Long-term effects of clenbuterol on diaphragm morphology and contractile properties in emphysematous hamsters


Long-term effects of clenbuterol on diaphragm morphology and contractile properties in emphysematous hamsters. J. Appl. Physiol. 85(1): 215–222, 1998.—The aim of the present study was to investigate the effect of chronic long-term clenbuterol treatment (1 mg/kg subcutaneously twice a day for 12 wk) on diaphragm morphology and function in emphysematous (EH) and normal hamsters (NH). Clenbuterol increased body weight, diaphragm weight, and skeletal muscle weight in both EH and NH to a similar extent. In the diaphragm, clenbuterol significantly increased myosin heavy chain type I, IIa, and IIx muscle fiber cross-sectional areas by ~35–55% in both EH and NH. This response to clenbuterol treatment was not significantly different between EH and NH diaphragm. In EH, twitch force (Pt), maximal tetanic force, and force-frequency curve were significantly reduced compared with NH. In EH, clenbuterol increased Pt by ~10%, restoring Pt to NH level. A similar improvement was observed in the force-frequency characteristics. Clenbuterol did not alter contractile properties in NH. In conclusion, long-term clenbuterol treatment resulted in an increased size of all diaphragm muscle fiber types in both EH and NH. Clenbuterol completely abolished the reduced force generation induced by emphysema.

contractile properties; β-adrenergic agonists; respiratory muscles; myosin heavy chains; emphysema

DYSFUNCTION of the respiratory muscles frequently occurs in patients with severe chronic obstructive pulmonary disease (COPD) (20). Those COPD patients with emphysema in particular are also often malnourished and underweight and have a reduced fat-free body mass, which may indicate muscle wasting (6). This condition is associated with a decrease in skeletal and respiratory muscle function (6) and with an increase in morbidity and mortality (32). Besides dietary supplementation and training, pharmacological interventions such as anabolic steroids, growth hormone therapy, or clenbuterol treatment may be considered to increase respiratory muscle mass and function.

Previous research in healthy animals has indicated that diaphragm contractile properties can be improved pharmacologically by administering β2-adrenoceptor agonists (1, 19, 29, 30). Within this group of β2-adrenoceptor agonists, clenbuterol has a special position because it is known to increase muscle cell growth and protein content both in vitro (16) and in vivo (5, 23). This effect was mediated by activation of the β2-adrenoceptor (3, 26) and was accompanied by increased cAMP levels (17).

In contrast to the peripheral skeletal muscles, little is known about the effect of clenbuterol on function and morphology of respiratory muscles. A single dose of clenbuterol (10 and 20 µg/l iv) increased transdiaphragmatic pressure (Pdi) in dogs in a dose-dependent fashion (19). Preliminary studies reported that administration of clenbuterol resulted in hypertrophy of both type I and type II muscle fibers in normal rat diaphragm after 2 and 10 wk of treatment (10, 22) or of type II muscle fibers after 2 wk of treatment (25). Functionally, however, these morphological changes were accompanied by either a reduction in force (22), or no effects on force generation and fatigue resistance (25), or a better preservation of Pdi after inspiratory resistive loading (10).

Whether clenbuterol can induce morphological and/or functional changes in the diaphragm of emphysematous animals is unknown. Induction of emphysema by intratracheal instillation of elastase in hamsters resembles the alterations found in patients with COPD to some extent. In the hamsters, lung volume and compliance were increased and diaphragm function was impaired (7, 13). We hypothesized that clenbuterol treatment will increase diaphragm muscle mass and cross-sectional area (CSA) and that this may (partly) restore the impairment of diaphragm function found in emphysema.

The aim of the present study was to investigate the effects of chronic clenbuterol treatment on diaphragm morphology in the elastase-induced emphysematous hamster (EH) model and to investigate whether these morphological changes are accompanied by improvement of contractile properties. We also wanted to investigate whether these effects are different from those found in normal, age-matched hamsters (NH). Because the actual presence of clenbuterol in the diaphragm will have a direct positive inotropic effect on diaphragm contractile properties, measurements of contractile properties were performed at least 24 h after the last dose of clenbuterol had been given.

