Effects of \(\beta_2\)-agonist administration and exercise on contractile activation of skeletal muscle fibers

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Lynch, Gordon S., Alan Hayes, Siun P. Campbell, and David A. Williams. Effects of \(\beta_2\)-agonist administration and exercise on contractile activation of skeletal muscle fibers. J. Appl. Physiol. 81(4): 1610–1618, 1996.—Clenbuterol, a \(\beta_2\)-adrenoceptor agonist, has therapeutic potential for the treatment of muscle-wasting diseases, yet its effects, especially at the single-fiber level, have not been fully characterized. Male C57BL/10 mice were allocated to three groups: Control-Treated mice were administered clenbuterol (2 mg·kg\(^{-1}\)·day\(^{-1}\)) via their drinking water for 15 wk; Trained-Treated mice underwent low-intensity training (unweighted swimming, 5 days/wk, 1 h/day) in addition to receiving clenbuterol; and Control mice were sedentary and untreated. Contractile characteristics were determined on membrane-permeabilized fibers from the extensor digitorum longus (EDL) and soleus muscles. Fast fibers from the EDL and soleus muscles of Treated mice exhibited decreases in Ca\(^{2+}\) sensitivity. Endurance exercise offset clenbuterol’s effects, demonstrated by similar Ca\(^{2+}\) sensitivities in the Trained-Treated and Control groups. Long-term clenbuterol treatment did not affect the normalized maximal tension of fast or slow fibers but increased the proportion of fast fibers in the soleus muscle. Training increased the proportion of fibers with high and intermediate succinate dehydrogenase activity in the EDL and soleus muscles, respectively. If clenbuterol is to be used for treating muscle-wasting disorders, some form of low-intensity exercise might be encouraged such that potentially deleterious slow-to-fast fiber type transformations are minimized. Indeed, in the mouse, low-intensity exercise appears to prevent these effects.

纤维类型；肌肉；肌肉收缩；去天线纤维

长期治疗具有 \(\beta_2\)-肾上腺素受体激动剂药物，如 sympathomimetic 胺类化合物 clenbuterol，已被证明可导致肌肉质量在正常肌肉和减少肌肉在 dystrophic (mdx) 小鼠，以及降低肌肉萎缩性与肾上腺素刺激性及运动症候群 (16, 23, 25, 27, 33, 34, 42, 44)。机制作用 action of clenbuterol for this hypertrophy of skeletal muscle is thought to involve increased protein synthesis and/or decreased protein degradation (5, 27, 32). Given its reported effects on skeletal muscle, it is not surprising that several authors have proposed administering clenbuterol to patients suffering muscle-wasting diseases (1, 4, 22, 41, 44). Interestingly, due to its muscle anabolic effects, clenbuterol also widely used in largely circles, particularly among athletes involved in strength- and power-related sports and bodybuilding activities (6, 31).

Previous studies indicate that \(\beta_2\)-adrenoceptor agonists clearly affect fast-twitch muscle fibers, their effect on slow-twitch fibers being less consistent (16). Zeman et al. (42) showed that clenbuterol caused slow- to fast-twitch fiber type conversions within the rat soleus, with accompanying increases in contractile speed. Similarly, Hayes and Williams (12) showed that clenbuterol significantly altered the contractile properties of intact fast- and slow-twitch muscles of the mouse. No studies have yet investigated the long-term effects of this \(\beta_2\)-agonist on skeletal muscle function at the cellular level. Earlier studies have demonstrated direct toxic effects of high dosages of \(\beta_2\)-agonists on cardiac muscle, including myocardial necrosis, ischemia, and arrhythmia (15). Importantly, it has not been determined whether long-term usage of \(\beta_2\)-agonists such as clenbuterol may have deleterious effects on skeletal muscle, especially in large dosages. Considering its reported therapeutic potential and its use in athletic competition (6), it is imperative that the effects of clenbuterol on skeletal and cardiac muscles are fully characterized (31). In this study, we investigated the effect of long-term clenbuterol administration on the contractile function of single skinned fast- and slow-twitch skeletal muscle fibers. Furthermore, we examined whether exercise modified the effects of clenbuterol treatment on skeletal muscle.

