

Chronic administration of therapeutic levels of clenbuterol acts as a repartitioning agent

CHARLES F. KEARNS,¹ KENNETH H. MCKEEVER,¹ KARYN MALINOWSKI,¹
MAGGIE B. STRUCK,¹ AND TAKASHI ABE²

¹Department of Animal Sciences, Rutgers, The State University of New Jersey,
New Brunswick, New Jersey 08901; and ²Department of Exercise and Sport Science,
Tokyo Metropolitan University, Tokyo 192-0397, Japan

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Kearns, Charles F., Kenneth H. McKeever, Karyn Malinowski, Maggie B. Struck, and Takashi Abe. Chronic administration of therapeutic levels of clenbuterol acts as a repartitioning agent. *J Appl Physiol* 91: 2064–2070, 2001.—The purpose of this study was to examine the effect of therapeutic levels of clenbuterol, with and without exercise training, on body composition. Twenty-three unfit Standardbred mares were divided into four experimental groups: clenbuterol (2.4 $\mu\text{g}/\text{kg}$ body wt twice daily) plus exercise (ClenEx; 20 min at 50% maximal oxygen consumption 3days/wk; $n = 6$), clenbuterol only (Clen; $n = 6$), exercise only (Ex; $n = 5$), and control (Con; $n = 6$). Rump fat thickness was measured at 2-wk intervals by using B-mode ultrasound, and percent body fat (%fat) was calculated by using previously published methods. For Ex, body fat decreased ($P < 0.05$) at week 4 (–9.3%), %fat at week 6 (–6.9%), and fat-free mass (FFM) increased ($P < 0.05$) at week 8 (+3.2%). On the other hand, Clen had significant changes in %fat (–15.4%), fat mass (–14.7%), and FFM (+4.3%) at week 2. ClenEx had significant decreases in %fat (–17.6%) and fat mass (–19.5%) at week 2, which was similar to Clen; however, this group had a different FFM response, which significantly increased (+4.4%) at week 6. Con showed no changes ($P > 0.05$) in any variable at any time. These results suggest that exercise training and clenbuterol have additive effects with respect to %fat and fat mass but antagonistic effects in terms of FFM. Furthermore, chronic clenbuterol administration causes significant repartitioning in the horse, even when administered in therapeutic doses.

ultrasound; equine; β_2 -agonist

CLENBUTEROL, A β_2 -AGONIST, is a potent selective bronchodilator that initially was used as a drug to treat bronchospasm and to alleviate the symptoms of chronic obstructive pulmonary disease (COPD) in the horse (38). Several investigators have studied the effect of short-term (either acute or 5.5 days) clenbuterol treatment on various cardiorespiratory functions in a variety of horse breeds (15, 36, 37, 40). Clenbuterol has also been shown to improve clinical signs of bronchitis and pneumonia (38) and to increase mucociliary transport rate in both normal horses and horses with COPD (18,

19). But clenbuterol has not been shown to cause any major cardiorespiratory or lactate effects in healthy horses during submaximal exercise (15). Recently, investigators looked at incremental doses (0.8–3.2 $\mu\text{g}/\text{kg}$ body wt twice daily) of clenbuterol in 239 heaves-affected horses (9). Seventy-five percent of the horses demonstrated clinical improvement in their COPD symptoms. It was on the basis of this study that the Federal Drug Administration approved clenbuterol for the management of horses with airway obstructions.

Interestingly, chronic (1–8 wk) clenbuterol administration has also been shown to elicit a muscle-directed protein anabolic response in a variety of species when used in higher than therapeutic doses (5, 6, 22, 24, 34). Specifically, clenbuterol has been used as an agent to increase muscle mass, body weight, and muscle protein synthesis rate (22). This repartitioning of nutrients to alter body composition has been shown to have a profound effect on the production of meat animals. Lambs fed clenbuterol demonstrated improved feed conversion, reduced fat deposition, and increased muscle deposition (1). Similar improvements in muscle accretion and fat reduction were seen in both steers (34) and broilers fed clenbuterol (6).