METHODS

Animals, Induction of Emphysema, and Study Design

Adult male outbred golden hamsters (n = 80) were studied. At the age of ~40 wk, the hamsters were anesthetized with a mixture of halothane and N2O, vaporized in air. A polyethylene cannula was inserted into the trachea with the tip located above the carina, and a dose of 25 U/100 g body wt of elastase (type 1 elastase from porcine pancreas, Sigma Chemical, Bornem, Belgium) was injected intratracheally to induce emphysema (7, 13, 28). Control animals were treated with an equal volume of saline (0.5 ml/100 g body wt). To improve the distribution to the peripheral parts of the lung, 3 ml of room
air were injected through the tube. The hamsters were monitored carefully until spontaneous breathing was restored.

The animals were housed under standard conditions and were fed ad libitum. Six months after instillation of elastase or saline, the animals were randomly allocated to treatment groups. Thirty EH and 30 age- and weight-matched NH were treated for 12 wk with either clenbuterol (1 mg/kg subcutaneously twice a day) or saline control. Body weight was measured every 2 wk. Because clenbuterol may have a direct inotropic effect on the diaphragm (19), this could interfere with the assessment of the contractile properties in relation to morphological changes in the diaphragm. There are no data on the exact serum half-life of clenbuterol in hamsters. In other species this pharmacokinetic parameter has been reported to range from 2 to 48 h (27). Incubation with these MHC antibodies was performed at 4°C for 2 h, and subsequently postfixation lung volume was inflated with 4% Formalin (pH 7.4) to a pressure of 25 cm H2O and fixed in 4% Formalin for at least 5 days.

Verification of Emphysema and Total Diaphragm Weight

In a separate experiment the presence and severity of emphysema was evaluated in 20 animals (NH + saline: n = 5; NH + clenbuterol: n = 5; EH + saline: n = 5; EH + clenbuterol: n = 5). After the hamsters were anesthetized as described in General Procedure and Treatment, the lungs were excised and inflated with 4% Formalin (pH 7.4) to a pressure of 25 cm H2O for 2 h, and subsequently postfixation lung volume was determined by fluid displacement. The lungs were fixed without external pressure in 4% Formalin for at least 5 days.
Subsequently, the left lung was embedded in paraffin, and sagittal sections (6-µm thickness) were cut and stained with hematoxylin-eosin. To determine the extent of emphysema changes in the lung, alveolar CSA of at least 200 alveoli was measured by using a Snyrty-based, personal computer-image digital analysis system (Bos). In addition, in this experiment the complete diaphragm was dissected, blotted dry, and weighed to determine the effects of clenbuterol treatment on diaphragm weight. These measurements were performed in separate groups of animals to avoid procedural bias like introducing extra time needed for the careful excision of the lungs and to prevent surgical or handling damage of the diaphragm needed for force measurements.

Data Analysis

Statistical analysis was performed by using a two-way ANOVA. The two experimental factors were disease state (presence or absence of emphysema) and treatment (clenbuterol or saline). In comparing the force-frequency relationship and body weight curves, a repeated-measurements design was used within this two-way ANOVA design. Post hoc analysis (Student-Newman-Keuls test) was used to compare differences in independent groups. The SPSS package (version 6.1.3, Chicago, IL) was used for statistical analysis. Results were considered significant at P < 0.05. All data are expressed as means ± SE.

RESULTS

Verification of Emphysema

Postfixation lung volume, determined by fluid displacement, was increased by ~50%. In saline-treated animals, lung volume increased from 7.8 ± 0.4 ml in NH to 11.6 ± 0.7 ml in EH (P < 0.05). In clenbuterol-treated animals, these values were 7.9 ± 0.5 and 11.9 ± 0.8 ml, respectively. The alveolar CSA was also significantly increased in saline-treated EH (5,147 ± 335 µm² in EH vs. 2,698 ± 91 µm² in NH; P < 0.05). Clenbuterol treatment did not alter the increase in alveolar CSA in EH (5,209 ± 350 µm² in EH + clenbuterol and 2,735 ± 106 µm² in NH + clenbuterol (P < 0.05)).

