Chronic clenbuterol administration negatively alters cardiac function

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ABSTRACT

SLEEPER, M. M., C. F. KEARNS, and K. H. McKEEVER. Chronic clenbuterol administration negatively alters cardiac function. *Med. Sci. Sports Exerc.*, Vol. 34, No. 4, pp. 643–650, 2002. **Purpose:** Chronic administration of pharmacological levels of β2-agonists have been shown to have toxic effects on the heart; however, no data exist on cardiac function after chronic clenbuterol administration. The purpose of this study was to examine the effect of therapeutic levels of clenbuterol on cardiac performance. **Methods:** Twenty unfit Standardbred mares were divided into four experimental groups: clenbuterol ($2.4 \ \mu g \cdot kg^{-1}$ twice daily 5 $d \cdot wk^{-1}$) plus exercise (20 min at 50% $\dot{V}O_{2max}$) (CLENEX; $N = 6$), clenbuterol (CLEN; $N = 6$), exercise (EX; $N = 4$), and control (CON; $N = 4$). M-mode and two-dimensional echocardiography (2.5-MHz sector scanner transducer) were used to measure cardiac size and function before and immediately after an incremental exercise test, before and after 8 wk of drug and/or exercise treatments. **Results:** After treatment, CLENEX and CLEN demonstrated significantly higher left ventricular internal dimension (LVD) at end diastole ($+23.7 \pm 4.8\%$; $+25.6 \pm 4.1\%$), LVD at end systole ($+29.2 \pm 8.7\%$; $+40.1 \pm 7.9\%$), interventricular septal wall thickness (IVS) at end diastole ($+28.9 \pm 11.0\%$; $+30.7 \pm 7.0\%$), IVS at end systole ($+29.2 \pm 8.7\%$; $+40.1 \pm 7.9\%$), and left ventricular posterior wall systolic thickness ($+43.1 \pm 14.\%$; $+45.8 \pm 14.1\%$). CLENEX and CLEN had significantly increased aortic root dimensions ($+29.9 \pm 6.1\%$; $+24.0 \pm 1.7\%$), suggesting increased risk of aortic rupture. **Conclusion:** Taken together, these data indicate that chronic clenbuterol administration may negatively alter cardiac function. **Key Words:** ECHOCARDIOGRAPHY, EXERCISE TESTING, EQUINE, CLENBUTEROL

The β2 sympathomimetic agent clenbuterol is a potent repartitioning agent that has been used to promote growth in cattle and sheep by increasing protein accretion and fat removal with little or no change in total body weight (5,29). It is a frequently abused drug in human athletes for this effect, especially in female athletes because it is not associated with the androgenic side effects of steroids (26). However, long-term treatment with high doses of β2-agonists ($mg \cdot kg^{-1}$) has been shown to be toxic to the heart in several species (15,18,24). Isoprenaline has caused severe necrosis in dog myocardium (15), salbutamol caused myocardial hypertrophy and necrosis in rats (18), and salbutamol, fenoterol, and isoprenaline caused multifocal myonecrosis of the left ventricle in sheep (24). Chronic clenbuterol treatment has been shown to cause cardiac hypertrophy (8,19) and resulted in elevated serum cardiac isoenzyme of creatine kinase (CKMB) and alpha hydroxy butyrate dehydrogenase in mice, perhaps due to cellular myocardial damage (7). According to Suzuki et al. (35), changes in heart and hind leg muscle after 10 d of clenbuterol therapy suggest that the drug may reduce oxygen supply to tissues and increase fatigability in mice. Furthermore, chronic clenbuterol therapy has been associated with sudden cardiac death in exercising rats (2).

In the horse, clenbuterol is used as a bronchodilator, much in the same way as albuterol and salbutamol in humans. However, it is administered in much lower doses ($\mu g \cdot kg^{-1}$) than in other species to achieve bronchodilatory effects (30,31,32). Clinical improvements have been shown in horses with chronic obstructive pulmonary disease (COPD), bronchitis, and pneumonia (30), but no significant effect has been detected on ventilatory function in normal horses given clenbuterol before an exercise test (32,34). Clenbuterol has also been shown to increase mucociliary transport rate in both the normal horse and horses with COPD (16). However, these studies have only assessed short-term dosing of no more than 5 d. In fact, the only chronic administration study performed in the horse evaluated clinical signs of COPD after 30 d of clenbuterol administration without assessment of cardiac function (9). Given the fact that long-term administration of β2-agonists in higher doses has been shown to have toxic effects on the heart (2,8,15,18,24), it may be considered unethical to evaluate long-term administration in humans. Furthermore, studies in rats (8) and mice (13) have demonstrated that exercise may be protective of clenbuterol's deleterious effects. Unlike the rodent, horses represent an animal model that readily exercise trains for athletic competition. In addition, horses frequently receive chronic systemic clenbuterol for the treatment of respiratory ailments. Therefore, the purpose of the present study was to test the hypothesis that chronic clenbuterol administration, with or without exercise training, would alter cardiac function and performance as assessed by ultrasound using the normal athletic horse.

MATERIALS AND METHODS

Animals and drug administration. Twenty untrained Standardbred mares (age: 10 ± 3 yr) were evaluated. The mares were unfit but accustomed to the laboratory and running on the treadmill before the start of the experiment. During the trial, the horses were housed as a group in 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 was 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 plus exercise (CLENEX; $N = 6$) and clenbuterol only (CLEN; $N = 6$) were orally administered 2.4 $\mu g \cdot kg^{-1}$ clenbuterol (Boehringer Inglelheim, U.K.) twice daily as a syrup (in an average volume of 20 mL) on a schedule of 5 d on and 2 d off for the duration of the study. The CLENEX group also aerobically trained for 3 $d \cdot wk^{-1}$. Four horses were used as the training group (EX) and aerobically trained for 3 $d \cdot wk^{-1}$, whereas another four horses were used as the control group (CON). Both EX and CON were administered similar volumes of molasses twice daily on a 5 d on and 2 d off schedule.