MATERIALS AND METHODS

Animals, clenbuterol Administration, and Training

All experiments were approved by the Animal Experimentation Ethics Committee of The University of Melbourne. Male C57BL/10 mice (5 wk of age) were separated into three groups: sedentary untreated (Control; n = 8), sedentary, clenbuterol treated (Control-Treated; n = 9) and endurance trained, clenbuterol treated (Trained-Treated; n = 10). Mice were housed in identical standard cages with no more than four animals per cage. Previous studies have already investigated the effect of endurance training on the activation characteristics of skeletal muscle fibers (19, 20), and, therefore, an experimental group testing only the effect of training on muscle fibers was deemed unnecessary. clenbuterol (2 mg·kg\(^{-1}\)·day\(^{-1}\)) was given in the drinking water every day for the first week. The effectiveness of clenbuterol administration via the drinking water has been well established (27). After week 1, a 2:2:3-day on-off cycle was followed, which has been shown to reduce the attenuation of the clenbuterol response (12). The duration of the clenbuterol treatment was 15 wk. A relatively high dosage of clenbuterol was administered to determine the maximum effects of the \(\beta_2\)-agonist on skeletal muscle and also to mimic the sort of excessive dosages used/abused by athletes in pursuit of maximum muscle development (6). The exercise group (Trained-Treated) underwent a 15-wk program consisting of low-intensity unweighted swimming 1 h/day, 5 days/wk (see Ref. 11 for further details regarding the swimming training protocol). All animals had free access to food (GX2PLUS, Barastoc Stockfeeds, Melbourne, Australia) and water.
Single Fiber Analysis

At 20 wk of age, the mice were killed by cervical dislocation, and the fast-twitch extensor digitorum longus (EDL) muscle and mixed slow- and fast-twitch soleus muscle were dissected tendon to tendon from one hindlimb. The muscles were tied to capillary tubes and placed in a skinnning solution [with the following composition (in mM): 125 K-propionate, 5 ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA), 2 ATP, 2 MgCl2, 20 imidazole, and 50% (vol/vol) glycerol, adjusted to pH 7.1 with 4 M KOH] and stored at −20°C for up to 6 mo until required. The contralateral muscles were frozen in isopentane cooled in liquid nitrogen and stored at −80°C for later histochemical analysis to determine fiber type proportions of the whole muscle.

On the day of an experiment, a muscle was cut from its ties on the capillary tubes and pinned to the bottom of a small Sylgard-based (Dow Corning, Midland, MI) petri dish filled with skinnning solution. The muscle bundle was carefully teased with fine forceps to obtain smaller bundles. From these smaller bundles, single chemically skinned fibers were randomly selected and then attached to a sensitive force transducer (AM801, SensonOr, Horten, Norway). The fibers were activated by Ca2+- and Sr2+-buffered solutions with techniques commonly employed in this laboratory (19, 20). At the end of the experiment, the muscle bundles were discarded. Fiber length and diameter were directly measured by means of a calibrated reticle in the eyepiece of a dissecting microscope (Nikon). Details regarding the determination of sarcomere length, which was set at 2.7–2.8 µm for all fibers, were activated by Ca2+- and Sr2+-activating solutions (9). fiber sensitivity to Ca2+- and Sr2+ [half-maximal tension (pCa50 and pSr50, respectively)]; fiber sensitivity to Ca2+- and Sr2+ (pCa50 - pSr50); and the relative steepness of the sigmoidal force-pCa -pSr) curves given by the associated Hill coefficients nCa and nSr, representing the number n in the Hill equation Pn = K[Xn] (1 + K[Xn]p), which provided the closest fit to the experimental points and which reflects the degree of cooperativity in the activation of tension. K is a constant associated with the [Ca2+] or [Sr2+] required for pCa50 or pSr50 by using the equation log10 K = nCa*pCa50 (nSr*pSr50), and [Xn] represents the [Ca2+] or [Sr2+]. A series of theoretical curves derived from the Hill equation for n values changing by 0.1 unit was used to fit the data points (18, 20). The maximal force response was taken as the first contractile response after activation in a maximal Ca2+-activated force (of the second Ca2+-activation sequence) to be within 10% of the initial maximal force level.

