Deleterious effects of chronic clenbuterol treatment on endurance and sprint exercise performance in rats

Noel D. DUNCAN, David A. WILLIAMS and Gordon S. LYNCH
Department of Physiology, The University of Melbourne, Parkville, Victoria 3052, Australia

A B S T R A C T

The β_2-adrenergic agonist, clenbuterol, has powerful muscle anabolic and lipolytic effects and is used by athletes to improve exercise performance; however, its use in conjunction with different forms of exercise training has received limited attention. Since previous studies have reported that chronic use of other β_2-adrenergic agonists has deleterious effects on cardiac muscle structure and function, the aim of the present study was to determine whether chronic clenbuterol administration would reduce the exercise capabilities of rats subjected to long-term treadmill sprint running, endurance swimming or voluntary wheel running training. The effect of clenbuterol treatment on exercise performance in rats was evaluated in three separate studies. Different groups of male rats were assigned to an endurance swimming (2 h/day, 5/7 days, 18 weeks) group, a treadmill sprint running (8 × 1 min bouts, 1.05 m/s, 20 weeks) group, or a voluntary wheel running (16 weeks) group. In each study, rats were allocated into either a treated group that received clenbuterol (2 $mg \cdot kg^{-1} \cdot day^{-1}$) in their drinking water or an untreated control group. In each of the three studies, treated rats exhibited a reduction in exercise performance compared with untreated rats. Treated rats ran ~ 57% less total distance than untreated rats in the voluntary running programme and were unable to complete the swimming and sprinting protocols performed by the untreated rats. In each of the studies, the treated rats exhibited cardiac hypertrophy, with absolute heart mass increased by ~ 19% and heart mass relative to body mass increased by ~ 20%. The hearts of sedentary rats treated with clenbuterol exhibited extensive collagen infiltration surrounding blood vessels and in the wall of the left ventricle. The results indicate strongly that chronic clenbuterol administration deleteriously affects exercise performance in rats, potentially due to alterations in cardiac muscle structure and function.

INTRODUCTION

The β_2-adrenoceptor agonist, clenbuterol, has potent muscle anabolic and lipolytic actions [1–4], and is reportedly used by some athletes, especially those involved in strength- and power-related sports [5,6], despite it being a banned substance by the International Olympic Committee. The gain in lean muscle mass has been shown in animals to result from an increase in protein synthesis [1,7,8] and a possible concomitant decrease in protein degradation [9].

Despite numerous studies on the effects of chronic clenbuterol administration on the skeletal muscle properties of sedentary animals, few have investigated its effects on exercise performance directly. Ingalls and colleagues [10] subjected mice to a combination of 8 weeks of

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Correspondence: Dr Gordon S. Lynch (e-mail g.lynch@physiology.unimelb.edu.au).

treadmill running (three sets of 3 min, 36–40 m/min, 10–17% grade, 30-s recovery, 4 days/week) and clenbuterol treatment (1.6 mg/kg, 4 days/week) and found that clenbuterol treatment decreased total work performance. Although clenbuterol increased muscle mass, it decreased exercise performance, such that the combination of exercise and clenbuterol had antagonistic effects on running performance [10]. Other studies have investigated the effects of clenbuterol and exercise training on skeletal muscle, but have not reported changes in exercise performance directly [11,12].

Previous studies have shown that chronic use of β_2-agonists in high doses can have toxic effects on the heart [13]. Histological examination of the myocardium of dogs following chronic treatment with isoprenaline in mg/kg doses revealed severe necrosis [13]. Congestion, interstitial oedema, hypertrophy of muscle fibres and myocardial necrosis were evident in rats given very large doses (between 17 and 150 $mg \cdot kg^{-1} \cdot day^{-1}$) of another β_2-agonist, salbutamol, for 1 month [14]. Cardiac mass increased by up to 27% in that study, but no change in the ECG was reported [15]. Severe myocardial lesions were found in the hearts of sheep given intravenous doses of salbutamol, fenoterol or isoprenaline (128 μg/kg at 15 min intervals) for 4 days [16].

Considering that the side effects of long-term clenbuterol use have not been fully determined and that clenbuterol is being used illegally by athletes to improve sports performance [5,6], it is important to determine the effects of long-term clenbuterol treatment on exercise training directly. To this end, we conducted three separate studies that investigated whether chronic clenbuterol treatment affected the performance (and training capabilities) of rats subjected to three different forms of exercise. Specifically, we investigated the effects of clenbuterol on treadmill sprint training, endurance swim training and voluntary wheel running. We tested the hypothesis that long-term clenbuterol administration would adversely affect the exercise performance of rats. Assessments of training performance of the rats were complemented with morphometric measurements of the isolated myocardium, analysis of the citrate synthase (CS) activity of cardiac muscle, and histological and biochemical assessments of collagen content within the heart.