Graded exercise test (GXT). Before the test, the horses were weighed and walked onto the treadmill. During incremental exercise tests the animals ran on the high speed horse treadmill (Sato I, Equine Dynamics, Inc., Lexington, KY) at a fixed 6% grade. 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 completed the previously determined speed that elicited $\dot{V}O_{2max}$ (14). Horses were immediately walked off the treadmill at the completion of the test and echocardiography was then performed within 30 s.

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 of 50% $\dot{V}O_{2max}$ (17). After 1 wk, the duration was increased to 20 $min \cdot d^{-1}$ and was held at this duration for the entirety of the study. During the exercise training, the high-speed horse treadmill was set at a fixed 6% grade.

Echocardiography. M-mode and two-dimensional echocardiographic data was obtained with a 2.5-MHz sector scanner transducer before (at rest) and immediately after horses performed a GXT (i.e., while the heart rate was still greater than 100 bpm; Fig. 1). Left ventricular diameter (LVD) at end diastole and end systole, interventricular septal wall thickness (IVS) at end diastole and end systole, left ventricular free wall thickness (LVFW) at end diastole and end systole, shortening fraction (SF), aortic root diameter (AO), diameter of the left atrial appendage (LA), ejection time (ET), and E point to septal separation of the mitral valve (EPSS) were determined according to criteria established by the American College of Echocardiography (10,21,27). These values were used in the Teichholtz equa-
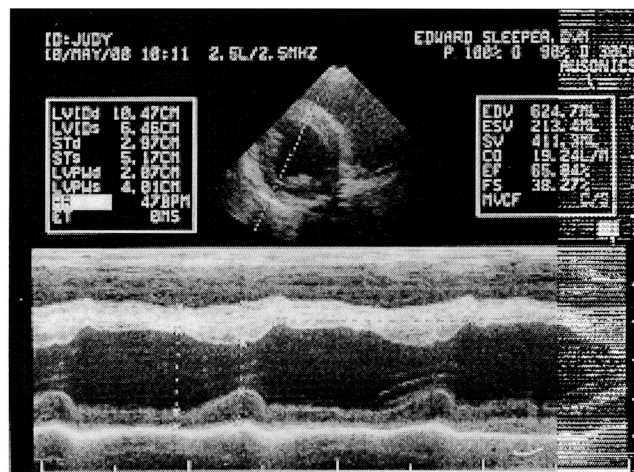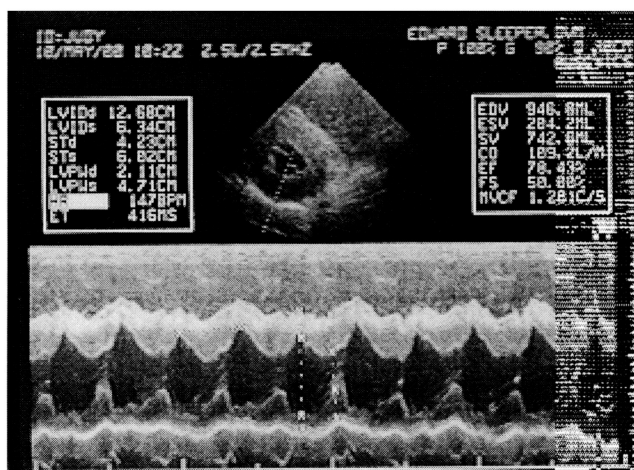

FIGURE 1—M-mode echocardiograms from the right parasternal window derived from a short axis plane. A, ventricular M-mode at rest. B, ventricular M-mode immediately after exercise in the same Standardbred mare. Note the increased thickening of the IVS and LVFW post exercise, which is a normal exercise-induced change.

tions for calculating stroke volume (SV), cardiac output ($\dot{Q}$), and ejection fraction (EF) pre- and post-exercise. All measures were repeated after 8 wk of drug and/or exercise treatments. M-mode measurements were made on three heart cycles with a representative one being used for this data. Two-dimensional echocardiography was also performed; however, all measurements were obtained from the M-mode views. The calculated average coefficient of variation (CV) based on four animals for LVID at end diastole and end systole, measured by electronic calipers and by hand, was less than 1%.

Left ventricular (LV) mass was calculated using the echocardiographic measurements of LVD, IVS and LVFW at end diastole with the following formula:

$$LV mass (g \cdot 1000g^{-1} bodyweight) =$$

$$([(LVD+IVS+LVFW)^3-LVD^3]\times1.05)/1000 [25]$$

Plasma cardiac troponin I (cTnI). Venous blood samples were collected at rest (before the start of the GXT) and 2 h after exercise and plasma cTnI concentration was measured by use of an automated analyzer (Stratus® CS by Dade Behring). The sensitivity of this analyzer for the measurement of cTnI concentration was 0.03 ng·mL^{-1}.

Statistical analysis. Results are expressed as means ± standard error of the estimate (SEM). For comparison by group and time a two-way ANOVA was used with the *a priori* level of statistical significance set at $P < 0.05$. *Post hoc* differences were determined using the Tukey test (Sigma Stat 2.0).

RESULTS

Chronic clenbuterol administration resulted in significant alterations in echocardiographically measured cardiac function, particularly evident immediately after exercise.

Indices of cardiac function. Acute exercise resulted in a significant heart rate (HR) elevation immediately after maximal exercise when compared to resting HR, both before and after treatment. There were no differences in HR between groups, except that HR was significantly higher in CON than EX immediately after maximal exercise during posttreatment testing (Fig. 2A).