Given the fact that clenbuterol may be prescribed for extended periods to alleviate bronchospasm or COPD in horses (9) and that chronic use of clenbuterol alters body composition in other species (5, 6, 22, 24, 34), it is reasonable to predict that clenbuterol may act as a repartitioning agent in the horse. Horses using clenbuterol may still be physically active or training while being treated with clenbuterol. Unfortunately, clenbuterol has been shown to be antagonistic to exercise capacity (7, 14, 43). Furthermore, there are no data examining the interaction of exercise training and clenbuterol with regard to body composition in any species, including humans and horses (35). Clenbuterol is frequently abused by human athletes, especially females, because its repartitioning effects are not associated with the androgenic side effects of steroids (31). Therefore, the purpose of this study was to test

Address for reprint requests and other correspondence: K. H. McKeever, Equine Exercise Physiology Laboratory, Dept. of Animal Sciences, 84 Lipman Drive, New Brunswick, NJ 08901-8525 (E-mail: mckeever@aesop.rutgers.edu).

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the hypothesis that 8 wk of clenbuterol, administered at therapeutic levels, with and without exercise training, would produce repartitioning effects in horses.

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 pasture. Each mare was fed ~ 6 kg/day of alfalfa and grass hay (Semican, Pessiville, Quebec) and ~ 3 kg/day of a commercially available grain ration (Browns & Sons; 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. To assess weight stability before the start of the study, each mare was weighed every morning for 3 mo. The average coefficient of variation in each mare's body weight over that time period was $<1\%$.

Horses were divided into four experimental groups. Clenbuterol plus exercise (ClenEx; $n = 6$) and clenbuterol only groups (Clen; $n = 6$) were orally administered $2.4 \mu\text{g}$ clenbuterol (Boehringer Ingelheim) per kilogram body weight twice daily as a syrup (for an average volume of 20 ml) on a schedule of 5 days on and 2 days off for the duration of the study. In addition to being given the drug, ClenEx was also trained aerobically for 3 days/wk. Five horses were assigned to the aerobic exercise training group (Ex) and aerobically trained for 3 days/wk. Another group of horses served as the sedentary, nondrug-treated control group (Con; $n = 6$). Both Ex and Con were administered volumes of molasses similar to the volume of clenbuterol twice daily on a 5 day on and 2 day off schedule.

Training program. The exercise program consisted of continuous treadmill running 3 days/wk for 8 wk. Horses initially ran for 15 min/day at a work rate of 50% maximal oxygen consumption ($\dot{V}O_{2 \text{ max}}$; determined before the study). After 1 wk, the duration of run time was increased to 20 min/day and was held at this duration for the entirety of the study. During exercise training, the high-speed horse treadmill (Sato I, Equine Dynamics, Lexington, KY) was set at a fixed 6% grade.

Body composition measurement. Rump fat thickness was measured by using B-mode ultrasonography (Aloka SSD-500, Tokyo, Japan) and was used to calculate percent body fat (%fat). The site was determined by placing the probe over the rump at ~ 5 cm lateral from the midline at the center of the pelvic bone (44). The region was scanned, and the position of maximal fat thickness was used as the measured site (Fig. 1). The calculated average coefficient of variation based on six animals for this rump fat thickness determination was $3.6 \pm 0.7\%$. Percent fat was estimated from the equations of Kane et al. (16)

$$\% \text{fat} = 2.47 + 5.47 [\text{rump fat (in cm)}]$$

Fat mass was determined by multiplying %fat and total body mass. Fat-free mass (FFM) was derived by subtracting fat mass from total body mass.

Rump fat was measured before the start and then every 2 wk until the completion of the study. Therefore, rump fat was measured at 0 (pretreatment), 2, 4, 6, and 8 wk of clenbuterol treatment to determine the time course of repartitioning.