Fiber Type Classification

On the basis of their relative force-pCa and force-pSr data, individual fibers were classified as fast or slow twitch by using the criteria detailed previously (see Refs. 18, 19, 40). To verify the physiological fiber type classification, individual fiber segments that had undergone contractile activation and for which complete force-pCa (-pSr) characteristics had been determined were also stored for sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis analysis. The procedure for separation of the myosin heavy chain (MHC) isoforms within single muscle fiber segments was slightly modified from that described by Talmadge and Roy (37) for homogenized rat skeletal muscles. Briefly, fiber segments were stored individually in small centrifuge tubes with a small volume (15 µl) of sample buffer [62.5 mM tris(hydroxymethyl)aminomethane (Tris), pH 6.6, 1% SDS, 0.01% bromophenol blue, 15% glycerol, and 5% β-mercaptoethanol] at −20°C for up to 4 wk. The separating gels were composed of 35% glycerol, 10% acrylamide-N,N' -methylene-bis-acrylamide (25:1), 0.375 M Tris (pH 8.8), and 0.4% SDS. The stacking gels were composed of 30% glycerol, 4% acrylamide-N,N'-methylene-bis-acrylamide (25:1), 0.5 M Tris (pH 6.7), 4 mM EDTA, and 0.4% SDS. The gel constituents were prepared from stock solutions, and polymerization was initiated with 0.2% N,N,N',N'-tetramethylethylenediamine and 1.25% ammonium persulfate. The gels were run on a Mighty Small II mini-gel electrophoresis unit (Hoefer Scientific Instruments, San Francisco, CA) and stained with Coomassie blue.
TABLE 1. Comparison of muscle and body mass measurements among groups

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Control-Treated</th>
<th>Trained-Treated</th>
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<tbody>
<tr>
<td></td>
<td>(n = 8)</td>
<td>(n = 9)</td>
<td>(n = 10)</td>
</tr>
<tr>
<td>BM, g</td>
<td>30.4 ± 0.5</td>
<td>30.5 ± 0.6</td>
<td>28.7 ± 0.5</td>
</tr>
<tr>
<td>EDL, mg</td>
<td>15.2 ± 0.3</td>
<td>16.6 ± 0.6</td>
<td>15.2 ± 0.4</td>
</tr>
<tr>
<td>EDL/BM, ×10⁻³</td>
<td>0.50 ± 0.01</td>
<td>0.55 ± 0.02</td>
<td>0.53 ± 0.01</td>
</tr>
<tr>
<td>Soleus, mg</td>
<td>13.7 ± 0.6</td>
<td>16.0 ± 0.7*</td>
<td>14.3 ± 0.5</td>
</tr>
<tr>
<td>Soleus/BM, ×10⁻³</td>
<td>0.45 ± 0.01</td>
<td>0.54 ± 0.02†</td>
<td>0.50 ± 0.01</td>
</tr>
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</table>

Values are means ± SE; n, no. of animals. Control, sedentary untreated; Control-Treated, sedentary clenbuterol treated; Trained-Treated, endurance trained, clenbuterol treated; BM, body mass; EDL, extensor digitorum longus. Significant difference between Control and Control-Treated groups: *P < 0.05; †P < 0.01.

RESULTS

Effect of Clenbuterol on Muscle and Body Mass

The effect of clenbuterol treatment on skeletal muscle and body mass is presented in Table 1. After the 15 wk of treatment, body mass was not different among groups. Muscle mass (absolute and relative to body mass) was significantly greater in the soleus but not in the EDL of the Control-Treated animals compared with the other groups. Interestingly, in another fast-twitch muscle, the plantaris, muscle mass was significantly increased both in absolute and relative terms in the Control-Treated animals compared with the other groups (data not shown).

Effect of Clenbuterol and Exercise on Single-Fiber Contractile Characteristics

EDL. All chemically skinned fibers from the EDL exhibited similar contractile characteristics and were not able to be clearly distinguished as either EDL population I (type IIA) or EDL population II (type IIB) as has been described previously for fresh mechanically skinned fibers (20) or of fibers liberated from partial enzyme digestion of whole muscles (19). Hence all EDL fibers were designated as fast twitch, a classification we have also used previously when describing mouse EDL fibers (40). Gel electrophoresis did not always fully separate the bands corresponding to the type IIA and type IIB varieties but nevertheless verified these fibers as being composed of fast MHC. Typical force-pCa (-pSr) curves for these fast-twitch fibers have been presented elsewhere (see Refs. 10, 20) and are not repeated here.