After 8 wk, both drug treatment groups had significantly elevated calculated SV immediately after maximal exercise due to increased LV size. These values were higher than the posttesting calculated SV for CON and EX. However, there were no differences ($P > 0.05$) in SV immediately after maximal exercise between pre- and post-treatment CON and EX (Fig. 2B).

Acute exercise resulted in an elevated ($P < 0.05$) calculated Q̇ immediately after maximal exercise in all groups. However, both drug groups demonstrated a significantly larger Q̇ immediately after maximal exercise during posttesting when compared with their pretreatment values and the posttesting values of CON and EX (Fig. 2C).

As expected, acute exercise resulted in a larger ($P < 0.05$) calculated ejection fraction and fractional shortening immediately after maximal exercise before and after treatment. There were no differences ($P > 0.05$) between groups or differences due to clenbuterol and/or exercise treatment (data not shown). Ejection time was significantly decreased
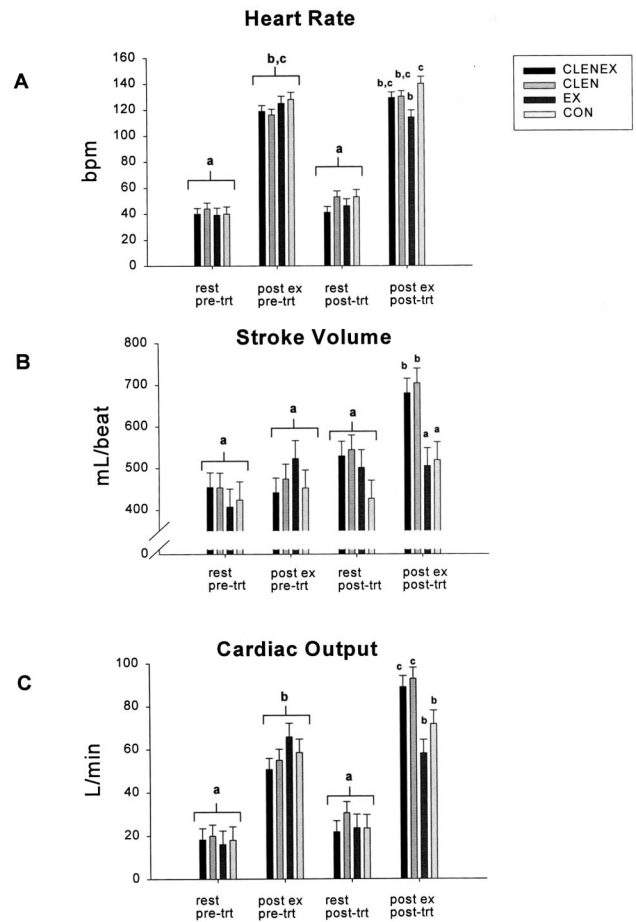

FIGURE 2—Echocardiographically measured values for (A) heart rate (bpm) and calculated values using the Teichholtz equations for (B) stroke volume (mL·beat^{-1}), and (C) cardiac output (L·min^{-1}) in unfit Standardbred mares at rest and immediately after exercise both before and after 8 wk of clenbuterol and/or exercise treatment (trt). All values are mean ± SEM for CLENEX (clenbuterol plus exercise), CLEN (clenbuterol), EX (exercise), and CON (control). a, b, c—letters are significantly different from each other ($P < 0.05$).

immediately after maximal exercise in all groups during both testing periods and there were no group differences (data not shown).

Echocardiographic parameters at rest. Neither exercise training nor clenbuterol treatment resulted in significant changes in the resting values of AO, EPSS, LVD (diastolic or systolic), LVFW (diastolic or systolic), IVS

TABLE 1. Echocardiographic parameters at rest.

	CLENEX		CLEN		EX		CON	
Parameter	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*
AO (cm)	8.29 ± 0.24	8.30 ± 0.29	8.28 ± 0.26	8.52 ± 0.18	8.53 ± 0.40	8.37 ± 0.23	8.17 ± 0.07	7.89 ± 0.08
EPSS (cm)	0.65 + 0.06	0.65 ± 0.10	0.61 + 0.08	0.77 ± 0.24	0.52 ± 0.14	0.78 ± 0.22	0.64 ± 0.05	0.58 ± 0.10
LVDd (cm)	10.96 ± 0.38	11.44 ± 0.37	11.13 ± 0.25	11.62 ± 0.20	11.13 ± 0.47	11.25 ± 0.14	10.89 ± 0.20	10.54 ± 0.31
LVDs (cm)	6.80 ± 0.28	6.66 ± 0.37	7.06 ± 0.17	6.66 ± 0.29	7.07 ± 0.46	6.56 ± 0.52	7.09 ± 0.45	6.33 ± 0.41
LVFWd (cm)	2.13 ± 0.16	2.13 ± 0.11	2.24 ± 0.13	2.20 ± 0.11	2.04 ± 0.18	2.29 ± 0.20	2.22 ± 0.05	1.94 ± 0.04
LVFWs (cm)	3.82 ± 0.29	3.94 ± 0.22	3.79 ± 0.20	4.42 ± 0.45	3.90 ± 0.26	4.14 ± 0.25	3.74 ± 0.09	3.65 ± 0.06
IVSd (cm)	2.72 ± 0.13	2.69 ± 0.18	2.76 ± 0.21	2.54 ± 0.18	2.79 ± 0.32	2.78 ± 0.30	2.69 ± 0.04	2.39 ± 0.08
IVSs (cm)	4.32 ± 0.11	4.67 ± 0.16	4.59 ± 0.09	4.93 ± 0.20	4.41 ± 0.19	4.78 ± 0.14	4.32 ± 0.25	4.69 ± 0.24
LA (cm)	6.51 ± 0.29	6.46 ± 0.36	5.81 ± 0.23	6.10 ± 0.29	6.37 ± 0.43	6.24 ± 0.24	5.91 ± 0.32	5.98 ± 0.11

Echocardiographic parameters (mean ± SEM) in unfit Standardbred mares pre- and post-treatment. CLENEX (clenbuterol plus exercise), CLEN (clenbuterol), EX (exercise) and CON (control). AO (Aortic root dimension), LVDd (left ventricular diameter at end diastole), LVDs (left ventricular diameter at end systole), IVSd (interventricular septal wall thickness at end diastole), IVSs (interventricular septal wall thickness at end systole), LVFWd (left ventricular free wall diastolic thickness), LVFWs (left ventricular free wall systolic thickness) and LA (left atrial appendage).