METHODS

Animal groups and drug administration

Male Wistar rats (4 weeks old), obtained from the Monash University Animal Services Department (Victoria, Australia), were housed in pairs in standard cages and provided with food and water *ad libitum*. They were maintained in an animal housing facility under a 12 h/12 h light/dark cycle (light 0.600–18.00 hours), at a constant room temperature of 22 °C. All experimental procedures were approved by the Animal Experimentation Ethics Committee of The University of Melbourne and complied with the Australian Code of Practices Act, relating to the care and use of animals for scientific purposes. Young rats were used in each of the three studies because of the greater ease of training young adult compared with older adult rats.

The effects of clenbuterol treatment on the exercise performance capabilities of rats were assessed from the results of three different training protocols: treadmill sprint running; endurance swimming and voluntary running. The studies were conducted at different times and each study was considered separately.

Clenbuterol treatment

The clenbuterol dose and treatment protocol were based on those described by Yang and McElligott [17]. Clenbuterol hydrochloride powder (Sigma Chemical Co., St. Louis, MO, U.S.A.) was added to the drinking water at a concentration of 10 mg/litre for the first week, which equated to an approximate working dose of $5\ mg \cdot kg^{-1} \cdot day^{-1}$ based on the volume of water consumed daily by the rats. In the second and subsequent weeks the concentration of clenbuterol in the drinking water was reduced to 5 mg/litre, which equated to a working dose of approx. $2\ mg \cdot kg^{-1} \cdot day^{-1}$ [18]. The clenbuterol was routinely replaced every 7 days to minimize oxidation. The effectiveness of clenbuterol administration via the drinking water has been well established [19]. Clenbuterol treatment began at week 4 of each of the three exercise training programmes. In all groups, clenbuterol treatment followed a staggered 2 days on/2 days off protocol, which has been shown to be effective in reducing attenuation of the clenbuterol response [17]. A high dose of clenbuterol was used in order to determine the maximum effects of the β_2-agonist and also to mimic the excessive doses used/abused by athletes in pursuit of maximum muscle development.

Sprint training

Rats were allocated into control untreated ($n = 5$ animals), control treated ($n = 6$), trained untreated ($n = 8$) or trained treated ($n = 6$) groups. The control rats remained in their cages for the duration of the study. The trained rats were subjected to a 20-week programme of sprint running on a motorized treadmill (Columbus Instruments, Columbus OH, U.S.A.). The rats were trained progressively, by either running more sprint bouts or increasing the speed or angle of inclination each week. By week 18, the untreated rats were capable of completing six 1 min sprint bouts running at a treadmill speed of 70 m/min on an incline of 15°. The animals trained for 4 days in every 7, i.e. Monday, Tuesday,

Thursday and Friday. The rats rested for 2.5 min between sprint bouts, during which time they walked at a treadmill speed of 10 m/min to promote the clearance of waste products and metabolites that may accumulate during the sprint exercise [20]. Rats were removed from the treadmill when they could no longer run despite continued gentle prodding by the supervising trainer. Control (unexercised) rats were also handled by the supervisor on each of the training days.

Endurance swimming

Rats were allocated into control untreated ($n = 8$), control treated ($n = 8$), trained untreated ($n = 8$) or trained treated ($n = 9$) groups. All rats swam together in a large Plexiglass tank (140 cm × 60 cm × 64 cm) in water thermostatically regulated at 35 ± 1 °C. Initially, the rats swam for short periods only so as to become familiar with the exercise. The duration of the swim bout was increased by 15 min each session. In order to standardize and increase the intensity of the swimming exercise, a weight corresponding to 2% of each rat's body mass was attached to the base of the tail before each session. The tail weight was added after the fourth week of training and was increased weekly in accordance with increases in body mass. Eventually, the untreated rats were capable of swimming 120 min/day (carrying a 2% load), 5 days/week. A rat was removed from the tank if it appeared to be struggling and failed to return to the surface within 5 s of being submerged. The person supervising the training sessions was varied each week so as to minimize any potential bias in the determination of absolute exercise capacity. The swimming programme was of 18 weeks' duration. The control (unexercised) rats remained in their cages but were handled daily by the person supervising the training sessions.