Effects of Clenbuterol Treatment on Body and Muscle Weight

Body weight and skeletal muscle weight were not significantly different at the start of the protocol. Clenbuterol treatment increased hamster body weight in both EH and NH significantly (P < 0.05; Table 1). After 12 wk of clenbuterol treatment, body weight was increased by ~9 and 12% in EH and NH, respectively. This response to clenbuterol treatment was not significantly different between EH and NH. In Fig. 1 the relative increase in body weight is presented; in both NH and EH clenbuterol treatment significantly increased relative body weight (P < 0.001, repeated-measurements ANOVA).

Soleus and EDL muscle weights were also significantly increased by clenbuterol treatment (P < 0.05; Table 1). When expressed as a percentage of body weight also, these muscle weights were significantly increased. Similar effects were found on diaphragm muscle weight (Table 1). No differences were found between the absence or presence of emphysema, and no interaction was found between the presence of emphysema and treatment.

Diaphragm Muscle Morphology (Fig. 2, A–C, Table 2)

Emphysema did not significantly alter muscle fiber type CSA, frequency, or relative contribution to total diaphragm CSA (percent CSA) of any muscle fiber type in the hamster diaphragm (Table 2, Fig. 2). Although type I, IIa, and IIx muscle fiber CSA tended to be smaller in EH diaphragm, the factor emphysema was not significant for any of the fiber types. In addition, no interaction was found between the factors emphysema

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**Table 1. Anabolic effects of clenbuterol treatment on hamster body and peripheral skeletal muscle weight**

<table>
<thead>
<tr>
<th></th>
<th>Emphysematous Hamsters</th>
<th>Normal Hamsters</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control (n = 15)</td>
<td>Clenbuterol (n = 15)</td>
</tr>
<tr>
<td>Body weight (start), g</td>
<td>148 ± 2.7</td>
<td>149 ± 3.7</td>
</tr>
<tr>
<td>Body weight (end), g</td>
<td>149 ± 2.7</td>
<td>164 ± 3.7*</td>
</tr>
<tr>
<td>Body weight increase, %initial</td>
<td>0.2 ± 0.7</td>
<td>8.7 ± 1.6*</td>
</tr>
<tr>
<td>Soleus weight, mg</td>
<td>40.1 ± 0.9</td>
<td>48.9 ± 1.4*</td>
</tr>
<tr>
<td>%body wt</td>
<td>0.27 ± 0.00</td>
<td>0.30 ± 0.01*</td>
</tr>
<tr>
<td>EDL weight, mg</td>
<td>33.3 ± 0.8</td>
<td>45.1 ± 0.9*</td>
</tr>
<tr>
<td>%body wt</td>
<td>0.22 ± 0.00</td>
<td>0.27 ± 0.01*</td>
</tr>
<tr>
<td>Diaphragm weight, %body wt</td>
<td>2.06 ± 0.04</td>
<td>2.50 ± 0.05*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of hamsters. Diaphragm weight was assessed in a separate experiment (n = 5). EDL, extensor digitorum longus. *Significant increases as compared with saline treatment, P < 0.05.

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![Fig. 1. Percentage of increase in body weight during long-term treatment.](http://jap.physiology.org/)
Clenbuterol treatment significantly increased CSA of types I and IIa in both EH and NH and IIx muscle fiber CSA in EH (Fig. 2, A–C). No differences in response to clenbuterol treatment were found between NH and EH.

Clenbuterol treatment slightly altered the frequency of muscle fiber types (Table 2). Significant effects of clenbuterol treatment on fiber frequency were found in type IIa and type IIx muscle fibers. The relative contribution of each fiber type to the total CSA (percent CSA) was not altered by clenbuterol treatment compared with saline treatment (Table 2).