Group	Left Ventricular Mass (At rest)	
	Pretesting	Posttesting
CLENEX	2.80 ± 0.24	2.99 ± 0.31
CLEN	2.96 ± 0.20	2.95 ± 0.17
EX	2.68 ± 0.30	3.13 ± 0.43
CON	2.79 ± 0.16	2.23 ± 0.14

Echocardiographically calculated left ventricular mass (grams) in unfit Standardbred mares, before and after clenbuterol and/or exercise treatment. LV mass (g·1000 g⁻¹ body weight) = ([(LVD + IVS + LVF)³ −LVD³] × 1.05)/1000. CLENEX (clenbuterol plus exercise), CLEN (clenbuterol), EX (exercise); and CON (control).

(diastolic or systolic), or LA (Table 1). There were no differences ($P > 0.05$) in echocardiographically calculated LV mass between any group, either before or after clenbuterol and/or exercise treatment (Table 2).

Echocardiographic parameters after exercise. Absolute aortic root diameter (AO) immediately after exercise was larger ($P < 0.05$) in both the CLENEX (+30%) and CLEN (+24%) groups after treatment (Fig. 3). Both CLENEX and CLEN demonstrated significantly ($P < 0.05$) larger posttreatment LVD at end diastole (+24 and +26% respectively; Fig. 4A) and end systole (+29 and +40%, respectively; Fig. 4B) immediately after exercise. Similarly, CLENEX and CLEN demonstrated ($P < 0.05$) larger post-treatment IVS at end diastole (+29 and +31% respectively; Fig. 5A) and end systole (+27 and +12%, respectively; Fig. 5B) immediately after exercise. There were no differences ($P > 0.05$) in LVFW immediately after exercise at end diastole after treatment (Fig. 6A); however, CLENEX and CLEN demonstrated larger ($P < 0.05$) posttreatment LVFW at end systole (+43 and +45% respectively; Fig. 6B) immediately after exercise. Mitral valve E point to septal separation after clenbuterol treatment was greater ($P < 0.05$) in CLENEX (+118%) and CLEN (+71%) immediately after exercise (Fig. 7A). The left atrial appendage was also larger ($P < 0.05$) posttreatment in CLENEX (+32%) and CLEN (+25%) immediately after exercise (Fig. 7B).

Cardiac troponin I (cTnI). There were no differences ($P > 0.05$) in plasma cTnI concentrations between groups either before or after drug and/or exercise treatment (data not shown). Acute exercise did not result in any plasma cTnI concentration changes at two hours postexercise in any group.

DISCUSSION

The significant finding of the present study was that chronic administration of therapeutic levels of clenbuterol adversely affected cardiac function immediately after exercise. Horses treated with clenbuterol had substantially elevated Q and SV immediately after a maximal exercise test compared to their pretreatment values. These calculations were due to increased LVD measurements on M-mode; CLENEX and CLEN mares demonstrated larger LVD and IVS at end diastole and systole immediately after exercise. Remodeling of the left ventricle can be reflected in abnormalities at rest, which may be worsened with exercise; however, the horses in the present study most likely were only in the initial or prodromal stages of cardiac remodeling where the changes in cardiac structure may not have been severe enough to be identified at rest and only manifest
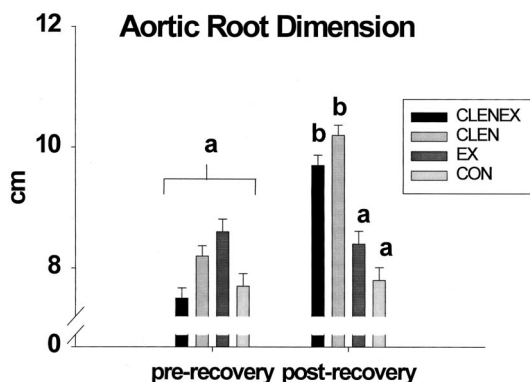