Body condition score. Body condition score was assessed by using the method of Hennek et al. (12), with individual scores recorded for the neck, withers, shoulders, ribs, loin, and



Fig. 1. Ultrasonic images representing rump fat thickness in healthy, untrained Standardbred horses.

tailhead. Body condition scores were assessed at the beginning of the experiment before clenbuterol or exercise treatments and again during the posttreatment testing period. The condition scores for all body areas for each individual horse were averaged to calculate a mean condition score.

Statistical analysis. Results are expressed as means \pm SE. For comparison by group and time, a two-way ANOVA with repeated measures was used with the a priori level of statistical significance set at $P < 0.05$. Post hoc differences were determined with Tukey's test.

RESULTS

%Fat and fat mass. There were no differences ($P > 0.05$) in %fat or fat mass between groups during pretreatment testing. Both exercise training and chronic clenbuterol treatment resulted in significant reductions in %fat and fat mass (Figs. 2, A–D, and 3, A–D, respectively). At week 2, there was a ($P < 0.001$) reduction in %fat and fat mass for ClenEx and Clen. Fat mass continued to decrease in ClenEx, but this decrease was not significant. Ex demonstrated a significant ($P < 0.05$) decrease in %fat at week 4 and in fat mass at week 6. There were no changes in %fat or fat mass in Con.

FFM. There were no group differences ($P > 0.05$) in FFM during pretreatment testing. Both exercise training and chronic clenbuterol treatment resulted in significant increases in FFM (Fig. 4, A–D). There was an increase ($P < 0.01$) in FFM in ClenEx at week 6. FFM was increased ($P < 0.01$) in Clen at week 2 and further increased ($P < 0.05$) at week 8. There was an increase ($P < 0.05$) in FFM in Ex at week 8, but there were no changes in FFM in Con.