Finally, type IIb muscle fibers were very scarcely encountered in the diaphragms of these hamsters. These type IIb fibers were only observed in clenbuterol-treated animals. In 1 of 30 control-treated animals, type IIb fibers were found (in an EH diaphragm). After 12 wk of clenbuterol treatment, type IIb fibers were found in 5 of 15 NH and 4 of 15 EH diaphragm muscles. This may indicate that clenbuterol treatment appears to increase the expression of type IIb MHC. However, calculation of a mean CSA or fiber frequency from such a limited number of fibers does not result in reliable data. Therefore, we have not included these data in our analysis.

**Contractile Properties**

Twitch characteristics and $P_o$. Emphysema significantly reduced $P_t$, $P_o$, and the measured $L_o$ of the diaphragm strips compared with NH ($P < 0.05$; Table 3). No interactions were found between emphysema and treatment for any parameter of diaphragm function.

**Table 2. Effects of clenbuterol treatment on diaphragm muscle fiber frequency and contribution to total diaphragm CSA**

<table>
<thead>
<tr>
<th></th>
<th>Emphysematous Hamsters</th>
<th>Normal Hamsters</th>
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<tbody>
<tr>
<td></td>
<td>Control (n = 12)</td>
<td>Clenbuterol (n = 10)</td>
</tr>
<tr>
<td></td>
<td>Clenbuterol (n = 10)</td>
<td>Control (n = 12)</td>
</tr>
<tr>
<td></td>
<td>Normal Hamsters</td>
<td>Clenbuterol (n = 10)</td>
</tr>
<tr>
<td>% Type I fiber</td>
<td>27.8 ± 1.2</td>
<td>26.2 ± 0.9</td>
</tr>
<tr>
<td>% Type IIa fiber</td>
<td>43.6 ± 1.7</td>
<td>46.0 ± 1.5</td>
</tr>
<tr>
<td>% Type IIx fiber</td>
<td>28.5 ± 1.8</td>
<td>27.8 ± 1.7</td>
</tr>
<tr>
<td>% CSA Type I fiber</td>
<td>19.3 ± 1.0</td>
<td>19.1 ± 0.9</td>
</tr>
<tr>
<td>% CSA Type IIa fiber</td>
<td>34.6 ± 2.0</td>
<td>35.6 ± 1.9</td>
</tr>
<tr>
<td>% CSA Type IIx fiber</td>
<td>46.0 ± 2.0</td>
<td>45.3 ± 2.5</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of hamsters. CSA, cross-sectional area. *Significant increases compared with saline treatment, $P < 0.05$.

**Table 3. Effects of clenbuterol treatment on diaphragm contractile properties**

<table>
<thead>
<tr>
<th></th>
<th>Emphysematous Hamsters</th>
<th>Normal Hamsters</th>
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<tbody>
<tr>
<td></td>
<td>Control (n = 15)</td>
<td>Clenbuterol (n = 15)</td>
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<tr>
<td></td>
<td>Clenbuterol (n = 15)</td>
<td>Control (n = 15)</td>
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<tr>
<td></td>
<td>Clenbuterol (n = 15)</td>
<td></td>
</tr>
<tr>
<td>$P_t$, N/cm²</td>
<td>6.4 ± 0.25</td>
<td>7.0 ± 0.2</td>
</tr>
<tr>
<td>$P_o$, N/cm²</td>
<td>26.9 ± 0.65</td>
<td>29.9 ± 0.7</td>
</tr>
<tr>
<td>$P_t/P_o$ ratio, %</td>
<td>24.0 ± 0.6</td>
<td>24.0 ± 0.8</td>
</tr>
<tr>
<td>CT, ms</td>
<td>26.8 ± 0.3</td>
<td>26.6 ± 0.3</td>
</tr>
<tr>
<td>RT$_{1/2}$, ms</td>
<td>26.7 ± 0.6</td>
<td>27.4 ± 0.7</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of hamsters. $P_t$, maximal twitch force; $P_o$, maximal tetanic force; CT, contraction time; RT$_{1/2}$, half-relaxation time. *Significant increases compared with saline treatment, $P < 0.05$. §Significantly different from all other groups, $P < 0.05$. 