FIGURE 3—Echocardiographically measured values for aortic root dimension immediately after exercise before and after 8 wk of clenbuterol and/or exercise treatment. All values are mean ± SEM for CLENEX (clenbuterol plus exercise), CLEN (clenbuterol), EX (exercise), and CON (control). a, b, c— letters are significantly different from each other ($P < 0.05$).
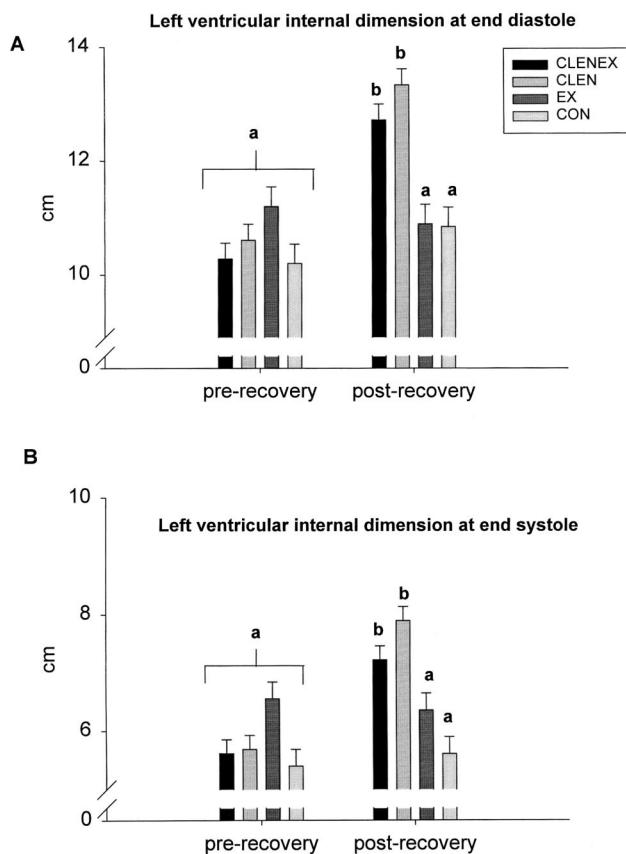

FIGURE 4—Echocardiographically measured values for left ventricular internal dimension at (A) end diastole and (B) end systole immediately after exercise before and after 8 wk of clenbuterol and/or exercise treatment. All values are mean ± SEM for CLENEX (clenbuterol plus exercise), CLEN (clenbuterol), EX (exercise), and CON (control). a, b, c—letters are significantly different from each other ($P < 0.05$).

FIGURE 5—Echocardiographically measured values for internal septal wall thickness at (A) end diastole and (B) end systole immediately after exercise before and after 8 wk of clenbuterol and/or exercise treatment. All values are mean ± SEM for CLENEX (clenbuterol plus exercise), CLEN (clenbuterol), EX (exercise), and CON (control). a, b, c—letters are significantly different from each other ($P < 0.05$).
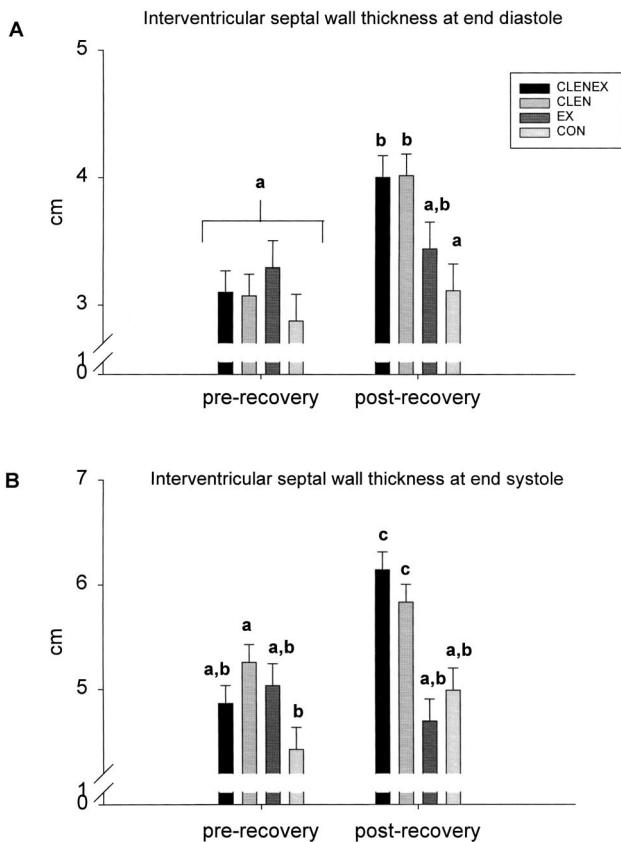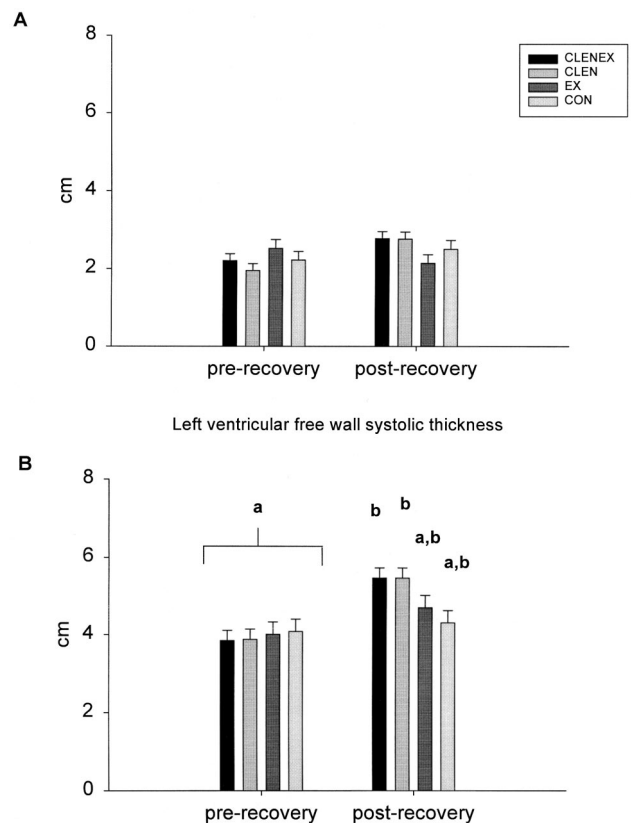

FIGURE 6—Echocardiographically measured values for left ventricular free wall thickness at (A) end diastole and (B) end systole immediately after exercise before and after 8 wk of clenbuterol and/or exercise treatment. All values are mean ± SEM for CLENEX (clenbuterol plus exercise), CLEN (clenbuterol), EX (exercise), and CON (control). a, b, c—letters are significantly different from each other ($P < 0.05$).

themselves after a physiological challenge such as exercise or severe stress. Secondary cardiac hypertrophy can theoretically result from chronically increased afterload (hypertension), and these data may be indicative of chronically increased afterload with secondary cardiac remodeling. Alternatively, the change may have been due to primary remodeling stimulated by clenbuterol therapy.