The Control-Treated fibers showed an increased threshold for contraction by and a decreased sensitivity to Ca²⁺ when compared with fibers from Control animals (see Table 2). However, fibers from the Trained-Treated mice exhibited similar values for the threshold for contraction and Ca²⁺ sensitivity to those of the Control group animals. The force-pCa relationships of fibers of the Control-Treated group were significantly enhanced.
Table 2. Effect of clenbuterol and exercise on contractile characteristics of fast-twitch fibers from EDL muscle

<table>
<thead>
<tr>
<th></th>
<th>Control-Treated (n = 18)</th>
<th>Trained-Treated (n = 31)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>pCa_{50.1}</strong></td>
<td>6.14 ± 0.04</td>
<td>6.03 ± 0.03*</td>
</tr>
<tr>
<td><strong>pCa_{50.2}</strong></td>
<td>5.83 ± 0.04</td>
<td>5.67 ± 0.04*</td>
</tr>
<tr>
<td><strong>n_{Ca}</strong></td>
<td>3.36 ± 0.14</td>
<td>3.20 ± 0.20*</td>
</tr>
<tr>
<td><strong>P_{so_{50.1}}</strong></td>
<td>4.83 ± 0.05</td>
<td>4.77 ± 0.04*</td>
</tr>
<tr>
<td><strong>P_{so_{50.2}}</strong></td>
<td>4.53 ± 0.04</td>
<td>4.53 ± 0.03*</td>
</tr>
<tr>
<td><strong>P_{so_{50.3}}</strong></td>
<td>3.41 ± 0.14</td>
<td>2.94 ± 0.10*</td>
</tr>
<tr>
<td><strong>P_{so_{50.4}}</strong></td>
<td>1.29 ± 0.02</td>
<td>1.23 ± 0.02</td>
</tr>
<tr>
<td><strong>P_{so_{50.5}}</strong></td>
<td>15.50 ± 3.57</td>
<td>16.91 ± 2.66</td>
</tr>
<tr>
<td><strong>Fiber diameter, µm</strong></td>
<td>42.95 ± 3.96</td>
<td>44.28 ± 2.95</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of fibers sampled. Fibers were sampled from 5 Control, 6 Control-Treated, and 6 Trained-Treated muscles. pCa_{50.1} and pSr_{50.1}, 10% relative force level for Ca and Sr, respectively; pCa_{50.2} and pSr_{50.2}, half-maximal tension for Ca and Sr; pCa_{50.3} and pSr_{50.3}, Hill coefficients for Ca and Sr, respectively; pCa_{50.4} – pSr_{50.4}, relative sensitivity of a fiber to activating ions; P_{so_{50}}, maximal force response. Significant difference (P < 0.05) between: *Control and Control-Treated; †Control-Treated and Trained-Treated; ‡Control and Control-Treated; ††Control-Treated and Trained-Treated.

Table 3. Effect of clenbuterol and exercise on contractile characteristics of slow- and fast-twitch fibers from soleus muscle