Voluntary running

Rats were allocated into control untreated ($n = 6$ animals), control treated ($n = 6$), trained untreated ($n = 6$ animals) or trained treated ($n = 6$) groups. Voluntary-run-trained animals were housed individually in polypropylene boxes (60 cm × 40 cm × 23 cm). Each box contained a polished, stainless steel exercise wheel (320 mm in diameter) which was attached to the cage roof. Trained animals had free access to the spinning wheels, which were equipped with bicycle ergometers (C-15 cycling computer, Vetta, Italy) to record running distances. The running programme was of 16 weeks' duration.

Whole-body, organ and muscle mass measurements

Following completion of each of the training programmes, all rats were killed by rapid cervical dislocation or diethyl ether overdose and weighed. The chest cavity was exposed and the heart carefully excised, blotted on filter paper, trimmed of large vessels and connective tissue, and weighed on an analytical balance, before being snap-frozen in isopentane, cooled in liquid nitrogen and then stored at −80 °C for later analysis. Heart mass measurements were used as a gross indicator of cardiac hypertrophy.

Histological analysis

For rats in the swim training programme, some hearts were excised, processed and embedded in wax, and paraffin sections of the mid-ventricular wall were cut and stained with Van Gieson's stain for determination of collagen localization. Stained sections were excited by an argon-ion laser (488 nm) and viewed with a laser scanning confocal microscope (Lasersharp MRC1024; Bio-Rad). Regions of collagen infiltration within the sections could be distinguished clearly from the adjacent regions of cardiac tissue, based on the intensity of the fluorescence emission.

Collagen biochemistry

The total collagen concentration of the left ventricle was assessed after the sprint and swim training programmes using a hydroxyproline assay [21,22]. The frozen hearts were thawed, the left ventricle was sliced into small pieces, and the wet mass of each tissue piece was determined. The tissue was lyophilized, ground and relyophilized to a constant weight (2–2.5 h). Total dry weight was measured and the concentration of collagen was determined by analysing four 10 mg samples of dry tissue from the mid-ventricular wall of each heart for 4-hydroxyproline content. The tissue was digested in 2 ml of HCl (5 M) and incubated at 100 °C for 4 h. Activated charcoal was added to each tube, which was shaken to decolorize the sample. After 2 min, the sample was gravity-filtered and rinsed with 800 μM NaOH and 500 μl of water.

Hydroxyproline was oxidized with chloramine-T solution and the sample was heated at 60 °C for 20 min with *p*-dimethylaminobenzaldehyde. Colorimetry was used to determine the collagen content. Full colour development was reached within 15 min and was stable for several hours. Fractions were read at 550 nm, and a conversion factor of 7.14 was used to convert 4-hydroxyproline into collagen content, assuming that collagen contains 14% 4-hydroxyproline [23,24].

CS activity

The cardiac muscles of rats from all groups were assayed for CS activity using the protocol described by Srere [25]. The tissue was homogenized (OMNI 1000; FSE) at

20000 rev./min for 30 s with a 5 mm stainless steel aggregate, in a buffer (0.175 M KCl/2 mM EDTA, pH 7.4) containing no sulphydryl compounds. Sulphydryl compounds are regularly added to other homogenizing buffers to prevent enzyme oxidation that reduces the concentration of 5,5′-dithiobis-(2-nitrobenzoic acid) in the enzyme buffer. The homogenate was frozen and thawed two or three times to ensure mitochondrial fracture and the release of enzyme into the surrounding medium. The reagents were prepared individually and comprised 100 mM Tris buffer (pH 8.3), 1 mM 5,5′-dithiobis-(2-nitrobenzoic acid), 10 mM oxalacetate and 3 mM acetyl-CoA. The kinetic assay was performed using a spectrophotometer plate reader running SOFTmax computer software (Version 2.3; Molecular Devices Corp., Menlo Park, CA, U.S.A.). The plate was read using the Kinetic L_1 wavelength setting, with a 405 nm filter for 5 min with automated mixing of the wells every 10 s. The absorbance limit was set at 0.5 and the V_{max} (mOD/min, where mOD is the maximum slope of the kinetic display of absorbance), i.e. the rate or maximum slope of the kinetic display of absorbance against time, was then determined.

Statistical analysis

All data are reported as means ± S.E.M. Analysis was carried out using NCSS (Number Cruncher Statistical System, Version 5.01) software (Dr. J. L. Hintze, Kaysville, UT, U.S.A.). Each of the training studies was considered separately. For each study, a two-way analysis of variance was used to test for the effects of clenbuterol treatment and exercise training. When significant differences were detected, the Student Newman–Keuls multiple comparison procedure was performed to identify specific differences between groups. Significance was set at $P < 0.05$.