Echocardiographic measurements. Although not the preferred method for measuring SV, $\dot{Q}$, or EF, M-mode echocardiography is considered the gold standard for measurements of cardiac structures (IVS, LVD, LVS, etc.). Accurate measurement of these structures allows one to calculate functional parameters by using the Teichholz formula. The Teichholz formula is a corrected cube formula that was used to calculate SV, $\dot{Q}$, and EF in this study from M-mode LV measurements. It attempts to account for the fact that the short axis of the LV widens more than the long axis when the ventricular chamber enlarges; however, the formula is most accurate in the undilated heart. Other groups have used this echocardiographic technique to evaluate cardiac output because it is noninvasive (25), and because all measurements were made similarly, significant trends should be apparent. Acute exercise significantly increased the Tiechholz calculated, postexercise SV and $\dot{Q}$ despite a lack of change in HR, in the CLENEX and CLEN groups;

whereas, HR, SV, and $\dot{Q}$ were similar to pretreatment values in the control group (Fig. 2A–C). The calculated SV and Q changes were secondary to changes in the echocardiographically measured LV dimensions postexercise. This increase may reflect poor recovery from exercise with an elevated metabolic demand in the experimental groups or cardiac remodeling due to an altered load. Poor exercise capacity also was demonstrated in the experimental groups by a reduced $\dot{V}O_{2max}$ (14), an observation that also has been recognized previously in clenbuterol-treated rats (8,13,19).

Echocardiographic measurements made immediately after exercise, including the IVS, LVD (diastolic and systolic; Fig. 4A–B, Fig. 5A–B), and AO (Fig. 3) were substantially increased in both clenbuterol-treated groups. Other researchers have observed a protective effect of exercise on clenbuterol administration (8,13,19). This was not the case in the present study as the CLENEX group experienced negative adaptations similar to the CLEN group. The reasons for this are not clear; however, the exercise only group did appear to show mild improvements in most echocardiographic parameters in measurements made immediately after exercise (Figs. 4–7). These data suggest that, although the exercise training protocol used in the present study was strong enough to elicit a substantial increase in aerobic
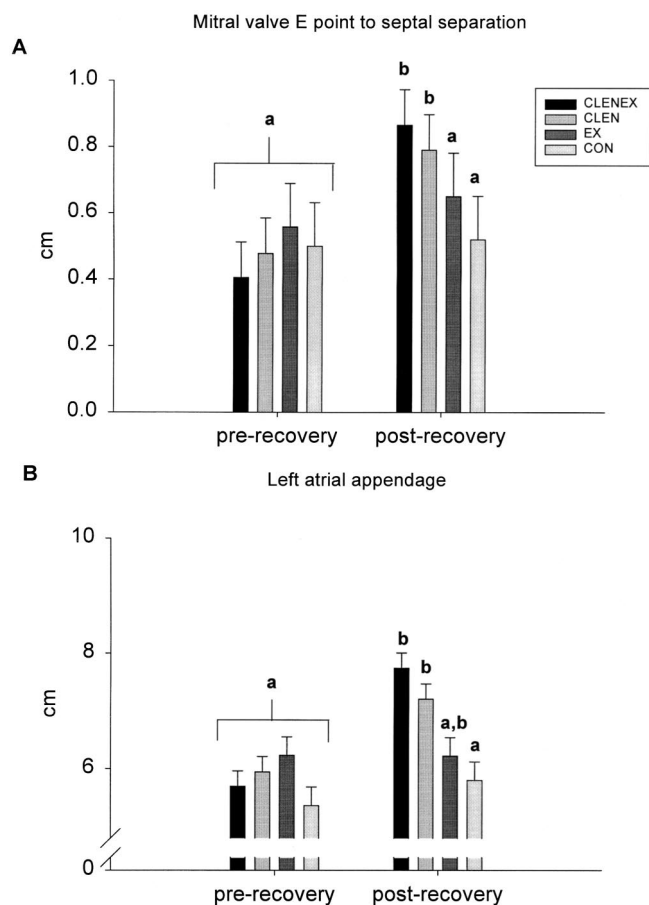
Mitral valve E point to septal separation

FIGURE 7—Echocardiographically measured values for (A) mitral valve E point to septal separation and (B) left atrial appendage immediately after exercise before and after 8 wk of clenbuterol and/or exercise treatment. All values are mean ± SEM for CLENEX (clenbuterol plus exercise), CLEN (clenbuterol), EX (exercise), and CON (control). a, b, c—letters are significantly different from each other ($P < 0.05$).

capacity (14), the intensity may not have been of a level protective against the deleterious effects of clenbuterol.

Cardiac remodeling. The thickened IVS and enlarged LVD (diastolic) seen in the present study may be indicative of cardiac muscle remodeling, a speculation that is supported by data from a study that evaluated clenbuterol in exercising rats (8). In that experiment, clenbuterol treatment resulted in myocardial hypertrophy and extensive collagen infiltration in the LV wall, a change which would be expected to decrease LV compliance and increase LV filling pressure. The effect may be too mild at this treatment protocol to be appreciable at rest. Definitive assessment would require myocardial histopathology after clenbuterol administration, and future research is needed.