<table>
<thead>
<tr>
<th></th>
<th>Slow Twitch</th>
<th>Fast Twitch</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Control (n = 12)</td>
<td>Control-Treated (n = 7)</td>
</tr>
<tr>
<td><strong>pCa_{10.1}</strong></td>
<td>6.56 ± 0.03</td>
<td>6.52 ± 0.04</td>
</tr>
<tr>
<td><strong>pCa_{50.1}</strong></td>
<td>6.07 ± 0.03</td>
<td>6.05 ± 0.05</td>
</tr>
<tr>
<td><strong>n_{Ca}</strong></td>
<td>1.83 ± 0.05</td>
<td>1.92 ± 0.07</td>
</tr>
<tr>
<td><strong>P_{so_{10.1}}</strong></td>
<td>6.25 ± 0.03</td>
<td>6.17 ± 0.04</td>
</tr>
<tr>
<td><strong>P_{so_{50.1}}</strong></td>
<td>5.72 ± 0.03</td>
<td>5.68 ± 0.04*</td>
</tr>
<tr>
<td><strong>n_{Sr}</strong></td>
<td>1.82 ± 0.05</td>
<td>2.03 ± 0.08</td>
</tr>
<tr>
<td><strong>P_{so_{50.2}}</strong></td>
<td>0.35 ± 0.03</td>
<td>0.37 ± 0.04</td>
</tr>
<tr>
<td><strong>P_{so_{50.3}}</strong></td>
<td>19.07 ± 3.39</td>
<td>14.05 ± 4.80</td>
</tr>
<tr>
<td><strong>Fiber diameter, µm</strong></td>
<td>40.08 ± 3.24</td>
<td>46.75 ± 4.59</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of fibers sampled. Fibers were sampled from 5 Control, 6 Control-Treated, and 6 Trained-Treated muscles. Significant difference (P < 0.05) between: *Control and Control-Treated; †Control-Treated and Trained-Treated.
To expand and clarify the histochemical classification of fiber types, muscle cross sections were also reacted for SDH activity, which separates fibers on the basis of their relative oxidative status. The results are presented in Fig. 3. In the EDL, the Control-Treated group displayed a significant reduction in the proportion of highly oxidative fibers compared with the Control and Trained-Treated groups. Similarly, this group had an increased percentage of fibers with low SDH activity (i.e., low-oxidative status) and a decreased percentage of fibers with intermediate SDH activity compared with the Control group. The Trained-Treated group also exhibited a reduction in the proportion of intermediate oxidative fibers compared with the Control group; however, the percentage of low-oxidative fibers was not different from control levels. For the soleus muscle, the Trained-Treated group displayed a significant reduction in the proportion of highly oxidative fibers and a significant increase in the percentage of intermediate fibers compared with both the Control-Treated and Control groups.

**DISCUSSION**

The most important findings of this study were 1) that long-term treatment with clenbuterol caused a decrease in the sensitivity to Ca$^{2+}$ in fast-twitch fibers from both the EDL and soleus muscles; 2) that low-intensity endurance exercise could prevent the decrease in Ca$^{2+}$-sensitivity resulting from clenbuterol administration; 3) that clenbuterol did not affect maximal tension development of fast- or slow-twitch fibers; and 4) that clenbuterol caused slow- to fast-twitch fiber conversion within the EDL and soleus muscles, which was also offset by exercise training.

In this study, a relatively high dosage of clenbuterol (2 mg·kg$^{-1}$·day$^{-1}$) was administered for two reasons: 1) to determine the maximum effects of the β$_2$-agonist on skeletal muscle fibers and 2) to mimic the high to excessive dosages that are commonly used/abused by strength and bodybuilding athletes in their quest for maximal muscle development (6). Much lower doses, on the order of 1 µg/kg, have been proposed for the treatment of muscle-wasting diseases in humans (22). However, there have been numerous reports in the literature concerning the undesirable effects of long-
Long-term clenbuterol treatment caused the force-pCa relationship of fast-twitch fibers from both the EDL and soleus to be right shifted, resulting in an increased threshold for contraction by Ca\(^{2+}\) and decreased fiber sensitivity to Ca\(^{2+}\). No change was observed in the slow-twitch fibers from the soleus in response to clenbuterol treatment. The decreased sensitivity to Ca\(^{2+}\) exhibited by the clenbuterol-treated (Control-Treated) fibers is most likely mediated by elevation of the cytosolic adenosine 3',5'-cyclic monophosphate (cAMP) concentration and the resultant activation of protein kinases, which is the most commonly proposed mechanism of clenbuterol on skeletal muscle (17, 25, 41). It may be expected that chronic clenbuterol administration would maintain high levels of cAMP within skeletal muscle fibers, although this was not directly measured in this study. It has previously been demonstrated in skinned cardiac muscle that such elevations of cAMP had a direct desensitizing effect on the contractile proteins, causing a decreased sensitivity to Ca\(^{2+}\), as reflected by a rightward shift of the force-pCa relationship (13). This decrease in Ca\(^{2+}\) sensitivity was accompanied by an increase in phosphorylation of troponin-I by cAMP-dependent protein kinase, adding to the decreased responsiveness of the myofilaments to Ca\(^{2+}\) (13). Thus chronic administration of clenbuterol may decrease fiber sensitivity to Ca\(^{2+}\) by altering contractile and regulatory protein interactions, with a resultant alteration in the cooperative interactions between functional regulatory mechanisms. Direct measurements of the effect of clenbuterol on resting intracellular [Ca\(^{2+}\)], Ca\(^{2+}\) transients, and the release and reuptake of Ca\(^{2+}\) by the SR would help clarify these issues.