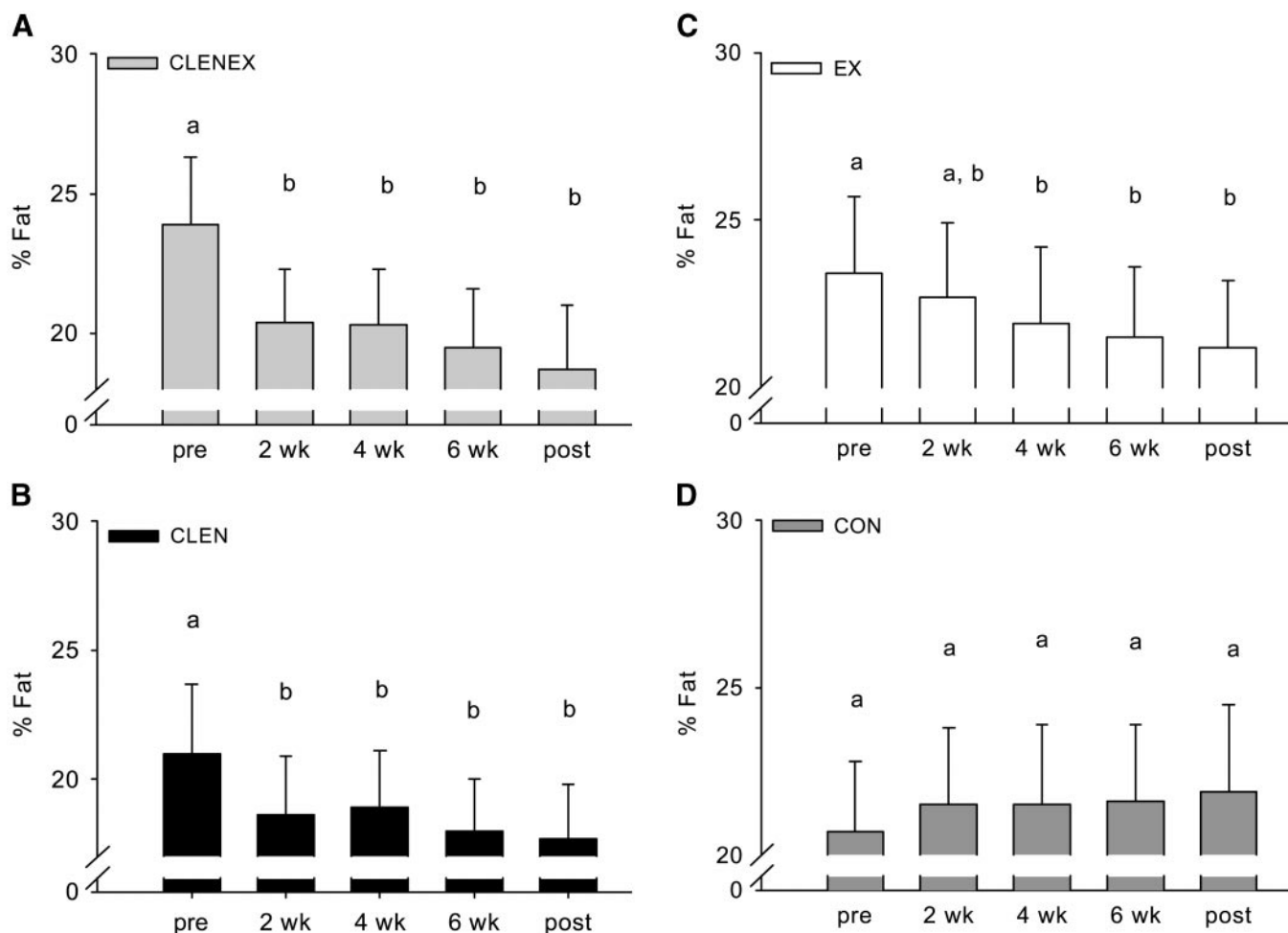


Fig. 2. Changes in percent body fat (%fat) over time in clenbuterol and exercise (ClenEx; A), clenbuterol only (Clen; B), exercise only (Ex; C), and control (Con; D) groups. Means with different letters (a and b) are significantly different.

Body condition score. Average body condition scores did not differ ($P > 0.05$) between groups during pretreatment testing. Chronic clenbuterol treatment, with or without exercise, did not result in any differences ($P > 0.05$) for average body condition score or any body condition score observed at any site of measurements (Table 1).

DISCUSSION

Repartitioning effects of clenbuterol. The unique finding in this paper was that even mild therapeutic doses of clenbuterol (2.4 $\mu\text{g}/\text{kg}$ body wt twice daily) produced dramatic repartitioning effects in the Standardbred mare. Clenbuterol is typically administered in doses between 0.8–3.2 $\mu\text{g}/\text{kg}$ twice daily (9) to alleviate bronchospasm in horses (18, 19, 38). The results of the present study are more dramatic than those reported in previous studies in which much higher doses of clenbuterol, typically in the hundreds of microgram per kilogram or milligram per kilogram range, were needed to produce repartitioning in other species (22–24, 34). Thus horses appear to be more sensitive to the effects of clenbuterol. Such a species-related increase in sensitivity to sympathomimetic drugs has been documented previously (26).

Although this was first paper to report body condition scoring in Standardbred horses (a major racing breed), there were no significant changes in body condition scores for any group, suggesting that the method of body condition scoring (12) may not be sensitive enough to detect quantifiable changes within the various body compartments.

Chronic clenbuterol administration resulted in significant repartitioning effects in the horse after only 2 wk. The time course of this change is in line with previous studies in other species (22, 23, 24, 33) that demonstrated clenbuterol-induced changes in carcass composition after 2 wk of administration. After 2 wk of treatment, there was a significant reduction in %fat and fat mass in both the drug treatment (with and without exercise) groups, with 20- and 15-kg reductions in ClenEx and Clen, respectively. However, neither drug treatment group demonstrated any further significant reductions in %fat or fat mass for the duration of the study. This rapid repartitioning and subsequent lack of change after 2 wk are consistent with receptor downregulation seen with chronic clenbuterol administration in other species (32, 39).