RESULTS

Exercise performance

Sprint training

The exercise performance of rats involved in the treadmill sprint training programme is indicated in Figure 1. Based on the ability of the untreated rats to maintain the imposed running speed, the exercise performance of treated rats was severely impaired. Before clenbuterol was administered at week 4 of the running programme, there was no difference in exercise performance between the treated and untreated rats, and this situation was maintained until week 8. The untreated rats were normally removed from the treadmill at the same time as the treated rats to ensure that all of the animals performed a similar exercise volume. On one occasion per week, the untreated rats continued to exercise at the intensity scheduled for that week, at the desired speed and incline, so that comparisons could be made between the exercise capabilities of the treated and untreated rats. After week 8 there was a steady decline in exercise performance of the treated rats that continued for the remainder of the programme. In addition to the inability to maintain the same running speed as untreated rats, the treated rats were also unable to run at the desired incline. By week 10, the untreated rats were capable of sprint running on an incline of 10 °, whereas the treated rats could only manage to run on an incline of 5 ° at a speed up to 23 % slower than that of the untreated rats. By week 16, the untreated rats were capable of running up to 70 m/min up a 15 ° incline, whereas the treated rats could only manage level (0 °) running at a speed 43 % slower than that of the untreated rats.

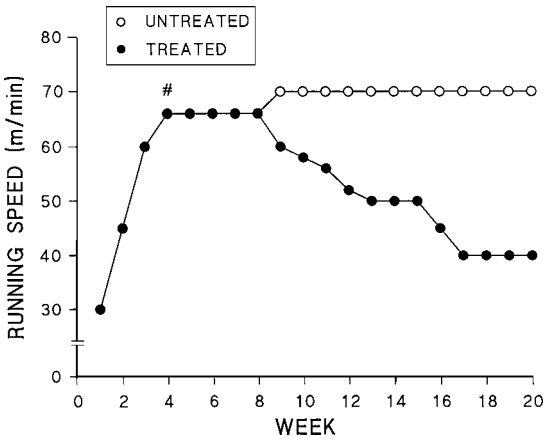

Figure 1 Effect of clenbuterol treatment on sprint exercise performance

Clenbuterol administration began at week 4 of the exercise programme, as indicated by #. After week 8, the clenbuterol-treated rats were unable to maintain the same sprint running speed as the untreated rats. The values represent the running speed at which the treadmill was set for each week.

Swimming

The exercise performance of rats involved in the swimming training programme is shown in Figure 2. As all untreated rats were able to swim for the imposed duration, it is clear that the exercise performance of the treated animals was severely impaired. Before clenbuterol was administered at week 4 of the swimming programme, there was no difference in exercise performance between the treated and untreated rats. At week 7 of the programme, the tail weights had to be removed from the treated rats in order for them to be able to complete the imposed exercise task. After week 8 there was a steady decline in exercise performance of the treated rats, such that by week 12 they could complete only 90 min of

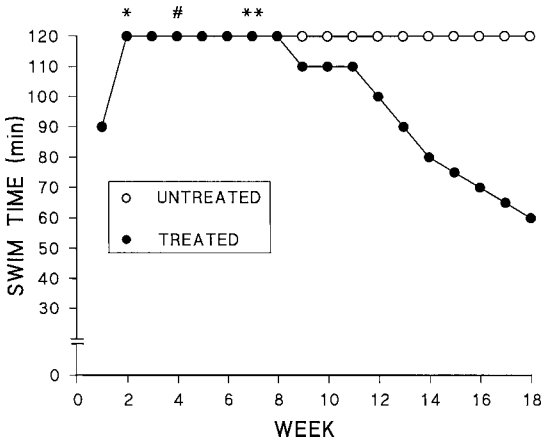

Figure 2 Effect of clenbuterol treatment on swim exercise performance

The values represent the swimming time that was set for the animals for that week. Tail weights (corresponding to 2% of body mass) were added after the second week of the programme, as indicated by *. Clenbuterol administration began at week 4 of the exercise programme, as indicated by #. After week 7, the tail weights had to be removed from the clenbuterol-treated rats, since the animals were otherwise unable to maintain the swimming exercise. Note the steady decline in swim time duration for the treated rats after week 8, as indicated by **.