Fig. 2. Diaphragm muscle fiber cross-sectional area. Open bars, saline-treated hamsters; solid bars, clenbuterol-treated hamsters. A: cross-sectional area of diaphragm muscle fibers expressing myosin heavy chain type I (or slow). B and C: type IIa and IIx muscle fiber cross-sectional area, respectively. *Significant increases compared with saline treatment, $P < 0.05$. 

and treatment for any fiber type or morphological parameter.
tion. $P_t$ and $P_o$ were $\sim 10\%$ lower in the saline-treated EH compared with NH. In these EH, clenbuterol increased $P_t$ by $\sim 10\%$, restoring $P_t$ to the level in the saline-treated NH. In NH, $P_t$ was not affected by clenbuterol. CT was not altered by clenbuterol treatment in either NH or EH. $RT_{1/2}$ was decreased in both NH and EH ($P < 0.05$; Table 3). Again, long-term clenbuterol treatment increased $P_o$ by $\sim 7\%$ in these EH, restoring force production to a level similar to saline-treated NH. In NH, $P_t$ was not affected by clenbuterol. CT was not altered by clenbuterol treatment in either NH or EH.

**Force-frequency relationship.** The force-frequency relationship was significantly reduced in EH compared with NH ($P < 0.05$, Fig. 3A). Clenbuterol treatment significantly increased the force-frequency relationship in EH, resulting in an upward shift of the force-frequency curve (Fig. 3A). However, a significant interaction was found between treatment and repeated measurements of absolute force. As a consequence, significant increases in force-frequency relationship were found for stimulation frequencies up to 80 Hz but not for higher stimulation frequencies. In EH, clenbuterol restored force production at these lower frequencies to the level similar to the force-generating capacity of NH diaphragm (EH treated with clenbuterol compared with saline-treated EH; $P < 0.05$). In contrast, clenbuterol treatment had no significant effects on the NH force-frequency relationship compared with saline treatment (Fig. 3A).

When the results found in this force-frequency protocol were expressed as a percentage of $P_o$, no differences were found between EH and NH (Fig. 3B). Clenbuterol treatment did not alter these relative force-frequency curves in EH. However, a significant interaction was again found between treatment and repeated force measurements ($P < 0.001$). Compared with saline treatment, clenbuterol caused a small but significant decrease in NH at stimulation frequencies of 80 and 120 Hz but not at the lower frequencies ($P < 0.05$; Fig. 3B).

**Isometric fatigue.** Fatigue index was not affected by either emphysema or clenbuterol treatment, and no interaction was found between these factors. In saline-treated animals, the fatigue index after 2 min was $58 \pm 1\%$ in NH and $59 \pm 1\%$ in EH. After clenbuterol these values were $61 \pm 2\%$ in NH and $59 \pm 2\%$ in EH. Also, after 5 min the fatigue index was not altered, with values ranging from 38 to 42%.

**DISCUSSION**

This study shows that long-term clenbuterol treatment increased body weight, diaphragm weight, and peripheral skeletal muscle weight in both NH and EH. Clenbuterol treatment resulted in hypertrophy of the MHC type I, IIa and IIx diaphragm muscle fibers in EH and of type I and IIa fibers in NH diaphragm. In EH, this hypertrophy was accompanied by an increase in $P_t$, $P_o$, and force-frequency relationship, restoring force in these animals to the level found in NH. In contrast, long-term clenbuterol treatment had no effect on functional properties in NH diaphragm. Fatigue index was not altered in either EH or NH.

**Anabolic Effects of Clenbuterol**

In line with the anabolic potency of clenbuterol described in previous studies (3–5, 23, 34), we found a significant increase in hamster body weight, fast- and slow-twitch peripheral skeletal muscle weight (EDL and soleus muscle), and diaphragm fiber CSA. Skeletal muscle weight was increased after 12 wk of clenbuterol treatment, also when expressed as percentage of body weight, which may indicate a specific effect on muscle growth. After $>3$ wk of treatment, the clenbuterol-mediated increase in body weight seemed to attenuate (Fig. 1). In previous studies a similar attenuation of clenbuterol effect on body weight gain in young rats was found within 14 days of treatment (15). This
appeared to depend on the continuity of clenbuterol treatment (15) and may be explained by a reduction of β₂-adrenoceptor density found after continued exposure to clenbuterol (26).