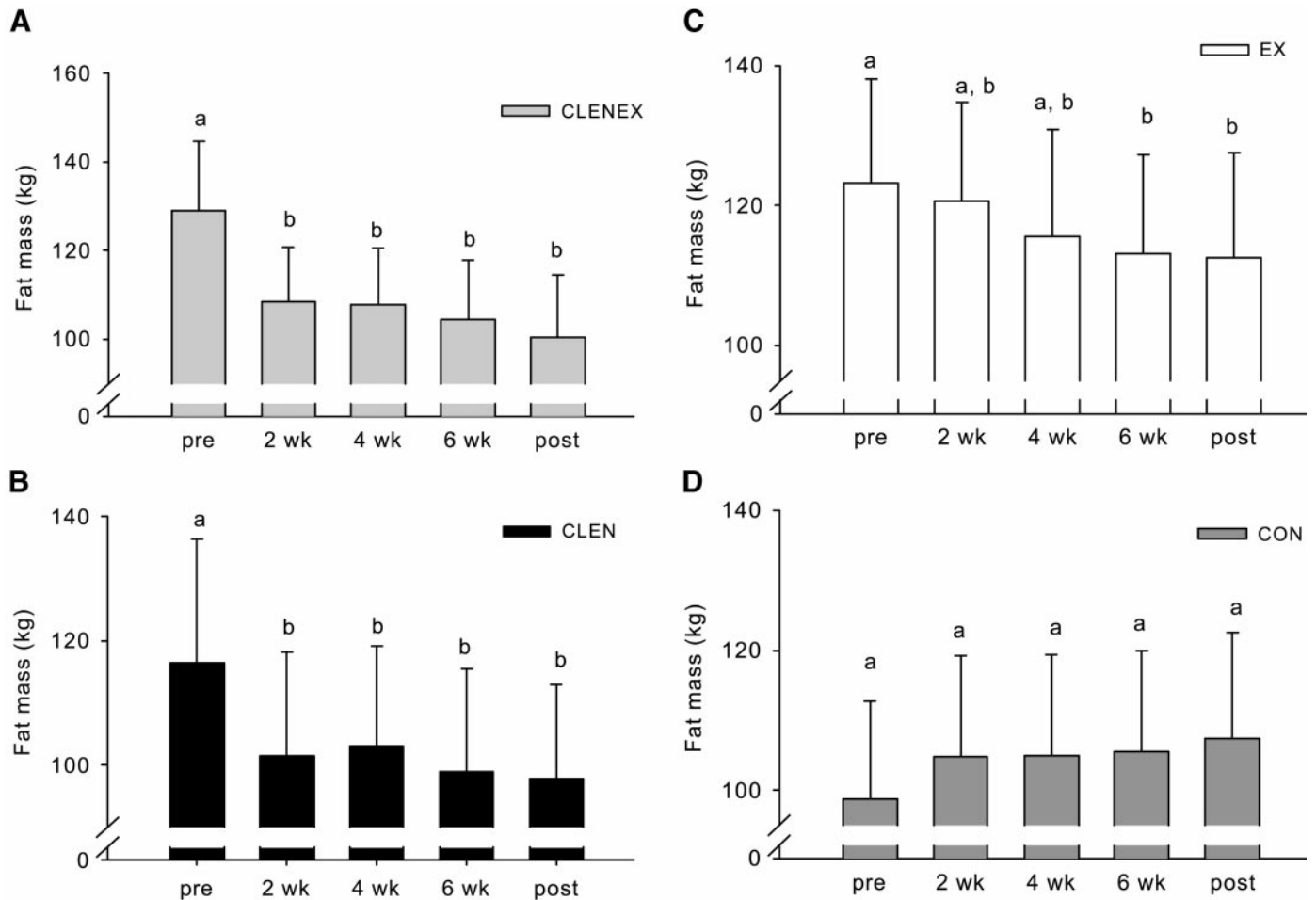


Fig. 3. Changes in fat mass over time in ClenEx (A), Clen (B), Ex (C), and Con (D). Means with different letters (a and b) are significantly different.