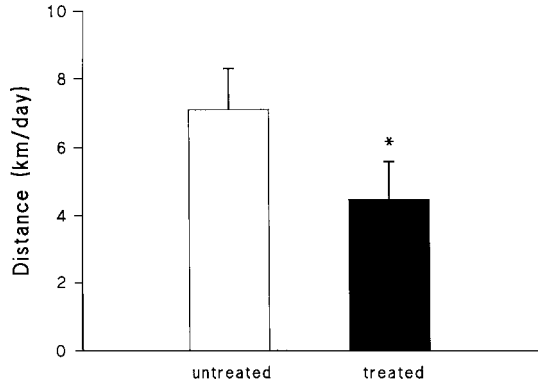

Figure 3 Effect of clenbuterol treatment on voluntary running exercise performance

The data are the average distances run for the duration of the 16-week voluntary running programme. On average, clenbuterol-treated rats ran 57% less total distance per day than the untreated rats.

unweighted swimming, and by week 18 they could only manage 60 min of unweighted swimming. Three treated rats died of sudden cardiac failure during swimming exercise. Autopsy revealed that the hearts from these animals contained large blood clots, especially in the aorta. No deaths occurred in the untreated rats subjected to endurance swimming exercise. The untreated rats were capable of maintaining a higher exercise intensity, swimming with a 2% tail weight for 2 h, for the duration of the training programme. The untreated rats were normally

Table 1 Effects of clenbuterol treatment and three different forms of exercise on heart mass, body mass and heart mass/body mass ratio in rats

Rats were subjected to a sprint training programme (Sprint), a swim training programme (Swim) or a voluntary running programme (Voluntary). Control rats were sedentary, whereas trained rats were subjected to the exercise training programme. Animals were treated or not with clenbuterol. The number of rats in each group is listed in the Methods section. HM, heart mass; BM, body mass. Significant differences ($P < 0.05$) are indicated by: * untreated compared with treated; † control compared with trained in the treated groups of rats; ‡ control compared with trained.

		Control	Trained
Sprint			
HM (g)	Untreated	1.23 ± 0.01	1.27 ± 0.03
	Treated	1.47 ± 0.02*	1.36 ± 0.04†
BM (g)	Untreated	534.6 ± 13.2	495.4 ± 18.3†
	Treated	603.9 ± 12.0*	505.3 ± 21.9
$10^3 \times$ HM/BM (g/g)	Untreated	2.30 ± 0.06	2.52 ± 0.10‡
	Treated	2.47 ± 0.06	2.70 ± 0.06*
Swim			
HM (g)	Untreated	1.11 ± 0.04	1.14 ± 0.04
	Treated	1.32 ± 0.04*	1.30 ± 0.05*
BM (g)	Untreated	462.8 ± 17.3	443.1 ± 21.9
	Treated	530.6 ± 18.5*	434.7 ± 17.0‡
$10^3 \times$ HM/BM (g/g)	Untreated	2.40 ± 0.05	2.58 ± 0.06‡
	Treated	2.49 ± 0.10	3.02 ± 0.24*
Voluntary			
HM (g)	Untreated	1.51 ± 0.04	1.66 ± 0.13
	Treated	1.88 ± 0.10*	1.91 ± 0.12*
BM (g)	Untreated	520.8 ± 17.0	524.8 ± 31.1
	Treated	585.5 ± 15.3	567.5 ± 34.4
$10^3 \times$ HM/BM (g/g)	Untreated	2.92 ± 0.13	3.15 ± 0.12
	Treated	3.16 ± 0.13	3.39 ± 0.17

removed from the swimming tank at the same time as the treated rats to ensure that all of the animals performed the same amount of swimming exercise. As for the sprint training study, once per week, when the treated rats were unable to continue swimming at the prescribed intensity, the untreated rats continued to exercise at the intensity scheduled for that week, enabling comparisons to be made between the swimming exercise capabilities of the treated and untreated rats.