Although there was no echocardiographic evidence of left ventricular hypertrophy in the present study (Table 2), other studies have demonstrated an increase in heart mass after either long-term treatment (19) or higher doses of clenbuterol (36). The reason that cardiac hypertrophy was not seen in the present study is not clear. It might be related to the lower doses used in our study compared with previous studies or it might be that compensated, decreased cardiac

function is present before secondary hypertrophy and necrosis. This proposed pattern is consistent with early, compensated LV hypertrophy (4).

Several investigators have suggested that increased cardiac mass and left ventricular hypertrophy might be beneficial to cardiac function (12,36); however, clenbuterol has not been shown to increase either specific force or normalized power output (19). Moreover, clenbuterol has been shown to increase passive Ca^{2+} leak from the sarcoplasmic reticulum (SR) of single-skinned mammalian skeletal muscle fibers (2), which could lead to calcium overload within the myocardial cell if similar leakage occurs from the cardiac SR. Interestingly, Ca^+/calmodulin-dependent protein kinase has been shown to regulate gene expression and this Ca^{2+} dependent pathway has been linked to skeletal muscle hypertrophy (22). Furthermore, β-adrenoreceptor-mediated hypertrophy in neonatal rat myocardial cells has been linked to SR Ca^{2+} release, not cAMP dependent Ca^{2+} (11). Therefore, clenbuterol administration could lead to muscle hypertrophy and Ca^{2+} overload in the myocardial cell.

In addition to its adverse affects on SR function, clenbuterol has been shown to increase collagen infiltration in the myocardium (8) and decrease total capillary density in rat myocardium (35). This alteration in cardiac capillary geometry may decrease oxygen supply to the tissue and thereby increase fatigability (35). Myocardial oxygen demand also may increase due to increased afterload and β-receptor stimulation (3). Therefore, clenbuterol administration may adversely affect the heart through several mechanisms. Intracellular elevations in calcium and cardiac hypoxia have been linked with arrhythmogenesis and subsequent sudden death (4), and could explain sudden death seen in a previous clenbuterol study (2).

Recently, Guldner and colleagues (12) evaluated a three-fold approach that combined electrical and dynamic training of skeletal muscle with clenbuterol therapy for a circulatory assist device (12). In this study, 10 goats (5 control and 5 experimental) were treated for 5–8 months with 150 μg clenbuterol three times weekly. Clenbuterol appeared to enhance several parameters of the circulatory assist device (skeletal muscle ventricles), such as SV volume displacement per minute, and maximum rates of pressure generation (12). The authors concluded that a clenbuterol-supported dynamic training system might have important implications for the treatment of end-stage heart failure. However, cardiac muscle may respond adversely to chronic clenbuterol administration, as suggested by the results of the current study. Human heart failure patients are typically prescribed β-blockers to reduce cardiac afterload and improve diastolic function. In fact, β-blockade is beneficial to heart failure patients by preventing the depletion of SR Ca^{2+} (20). Thus, it would seem potentially dangerous to prescribe a β-agonist to heart failure patients, especially in lieu of the other potentially cardiotoxic effects of clenbuterol seen previously and in this study.

Clinical implications. Cardiac troponin I is a highly specific and sensitive marker for myocardial damage in many mammalian species, with a highly conserved structure

(1,23). Cardiac troponin I concentration increases in the peripheral blood stream after myocardial necrosis and has been recognized to rise in the horse after rupture of a left ventricular outflow tract jet lesion (6). A significant increase was not seen in the present study, an observation that may have been associated with several possible factors. First, the blood samples in these horses were obtained 2 h after exercise, which may have been too early for significant cTnI release. This speculation is supported by the fact that cTnI reaches a plateau level in the bloodstream of other species after 12 h, with the initial rise taking 5–7 h (28,33). Alternatively, it is also possible that acute cellular damage does not result from clenbuterol therapy.

The increased aortic root diameter and systolic LVD measurements, as stated previously, could be secondary to increased afterload in the clenbuterol-treated groups. The change was most dramatic in the sedentary clenbuterol group. Experimentally, an acutely increased afterload, determined by vascular impedance and arterial blood pressure, results in increased systolic and diastolic volumes (4). This could have been the case in the CLENEX and CLEN groups. Furthermore, aortic dilation is often present with decreased arterial compliance and increased afterload (4). Afterload is clinically assessed by arterial systolic blood pressure. Unfortunately, systemic blood pressure was not measured in these subjects; however, if collagen infiltration occurred in the LV, as seen in the rat (8), myocardial stiffness and afterload would increase. Furthermore, an increase in myocardial diffusion distance, as has been seen in a previous study (35), may ultimately result in increased cardiac afterload. During exercise, systemic blood pressure rises and afterload increases despite a decrease in peripheral vascular resistance. Thus, in the CLEN and CLENEX horses, the exercise stress test may have resulted in altered postexercise echocardiographic parameters that were masked at rest due to compensatory mechanisms.

The increase in aortic root diameter (Fig. 3) seen in both drug treatment groups is of particular concern because the change may increase the risk of aortic root rupture, a potential cause of death in the exercising horse (6). Dilation of the pulmonary artery secondary to pulmonary hypertension is associated with an increased risk of pulmonary artery rupture (4). Moreover, sudden cardiac death has been associated with clenbuterol therapy and exercise in a previous study (2), although it is unclear whether death was associated with an arrhythmia or aortic rupture.

SUMMARY AND CONCLUSION

The abuse of clenbuterol as a repartitioning agent has increased in human athletes (26), with little consideration to its potential health risks. Data from the present study suggest that chronic administration of clenbuterol, even at low therapeutic levels, can cause changes in the structural dimensions of the equine heart that are consistent with cardiac remodeling. This remodeling negatively alters cardiac function. The changes in cardiac function were consistent with those shown to cause decreased aerobic capacity seen in other species (9,13,19). Exercise training was not protective against these deleterious alterations as the clenbuterol plus exercise group experienced responses similar to the clenbuterol only group. Finally, clenbuterol administration results in an enlarged aorta immediately after exercise, a change that could increase the risk of aortic rupture and sudden death, particularly in the athletic horse.

