2000.
15. Erichsen, D. F., A. D. Aviad, R. H. Shultz, and T. J. Kennedy. Clinical efficacy and safety of clenbuterol HCl when administered to effect in horses with chronic obstructive pulmonary disease (COPD). *Equine Vet. J.* 26:331–336, 1994.
16. Hachamovitch, R., D. S. Berman, L. J. Shaw, et al. Incremental prognostic value of myocardial perfusion single photon emission computed tomography for the prediction of cardiac death. *Circulation* 97:535–543, 1998.
17. Hahnau, S., and B. Julicher. Evaluation of commercially available ELISA test kits for the detection of clenbuterol and other β2-agonist. *Food Add. Contam.* 13:259–274, 1996.
18. Hinchcliff, K. W. Effects of furosemide on athletic performance and exercise-induced-pulmonary hemorrhage in racehorses. *J. Am. Vet. Assoc.* 215:630–635, 1999.
19. Ingalls, C. P., W. S. Barnes, and S. B. Smith. Interaction between clenbuterol and run training: effects on exercise performance and MLC isoform content. *J. Appl. Physiol.* 80:795–801, 1996.
20. Kallings, P., C. Ingvast-Larsson, S. Persson, L.-E. Appelgren, H. J. Forster, and K. L. Rominger. Clenbuterol plasma concentration after repeated oral administration and its effects on cardio-respiratory and blood lactate responses to exercise in healthy Standardbred horses. *J. Vet. Pharmacol. Therap.* 14:243–249, 1991.
21. Kearns, C. F., K. H. Mckeever, K. Malinowski, M. B. Struck, and T. Abe. Chronic administration of therapeutic levels of clenbuterol acts as a repartitioning agent. *J. Appl. Physiol.* 91:2064–2070, 2001.
22. Kendall, M. J., and C. A. Haffner. The acute unwanted effects of β2 receptor agonist therapy. In: *The Role of Beta Receptor Agonist Therapy in Asthma*, R. Beasley and N. E. Pearce (Eds.). London: CRC Press, 1993, pp. 163–199.
23. Kiely, R. G. Mucociliary transport rate in the horse and its modification with various phamacologic agents. *Proc. Am. Coll. Vet. Int. Med.* 126:1985.
24. Kiely, R. G., and W. L. Jenkins. The effect of clenbuterol chloride on the mucociliary transport rate in horses with a clinical diagnosis of chronic obstructive pulmonary disease (COPD). *Proc. Am. Coll. Vet. Int. Med.* 146:1985.
25. Kim, S. H., B. K. Kong, D. H. Kim, Y. H. Ju, S. L. Jung, and H. Y. Kang. Effect of treadmill training and clenbuterol treatment on body fat accumulation and muscle enzyme activities of rats. *Med. Sci. Sports Exerc.* S282:222, 2000.
26. Kuiper, H. A., M. Y. Noordam, M. M. H. Van Dooren-Flipsen, R. Schilt, and A. H. Roos. Illegal use of β-adrenergic agonist: European community. *J. Anim. Sci.* 76:195–207, 1998.
27. Lawrence, L. M., R. A. Kane, P. A. Miller, A. Reece, and C. Hartman. Urea space determination and body composition in horses. *J. Anim. Sci.* 63(Suppl. 1):233, 1986.
28. Lindinger, M. I., L. J. Mccutcheon, G. L. Ecker, and R. J. Geor. Heat acclimation improves regulation of plasma volume and plasma Na(+) content during exercise in horses. *J. Appl. Physiol.* 88:1006–1013, 2000.
29. Lynch, G. S., R. T. Hinkle, and J. A. Faulkner. Year-long clenbuterol treatment of mice increases mass, but not specific force or normalized power, of skeletal muscles. *Clin. Exp. Pharmacol. Physiol.* 26:117–120, 1999.
30. Maclennan, P. A., and R. H. T. Edwards. Effects of clenbuterol and propanol on muscle mass. *Biochem. J.* 264-:573–579, 1989.
31. Mckeever, K. H., R. Scali, S. Geiser, and C. F. Kearns. Plasma aldosterone concentration and renal sodium excretion are altered during the first days of training in horses. *Equine Veterinary Journal Suppl.* 34:524–531, 2002.
32. Mckeever, K. H., W. A. Schurg, and V. A. Convertino. A modified Evans blue dye method for determining plasma volume in the horse. *J. Equine Vet. Sci.* 8:208–212, 1988.
33. Mckeever, K. H., W. A. Schurg, S. H. Jarrett, and V. A. Convertino. Exercise training-induced hypervolemia in the horse. *Med. Sci. Sports Exerc.* 19:21–27, 1987.
34. Mckeever, K. H., W. A. Schurg, and V. A. Convertino. Exercise training-induced hypervolemia in greyhounds: role of water intake and renal mechanisms. *Am. J. Physiol.* 248(4 Pt 2):R422–425, 1985.