Voluntary running

The exercise performance of rats in the voluntary running programme, based on the distance run per day, is shown in Figure 3. On average, treated rats ran 57% less total distance than untreated rats. There was considerable variability in the distance run per day for both trained treated and untreated rats. Four of the six trained treated rats ran less than 4 km/day, while in the trained untreated

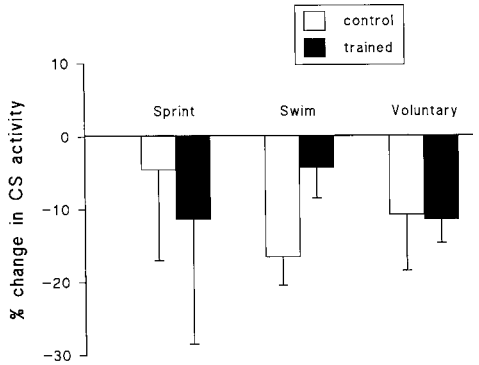

Figure 4 Percentage changes in cardiac CS activity following each of the three different exercise training programmes

Values for the untrained (control) treated and trained treated groups are expressed relative to those for the control untreated and trained untreated groups. The absolute values for CS activity (μmol · min^{-1} · g^{-1}) and the numbers of animals (n) in each group were as follows: sprint training study: control untreated, 78.3 ± 8.8, $n = 5$; control treated, 74.5 ± 10.0, $n = 8$; trained untreated, 95.4 ± 16.4, $n = 6$; trained treated, 84.5 ± 12.0, $n = 6$; swim training: control untreated, 86.7 ± 7.8, $n = 8$; control treated, 72.1 ± 3.5, $n = 8$; trained untreated, 92.2 ± 9.4, $n = 8$; trained treated, 88.2 ± 4.2, $n = 6$; voluntary running: control untreated, 95.6 ± 3.6, $n = 6$; control treated, 85.2 ± 7.5, $n = 6$; trained untreated, 95.1 ± 5.8, $n = 6$; trained treated, 84.3 ± 3.3, $n = 5$. The variability in cardiac CS activity meant that no significant differences were evident between the groups for each of the training studies.

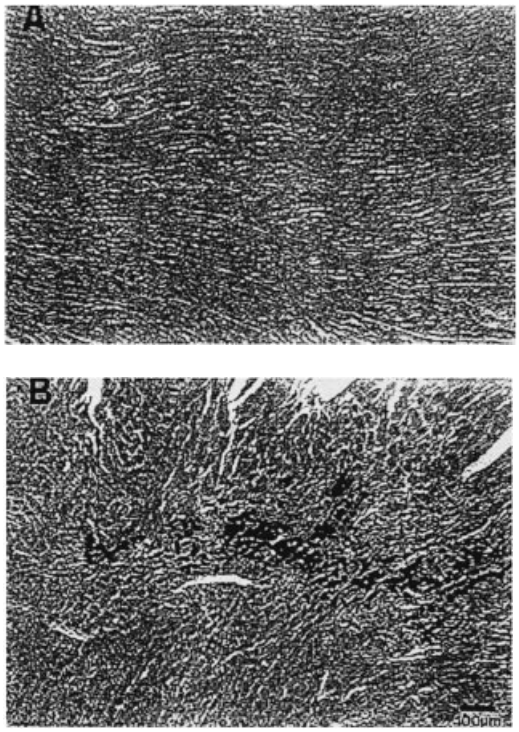

Figure 5 Examples of mid-left-ventricular wall of the heart from a control untreated rat (upper panel) and a control treated rat (lower panel)

Collagen infiltration appears as the dark areas in the ventricular wall shown in the lower panel.

group the range of distances travelled by the rats was 3.5–11.4 km/day.

Morphometric analysis

In each of the three studies, clenbuterol treatment increased the heart mass of sedentary rats (Table 1). In the sprint training study, treated rats that were subjected to sprint running had a lower heart mass than sedentary rats. However, in the swim and voluntary running studies, the heart mass of trained treated rats was not different from that of sedentary treated animals. When expressed relative to body mass, heart mass was greater in clenbuterol-treated rats compared with untreated rats in the sprint and swim training studies, but not in the voluntary running investigation. While absolute heart mass did not differ in the untreated rats subjected to any of the exercise programmes compared with that of untreated untrained rats, when expressed relative to body mass the heart mass of untreated rats was greater following sprint and swim training, but not voluntary running.

CS activity

Figure 4 shows the percentage change in CS activity of cardiac muscle tissue from sedentary treated and trained treated rats compared with sedentary untreated and trained untreated rats. In each study, a two-way analysis of variance revealed no significant difference in cardiac CS activity between treated and untreated groups of rats. CS activity was highly variable in cardiac muscle, especially that from the treated rats subjected to sprint training.