REFERENCES

1. ADAMS, J. E., D. R. ABERDSCHEIN, and A. F. JAFFE. Biochemical markers of myocardial injury. Is MB creatine kinase the choice of the 1990's? *Circulation* 88:750–763, 1993.
2. BAKKER, A. J., S. L. HEAD, A. C. WAREHAM, and D. G. STEPHENSON. Effect of clenbuterol on sarcoplasmic reticulum function in single skinned mammalian skeletal muscle fibers. *Am. J. Physiol.* 274: C1718–C1726, 1998.
3. BISHORIC, N. H., B. SATO, and K. A. WEBSTER. Beta-adrenergic regulation of a myocardial actin gene via a cyclic AMP-independent pathway. *J. Biol. Chem.* 267:20932–20936, 1992.
4. BROWN, A., A. TAYLOR, and A. SLANKER. Sudden and unexpected death in adult horses. *Comp. Cont. Educ.* 9:78–85, 1987.
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. CORNELISSE, C. J., H. C. SCHOTT, N. B. OLIVIER, et al. Concentration of cardiac troponin I in a horse with a ruptured aortic regurgitation jet lesion and ventricular tachycardia. *J. Am. Vet. Med. Assoc.* 217:231–235, 2000.
7. CUBRIA, J. C., R. REGUERA, R. BALANA-FOUCE, C. ORDONEZ, and D. ORDONEZ. Polyamine-mediated heart hypertrophy induced by clenbuterol in the mouse. *J. Pharm. Pharmacol.* 50:91–96, 1998.
8. DUNCAN, N. D., D. A. WILLIAM, and G. S. LYNCH. Deleterious effects of chronic clenbuterol treatment on endurance and sprint exercise performance in rats. *Clin. Sci.* 98:339–347, 2000.
9. 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.
10. FEIGENBAUMM, H. *Echocardiography*. Philadelphia: Lea & Febiger; 1981, pp. 189–329.
11. FLUCK, M., M. N. WAXMAN, M. T. HAMILTON, and F. W. BOOTH. Skeletal muscles Ca^{2+}-indepenent kinase activity increases during either hypertrophy or running. *J. Appl. Physiol.* 88:352–358, 2000.
12. GULDNER, N. W., P. KLAPPROTH, M. GROSSHERR, et al. Clenbuterol-supported dynamic training of skeletal muscle ventricles against systemic load: a key for powerful circulatory assist? *Circulation* 101:2213–2219, 2000.
13. 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.

14. KEARNS, C. F., and K. H. MCKEEVER. (submitted) Clenbuterol administration disrupts central mechanisms of aerobic performances in the horse. *Med. Sci Sports Exerc.*

15. 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.

16. 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.

17. KIGHT, P. K., A. K. SINHA, and R. J. ROSE. Effects of training intensity on maximum oxygen uptake. *Equine Ex. Physiol.* 3:77–82, 1991.

18. LIBRETTO, S. E. A review of toxicology of salbutamol (albuterol). *Arch. Toxicol.* 68:213–216, 1994.

19. 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.

20. MARKS, A. R. Cardiac intracellular calcium release channels: role in heart failure. *Circ. Res.* 87:8–11, 2000.

21. MARTIN, B. B., V. B. REEF, E. J. PARENTE, and A. D. SAGE. Causes of poor performance of horses during training, racing, or showing: 348 cases (1992–1996). *J. Am. Vet. Med. Assoc.* 216:554–558, 2000.

22. NAIRNE, A. C., and M. R. PICCIOTTO. Calcium/calmodulin-dependent protein kinases. *Semin. Cancer Biol.* 5:295–303, 1994.

23. O'BRIEN, P. J., Y. LANDT, and J. H. LANDERSON. Differential reactivity of cardiac and skeletal muscle from various species in a cardiac troponin I immunoassay. *Clin. Chem.* 43:2333–2338, 1997.

24. PACK, R. J., M. R. ALLEY, J. A. DALLIMORE, K. R. LAPWOOD, C. BURGESS, and J. CRANE. The myocardial effects of fenoterol, isoprenaline and salbutamol in normoxic and hypoxic sheep. *Int. J. Exp. Pathol.* 75:357–362, 1994.

25. PIPERS, F. S., and R. L. HAMLIN. Echocardiography in the horse. *J. Am. Vet. Med. Assoc.* 170:815–819, 1977.

26. 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.

27. REEF, V. B. Echocardiographic examination in the horse: the basics. *Comp. Cont. Ed. Pract. Vet.* 12:1312–1320, 1990.

28. RICE, M. S. and D. C. MACDONALD. Appropriate roles of cardiac troponins in evaluating patients with chest pain. *J. Am. Board Fam. Pract.* 12:214–218, 1999.

29. 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.

30. 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.

31. 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.

32. SHAPLAND, J. E., H. E. GARNER, and D. G. HATFIELD. Cardiopulmonary effects of clenbuterol in the horse. *J. Vet. Pharmacol. Ther.* 4:43–50, 1981.

33. SIEGEL, A. J., M. SHOLAR, J. YANG, E. DHANAK, and K. B. LEWANDROWSKI. Elevated serum cardiac markers in asymptomatic marathon runners after competition: is the myocardium stunned? *Cardiology* 88:487–491, 1997.

34. 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.

35. 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.

36. WONG, K., K. R. BOHELER, J. BISHOP, M. PETROU, and M. H. YACOUB. Clenbuterol induces cardiac hypertrophy with normal functional, morphological and molecular features. *Cardiovasc. Res.* 37:115–122, 1998.