Further evidence of clenbuterol modifying the contractile characteristics of single muscle fibers was evident in the EDL muscle, where the steepness of the force-pCa and force-pSr relationships of single fibers was significantly reduced in the clenbuterol-treated (Control-Treated and Trained-Treated) groups compared with the Control group. Such decreases in the steepness of the force-pCa relationship indicate a reduction in the cooperative interactions between functional regulatory groups within the thin filament in the activation process (2, 30).

Previous investigations into the effect of clenbuterol on the contractility of skeletal muscle have been at the whole muscle level. These studies have shown that clenbuterol (in doses similar to that used in the present study) decreased the time course of the isometric twitch response (12, 43). Clenbuterol has also been shown to cause significant muscle hypertrophy due to an increase in protein synthesis and possibly due to a decrease in protein degradation (16, 32). Indeed, in this study, clenbuterol caused a significant increase in the mass of the soleus but not of the EDL muscle. Swim training offset this clenbuterol-induced increase in muscle mass (see Table 1).
With intact in vitro muscle preparations, addition of sympathomimetic amines to the bathing solution has been shown to increase force production by a cAMP-dependent phosphorylation of Ca\(^{2+}\)-release channels to facilitate SR Ca\(^{2+}\) release during tetanic stimulation (3). However, in this study, muscle force assessed by maximal Ca\(^{2+}\) activation of skinned muscle fiber preparations showed that chronic clenbuterol administration did not affect force production at the single-fiber level. Although the diameters of the single muscle fibers sampled were also not different among groups (see Tables 2 and 3), the fact that fibers swell after skimming indicates that these measurements should be used with caution in the assessment of whether clenbuterol caused fiber hypertrophy. It is possible that the lack of a clenbuterol-induced increase in single-fiber diameter and force production was due to the fact that the increases in overall mass after treatment were too small to reach significance. For example, in the soleus, muscle mass was 17% higher in the Control-Treated compared with the Control group. However, the large variability in diameter of the single fibers sampled from that muscle would be likely to obscure differences in force production at the cellular level after long-term clenbuterol treatment. The increase in maximal isometric force production observed in intact muscle after long-term clenbuterol treatment (12, 43) can most likely be explained by the concomitant increases in overall muscle mass and muscle cross-sectional area.

Effect of Clenbuterol on Muscle Fiber Type Proportions

Clenbuterol has previously been shown to affect the relative fiber type distribution within skeletal muscle, causing type I to type II fiber conversions and even alterations within the type II fiber population (21, 29). Similarly, in this study, clenbuterol caused an increase in the percentage of fast-twitch type II fibers within the soleus. In the EDL, clenbuterol also appeared to modify the type II fiber population. By using the metachromatic dye-ATPase method, it was found that in the EDL of the Control, and even the Trained-Treated, group there were fibers that reacted as type I according to the criteria described by Ogilvie and Feeback (28). However, within the EDL of the Control-Treated group, no such fibers were identified, suggesting that clenbuterol-induced alterations had occurred within this fiber pool. To supplement the fiber type classification by mATPase histochemistry, muscle cross sections were also reacted for relative SDH activity. Generally, SDH activity will only give an estimate of muscle fiber proportions but is useful for detecting exercise and/or drug treatment effects on the oxidative properties of muscle. The results further supported the fiber type classifications obtained from mATPase histochemistry. In the EDL, the Control-Treated animals displayed a reduction in the number of fibers with high and intermediate levels of SDH activity and an increase in the number of fibers with low SDH activity. The clenbuterol-treated animals that were also subjected to the low-intensity swimming program exhibited a reduction in the percentage of intermediate-oxidative fibers but showed no change in the proportion of fibers of low-oxidative status. The percentage of fibers with high levels of SDH activity was similar to that found in the EDL of the Control group. This illustrates the effectiveness of the low-intensity exercise program in preventing the clenbuterol-induced shift in fiber type proportions within the EDL.