Collagen analysis

Qualitative histological assessment of the hearts from three rats from each of the four experimental groups in the swim training study indicated localized infiltration of collagen in the mid-ventricular wall of all of the sedentary rats treated with clenbuterol (Figure 5). Although not extensive, this abnormal collagen deposition was not evident in any of the untreated rats (control or exercised), nor in any of the treated rats subjected to swim training. In the clenbuterol-treated animals, collagen deposition was especially evident surrounding blood vessels within the ventricular wall. High-power confocal images of sections of ventricular wall taken from the hearts of control (untreated) rats showed normal cardiac tissue appearance (Figure 6, top panel). High-power confocal images of the collagen-infiltrated regions in the hearts of treated rats showed a complete absence of cardiac cells in

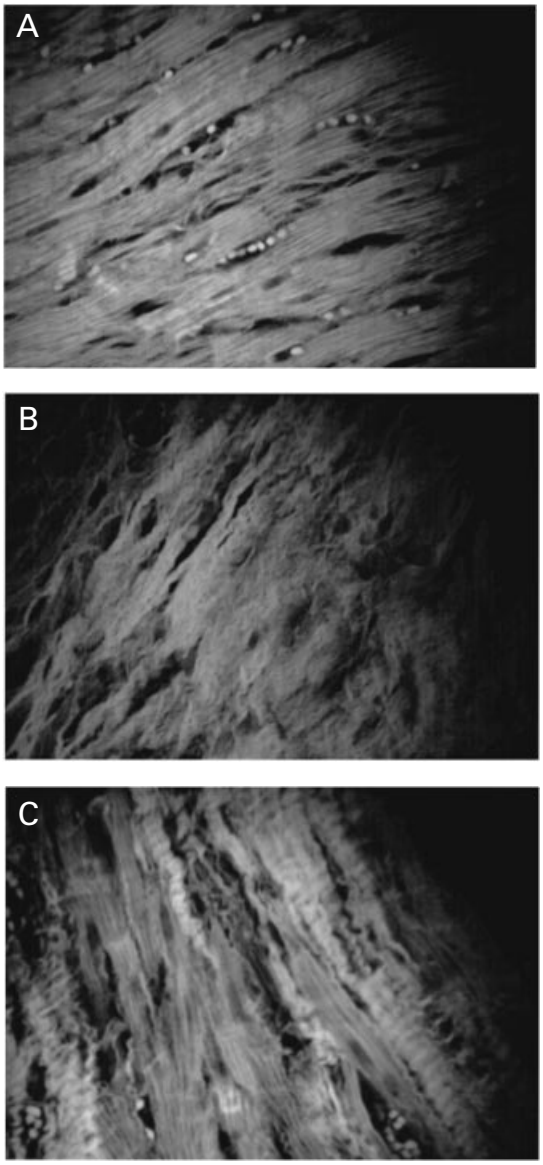

Figure 6 High-power (60× objective) confocal images of ventricular tissue from untreated and clenbuterol-treated rats

Top panel: normal cardiac muscle appearance in fixed sections of the heart from an untreated rat. Cross-striations and longitudinal bundles of contractile material and mitochondria are seen clearly. Middle panel: collagen-infiltrated region devoid of cardiac cells in a region of the heart from a rat treated chronically with clenbuterol. Bottom panel: regions of normal cardiac muscle tissue interspersed with collagen infiltration in the heart from a rat treated chronically with clenbuterol.

those areas (Figure 6, middle panel). Adjacent regions of collagen-infiltrated cardiac muscle cells from the hearts of treated rats are shown in the bottom panel of Figure 6.

To determine whether the concentration of collagen in the whole heart was altered following clenbuterol treatment, a biochemical assessment of the overall collagen content was performed on cardiac tissue from rats in the

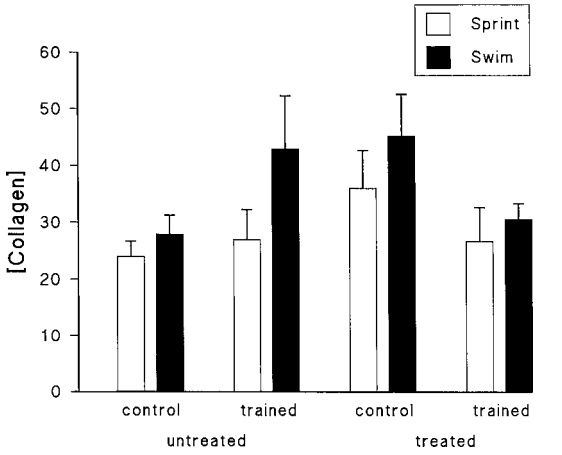

Figure 7 Total collagen concentration in cardiac muscles of rats in the sprint training and swim training studies

Collagen concentration is expressed in units of μg/mg of muscle tissue. No increase in collagen concentration was evident in either investigation. Number of animals (n) in each group: sprint training: control untreated, $n = 5$; control treated, $n = 8$; untreated trained, $n = 6$; trained treated, $n = 6$; swim training: control untreated, $n = 8$; control treated, $n = 8$; trained untreated, $n = 8$; trained treated, $n = 6$.

sprint and swim training groups (Figure 7). No significant differences in overall total collagen content in the hearts of control and treated rats were evident following either investigation.