FFM also increased dramatically in both drug treatment groups; however, the time course for the change was different for each of these groups. A major observation of the current study was that it took 6 wk for FFM to increase in ClenEx but only 2 wk in Clen. Additionally, Clen demonstrated a further significant increase at week 6. Increases in FFM were equal in magnitude to the respective fat mass reductions in both drug treatment groups, thereby demonstrating a true repartitioning effect (i.e., no change in body weight was seen in the present study). Clenbuterol has been shown to reduce the growth rate of several visceral organs, including the liver and kidney (33). Although the horses of this study were fully matured and not growing, clenbuterol has not been shown to increase organ or bone weight in any species studied to date. Therefore, we would suggest that the increases in FFM seen in the present study were a result of an increased muscle mass and not organ weight.

There appears to be an antagonistic interaction between aerobic exercise training and clenbuterol administration with respect to FFM. In contrast, there appears to be an additive interaction with respect to fat mass. ClenEx showed a time course for the reduction of %fat and fat mass (2 wk) that was similar to the time

course seen in Clen; however, ClenEx took an additional 4 wk before demonstrating a significant increase in FFM. These data suggest that exercise training may impede the anabolic properties of clenbuterol. The reasons for these interactions are not clear as the exact mechanism(s) of clenbuterol's action on muscle tissue has yet to be elucidated. Similar interactions between clenbuterol and training have been seen with regard to exercise capacity, in which exercise and clenbuterol also had antagonistic responses in rats (7, 14, 43).

Potential mechanism(s) of clenbuterol-induced repartitioning. The effects of clenbuterol on muscle are likely the result of stimulation of the β_2 receptors (20, 46). Stimulation of β -receptors has a marked effect on many muscle metabolic pathways, most notably regulation of cAMP levels and Na^+ and K^+ transport (4, 42). The activation of cAMP phosphorylates hormone-sensitive lipase, which in turn causes a large increase in adipocyte lypolysis (13, 41). However, clenbuterol has been shown to decrease β -adrenergic receptor (AR) density (32, 39), whereas aerobic training has been shown to increase β -AR (28, 45). Thus it might be speculated that clenbuterol decreases fat mass through β -AR stimulation while simultaneously causing β -AR downregulation. This may explain why, in the horses of