35. Mckenzie, D. C., E. C. Rhodes, D. R. Stirling, et al. Salbutamol and treadmill performance in non-atopic athletes. *Med. Sci. Sports Exerc.* 15:520–522, 1983.
36. Meeuwisse, W. H., D. C. Mckenzie, S. R. Hopkins, and J. D. Road. The effect of salbutamol on performance in elite nonasthmatic athletes. *Med. Sci. Sports Exerc.* 24:1161–1166, 1992.
37. Nishime, E. O., C. R. Cole, E. H. Blackstone, F. J. Pashkow, and M. S. Lauer. Heart rate recovery and treadmill exercise score as predictors of mortality in patients referred for exercise ECG. *JAMA* 284:1392–1398, 2000.
38. Persson, S. G. B. On blood volume and working capacity. *Acta. Vet. Scand. Suppl.* 19:1–189, 1967.

39. POLLOCK, M. L., A. WARD, and J. J. AYRES. Cardiorespiratory fitness: response to differing intensities and durations of training. *Arch. Phys. Med. Rehabil.* 58:467–476, 1977.
40. PRATHER, I. D., D. E. BROWN, P. NORTH, and J. R. WILSON. Clenbuterol: a substitute for anabolic steroids? *Med. Sci. Sports Exerc.* 27:1118–1121, 1995.
41. RICKS, C. A., R. H. DALRYMPLE, P. K. BAKER, and D. L. INGLE. Use of a β-agonist to alter fat and muscle deposition in steers. *J. Anim. Sci.* 59:1247–1255, 1984.
42. ROSE, R. J., J. R. ALLEN, and K. A. BROCK. Effects of clenbuterol hydrochloride on certain respiratory and cardiovascular parameters in horses performing treadmill exercise. *Res. Vet. Sci.* 35:301–305, 1983.
43. ROSE, R. J., and D. L. EVANS. Cardiorespiratory effects of clenbuterol in fit Thoroughbred horses during a maximal exercise test. In: *Equine Exercise Physiology*, Vol. 2, J. R. Gillespie and N. E. Robinson (Eds.). San Diego, CA: ICEEP Publications, 1984, pp. 117–131.
44. SASSE, H. H., and R. HAJER. NAB 365, a beta 2 receptor sympathomimetic agent: clinical experience in horses with lung disease. *J. Vet. Pharmacol. Ther.* 1:241–243, 1978.
45. SAWAYA, A. L., and P. G. LUNN. Increase in skeletal muscle protein content by the β-2 selective adrenergic agonist clenbuterol exacerbates hypoalbuminemia in rats fed a low-protein diet. *Braz. J. Med. Biol. Res.* 31:819–825, 1998.
46. SIGNORILE, J. F., T. A. KAPLAN, B. APPLEGATE, and A. C. PERRY. Effects of acute inhalation of the bronchodilator, albuterol, on power output. *Med. Sci. Sports Exerc.* 24:638–642, 1992.
47. SLEEPER, M., K. H. MCKEEVER, and C. F. KEARNS. Chronic clenbuterol administration negatively alters cardiac function. *Med. Sci. Sports Exerc.* 34:643–650, 2002.
48. SLOCOMBE, R. F., G. COVELLI, and W. M. BAYLY. Respiratory mechanics of horses during stepwise treadmill exercise tests, and the effect of clenbuterol pretreatment on them. *Aust. Vet. J.* 69: 221–225, 1992.
49. SMITH, D. J. The pharmacokinetics, metabolism, and tissue residues of β-adrenergic agonists in livestock. *J. Anim. Sci.* 76:173–194, 1998.
50. SUZUKI, J., M. GAO, Z. XIE, and T. KOYAMA. Effects of the beta 2-adrenergic agonist clenbuterol on capillary geometry in cardiac and skeletal muscles in young and middle-aged rats. *Acta Physiol. Scand.* 161:317–326, 1997.
51. TORGAN, C. E., G. J. ETGEN, H. Y. KANG, and J. L. IVY. Fiber type-specific effects of clenbuterol and exercise training on insulin-resistant muscle. *J. Appl. Physiol.* 79:163–167, 1995.
52. TORGAN, C. E., J. ETGEN, G. J., J. BROZINICK, J. T., R. E. WILCOX, and J. L. IVY. Interaction of aerobic exercise training and clenbuterol: effects on insulin-resistant muscle. *J. Appl. Physiol.* 75: 1471–1476, 1993.
53. ZEMAN, R. J., R. LUDEMANN, T. G. EASTON, and J. D. ETLINGER, Slow to fast alterations in skeletal muscle fibers caused by clenbuterol, a β2-receptor agonist. *Am. J. Physiol.* 254:E726–E732, 1988.