DISCUSSION

The most important finding of the present study was that long-term clenbuterol administration significantly decreased the sprint running, endurance swimming and voluntary running training performance of rats. This finding was consistent with our working hypothesis that chronic treatment with β_2-agonists would deleteriously affect cardiac muscle and hence reduce exercise capacity. The detrimental effects of clenbuterol on exercise performance has important implications for athletes involved in similar modes of training that are taking this and similar drugs to increase muscle mass and/or decrease body fat. In addition to the clenbuterol-induced decreases in exercise performance, the findings of cardiac hypertrophy and localized collagen infiltration in the left ventricular wall of the sedentary control treated rats (of the swim training protocol) also indicate undesirable adaptations following chronic clenbuterol treatment. Interestingly, despite the overall reduction in exercise performance, the hearts of the treated rats subjected to the swim training protocol did not exhibit localized collagen infiltration in the left ventricle, indicating that training reduced clenbuterol-induced collagen formation in the heart.

In the present study, the finding of increased cardiac mass, along with collagen infiltration surrounding blood vessels and in the mid-ventricular wall of hearts from clenbuterol-treated rats, provides further evidence that large doses of these β_2-agonists cause deleterious alterations to cardiac muscle structure. The hearts from treated rats that were subjected to swim training did not show evidence of collagen infiltration in the mid-ventricular wall. This indicates that, despite the overall reduction in exercise performance of treated compared with untreated rats, training did prevent the infiltration of collagen in the heart. Interestingly, the concentration of collagen in the hearts of clenbuterol-treated rats was not different from that in untreated rats. This was not surprising, given that the collagen deposition did not extend across the entire ventricular wall. Other studies have reported that alterations in the major non-reducible cross-link-stabilizing collagen fibrils in muscle may occur independently of changes in collagen concentration [26,27].

The mechanisms responsible for the $\sim 20\%$ increase in absolute cardiac mass and cardiac mass relative to body mass following chronic treatment with high doses of clenbuterol are not known. Unlike the cardiac hypertrophy that results from exercise, this growth is likely to reduce left ventricular chamber compliance (which would be further reduced because of an increased ventricular stiffness due to the localization of collagen infiltration), and reduce peak hyperaemic flow due to a decrease in coronary flow reserve [28]. Platelet aggregation in capillaries has been postulated as a possible mechanism to explain the myocardial necrosis associated with chronic adrenaline administration [29]. These adaptations may be responsible for, or contribute to, the reduction in exercise performance observed in the clenbuterol-treated rats observed in the present study. It has been postulated that cardiac muscle growth is mediated by either selective β_2-adrenoceptors or prostaglandins [1,30]. These effects on cardiac muscle can be blocked by a cyclo-oxygenase inhibitor [4,31], indicating that the powerful anabolic and/or lipolytic actions of clenbuterol may still be of potential clinical use for ameliorating skeletal muscle wasting [32].

Torgan and colleagues [33] reported that endurance training mitigated a clenbuterol-induced decrease in CS activity and that clenbuterol treatment was responsible for a reduction in skeletal muscle oxidative potential. The important findings of the present study are that chronic high-dose treatment with clenbuterol causes deleterious adaptations in cardiac muscle, including significant increases in absolute and relative cardiac mass as well as infiltration of collagen in the ventricular walls. We have demonstrated that collagen infiltration was not evident in the hearts of clenbuterol-treated rats that were subjected to swim training, indicating that the exercise performance (despite being of a lower capacity than for the untreated rats) was still sufficient to confer protection from the deleterious effects of clenbuterol on the heart. The clenbuterol-induced increase in cardiac mass was not offset by any of the training regimes, and this deleterious adaptation is likely to have contributed to the overall decrease in exercise training performance.

We conclude that chronic treatment with high doses of clenbuterol significantly affects the exercise capabilities of rats. Since clenbuterol is used by athletes [5], these deleterious adaptations need to be investigated further, specifically whether chronic clenbuterol administration adversely affects cardiac muscle mechanics.

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