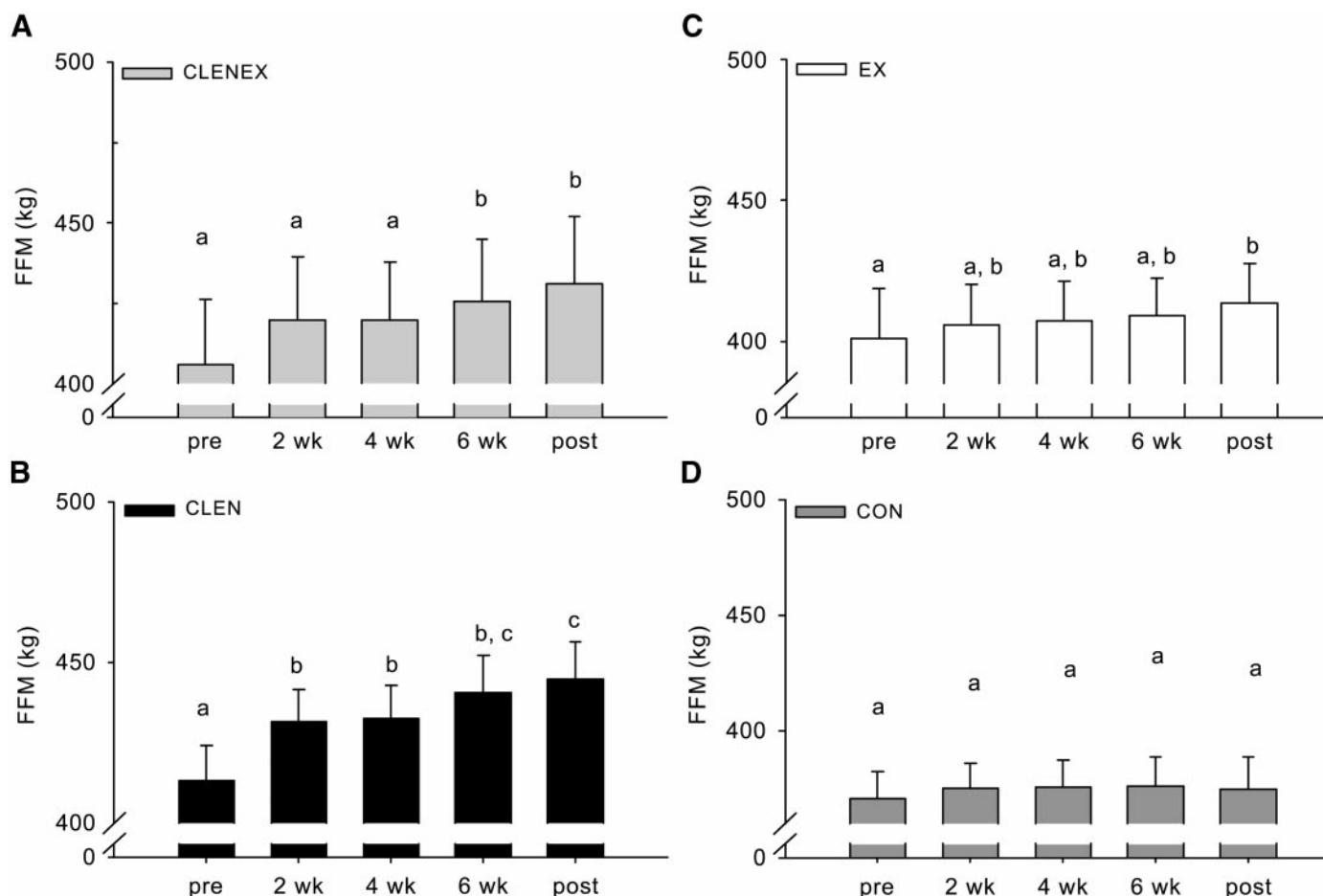


Fig. 4. Changes in fat free mass (FFM) over time in ClenEx (A), Clen (B), Ex (C), and Con (D). Means with different letters (a–c) are significantly different.

the present study, fat mass rapidly decreased by *week* 2 without further reductions. On the basis of studies of other species (28, 45), exercise training would be expected to act to maintain β -AR density, and this would allow for continued fat mass loss. Interestingly, the exercise program in the present study may not have been of a sufficient intensity to overcome the pharmacological-induced β -AR downregulation imposed by clenbuterol. This may explain why fat mass continued to decrease (but not significantly) after 2 wk in ClenEx.

The reasons for the further increase in FFM despite no changes in %fat or fat mass are unclear. It might be

speculated that clenbuterol increases FFM through another nonreceptor-mediated pathway. The plasma half-life of clenbuterol after repeated oral doses (5.5 days of 0.8 μ g/kg) was determined to be 10.4 days (15). However, clenbuterol is highly lipophilic and can enter muscle tissue (11). Clenbuterol has been shown to increase passive Ca^{2+} release from the sarcoplasmic reticulum of single fibers (21). A Ca^{2+} /calmodulin-dependent protein kinase has been shown to regulate gene expression (27). Furthermore, this Ca^{2+} -dependent pathway has been linked to skeletal muscle hypertrophy in rats (10). In addition, β -AR-mediated hy-