On the basis of mATPase activity, the proportion of type I and type IIA fibers within the soleus was not significantly different between the Trained-Treated and Control groups, indicating that training offset or prevented the clenbuterol-induced slow-to-fast fiber transformation exhibited in the soleus muscles of the Control-Treated group. Interestingly, we found a decrease in the percentage of fibers with high SDH activity and an increase in the percentage of fibers with intermediate levels of SDH activity within the soleus muscle of the Trained-Treated group compared with both the Control-Treated and Control groups, indicating an overall decrease in oxidative capacity. In a recent study (38), after 7 wk of clenbuterol administration in Zucker rats, citrate synthase activity was decreased in the red and white gastrocnemius but increased in the soleus muscle. Although the histochemical results of the present study cannot be directly correlated with the data of Torgan et al. (38), it is clear that clenbuterol affects enzyme activity differently in fast- and slow-twitch muscles. The reduction in the number of fibers within the soleus muscle exhibiting high levels of SDH activity after clenbuterol and training is interesting, considering that muscle oxidative status is often enhanced after endurance training (38). However, in a previous study, Hayes et al. (11) have shown that the fiber proportions (based on SDH activity) within the mouse soleus were not altered after long-term (15 wk) 2 h/day, 5 days/wk, high-intensity (weighted) swimming training. Therefore, we would expect that the changes in SDH activity observed in the fibers of the soleus muscle of the Trained-Treated group are probably caused by the combined effects of clenbuterol treatment and the swimming activity and not simply an effect of training alone. Although the low-intensity training was sufficient in offsetting the decrease in Ca\(^{2+}\) sensitivity in the sampled fibers, the histochemical data (SDH activity) indicate that this training did not completely prevent the clenbuterol-induced shift in fiber proportions. A comparison of the relative fiber proportions within the soleus based on the number of randomly sampled fibers (and separated into fiber types by their physiological characteristics) supports this notion. In both clenbuterol-treated groups (Control-Treated and Trained-Treated), a greater percentage of fast-twitch fibers were sampled (75 and 74%, respectively) compared with 60% from the Control group.

This study has shown that long-term clenbuterol treatment in mice caused a decrease in the sensitivity to Ca\(^{2+}\) of fast-twitch fibers within the EDL and soleus muscles. Muscle fibers from animals that received chronic clenbuterol treatment and also underwent an endurance training program did not exhibit this decrease in Ca\(^{2+}\) sensitivity. Clenbuterol does cause in-
creases in muscle mass, a desirable effect for athletes involved in sports requiring a larger muscle bulk, and its effects on the skeletal muscle contractile properties might also appear to be conducive to improvements in specific muscle performance, e.g., faster rates of contraction and relaxation. Interestingly, however, the effects on single muscle fiber contractility in isolation appear to be less desirable, as demonstrated by the decrease in Ca\(^{2+}\) sensitivity; thus higher [Ca\(^{2+}\)] values are required to achieve activation. The large doses of mass-building drugs taken by these athletes may also be life-threatening, considering the evidence for myocardial necrosis and decreased cardiac performance after chronic high-dose \(\beta_2\)-agonist therapy (15). Indeed, in other related experiments, Duncan and colleagues (7, 8) have observed a decrease in the exercise performance of chronic clenbuterol-treated rats during 2-h swimming sessions and short-term high-intensity treadmill-running bouts that appear to be attributed to decreased cardiac performance. In this study, 1 h of low-intensity unweighted swimming was comfortably managed by the clenbuterol-treated mice, and no attempt was made to subject the clenbuterol-treated mice to more intense training. It should also be stressed that the therapeutic dosage of clenbuterol would be considerably smaller than that employed in this study, on the order of 1 \(\mu\)g/kg, as proposed for the treatment of muscle-wasting diseases in humans (22). If, because of its powerful anabolic properties, clenbuterol is to be used as a therapeutic agent in the treatment of muscle-wasting disorders, it might also be suggested that some form of low-intensity exercise be encouraged such that the existing muscle mass be maintained but the potentially deleterious slow-to-fast fiber type transformations be minimized. It has been established that in muscular dystrophy the large type II fibers are more susceptible to necrosis than the smaller caliber type I fibers (14, 39). Although a clenbuterol-induced increase or maintenance of existing muscle mass would be desirable for the dystrophic condition, the slow-to-fast fiber transformations would be an unwanted side effect because the potential for further necrosis might be increased. In mice, the performance of even low-intensity endurance exercise (e.g., unweighted swimming) appears to prevent these deleterious effects.

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