Table 1. *Body condition scores*

Group	Neck		Withers		Shoulders		Ribs		Loin		Tailhead		Average	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
ClenEx (n = 6)	5.2 ± 0.79	5.5 ± 0.56	5.8 ± 0.75	4.8 ± 0.31	6.2 ± 0.31	5.3 ± 0.21	6.3 ± 0.80	5.8 ± 0.48	6.2 ± 0.40	5.7 ± 0.33	6.7 ± 0.67	6.2 ± 0.56	6.1 ± 0.47	5.6 ± 0.20
Clen (n = 6)	5.2 ± 0.40	5.7 ± 0.49	4.5 ± 0.22	5.0 ± 0.26	5.3 ± 0.48	4.9 ± 0.33	5.8 ± 0.54	6.2 ± 0.54	5.8 ± 0.31	5.8 ± 0.31	5.5 ± 0.56	5.8 ± 0.57	5.4 ± 0.26	5.6 ± 0.33
Ex (n = 5)	6.2 ± 0.58	6.2 ± 0.58	5.3 ± 0.80	5.2 ± 0.66	6.0 ± 0.45	5.5 ± 0.22	6.4 ± 0.93	7.4 ± 0.68	5.6 ± 0.24	5.7 ± 0.30	6.8 ± 0.97	6.3 ± 0.77	6.1 ± 0.59	6.1 ± 0.42
Con (n = 6)	4.8 ± 0.48	5.3 ± 0.21	4.7 ± 0.33	5.4 ± 0.37	5.7 ± 0.56	5.3 ± 0.21	6.8 ± 0.60	6.7 ± 0.49	5.7 ± 0.21	5.7 ± 0.33	5.0 ± 0.00	5.1 ± 0.37	5.5 ± 0.23	5.6 ± 0.16

Values are means \pm SE. Body condition scores for horses in the clenbuterol only (Clen), clenbuterol and exercise (ClenEx), exercise only (Ex), and control (Con) groups before (pre) and after (post) treatment.

hypertrophy in neonatal rat myocardial cells has been linked to sarcoplasmic reticulum Ca^{2+} release, not cAMP-dependent Ca^{2+} (3). Therefore, clenbuterol administration could lead to increased muscle mass via a Ca^{2+} -mediated pathway. However, whether the clenbuterol-mediated increase in muscle is a result of increased protein synthesis (8, 22) or decreased protein degradation (2, 33) is uncertain. Clearly, further research is needed to elucidate the exact mechanism of this phenomenon.

Another interesting finding of the current study was the exercise-induced increase in FFM in the horse. There was a significant increase in FFM of 13 kg in Ex at 8 wk. This is similar to a previous study (44) in horses that demonstrated a 10-kg increase in FFM after aerobic-type polo training. However, in that study, the amount of training the horses completed was not quantified. The reason for the increase in FFM in the present study is unclear but may be attributed to the fact that the horses of the present study were running at a fixed 6% grade and therefore were lifting their 500-kg body mass with each step. However, it is not uncommon to see losses in body fat accompanied by similar gains in FFM during the initial phases of aerobic training in humans (30). The changes in the present study are consistent with endurance training adaptations seen in humans (29). The magnitude of change in %fat in the current study is also very similar to that seen in human studies with comparable training intensities (29). Running at 50% $\dot{V}_{\text{O}_2 \text{max}}$ for 20 min 3 times per week significantly reduced %fat at week 4 and fat mass at week 6. Whether this training intensity reflects a minimum threshold or not is uncertain, and more work is warranted.

In conclusion, clenbuterol has been shown to be a powerful repartition agent in the horse, even when used at mild therapeutic levels (2.4 $\mu\text{g}/\text{kg}$). The time course for change is consistent with those seen in other species. Furthermore, the results of the present study would suggest that exercise training and clenbuterol have additive effects with respect to %fat and fat mass but antagonistic effects in terms of FFM.

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