The anabolic effects of clenbuterol are mediated via β₂-adrenoceptor activation with subsequent cAMP response (17). The precise mechanism of action of the clenbuterol-mediated growth-stimulating effect is not clear but appears not to be mediated by growth hormone or thyroid stimulation (5) or by elevated insulin levels (5). Several studies reported that increased muscle growth was accompanied by an increase in protein and RNA content and increased protein synthesis (indicated by an increased RNA-to-protein ratio) (3, 5). A reduction in protein degradation was suggested in another study (23).

Similar muscle-growth potentiating effects were found for salmeterol, another long-acting β₂-agonist (17). However, these effects were less potent than the clenbuterol effects and depended on the route of administration (17). Because the anabolic effects of short-acting β₂-adrenoceptor agonists like salbutamol, fenoterol or terbutaline were less potent (2, 3, 5, 24), it is likely that a long duration of action is needed to induce these anabolic effects.

Effects of Clenbuterol on Diaphragm Morphology and Function

The increase in specific force production found after long-term clenbuterol treatment in EH may, at least partly, be explained by the changes in diaphragm morphology. In EH the percentage of type IIx muscle fibers was increased, and CSA of every fiber type was increased in both EH and NH. This increase in type IIx muscle fiber CSA may influence force production because these muscle fibers generate the largest forces and represent the major part (~50%) of the total diaphragm CSA. The reduction in RT₁/₂ observed in the present study may be a reflection of the increase in type IIx muscle fiber CSA, because type IIx muscle fibers not only produce the largest forces but also have short CT and RT₁/₂. A reduction of RT₁/₂ is likely to have contributed to the changes in the shape of the force-frequency curve.

Besides these morphological alterations, other mechanisms may be involved in the clenbuterol-mediated improvements of P₁ and P₀. These may include alterations in biochemical processes involved in energy supply and consumption, changes in excitation-contraction coupling-related processes [including alterations in Ca²⁺ release from the sarcoplasmatic reticulum (SR)], or altered Ca²⁺ sensitivity (14). For example, the observation that clenbuterol-treated NH failed to reach 100% of P₁ during the force-frequency protocol (Fig. 3B) is in agreement with earlier observations from studies that used salbutamol in vitro (29, 30). This may be the result of a progressive depletion of the SR Ca²⁺ pool or may indicate that β-agonists have a predominant effect on type II muscle fibers (29, 30). It is possible that the activity of these processes was altered by chronic clenbuterol treatment, which may have influenced force generation in the present study.

Comparison of Effects of Clenbuterol on Peripheral Skeletal Muscle and Diaphragm

Because the diaphragm is a mixed skeletal muscle that is constantly active, it is difficult to compare our results with those found in peripheral skeletal muscles. Moreover, the diaphragm has a specific population of β-adrenoceptors, consisting solely of the β₂-subtype (9), whereas hindlimb muscles contain a mixture of both β₁- (15%) and β₂-adrenoceptors (85%) (26). Nevertheless, effects similar to the present study, were found in young rats after long-term clenbuterol administration (1.6 mg·kg⁻¹·day⁻¹ per os for 2–12 wk) (34). In this study, clenbuterol increased type I and II muscle fiber CSA in fast-twitch rat EDL (34) and type II or type IIx fibers in soleus muscle (4, 34). This was accompanied by an increase in P₁ and P₀ in soleus muscle and an increase in P₁ in EDL (34).

The results of the present study are in agreement with the effects of clenbuterol on respiratory muscle morphology reported in two preliminary studies (22, 25). However, these morphological changes were accompanied by a reduction in force production and fatigue resistance in female rats after long-term treatment (10 wk) (22). No effects on force generation and fatigue resistance were found in male rats after 10 wk of treatment (22) or in male and female rats after 2 wk of clenbuterol treatment (25). Also, in rabbit diaphragm, a 2-wk clenbuterol treatment induced hypertrophy of type I, IIa, and IIb muscle fibers without altering the relative contribution to total CSA (10). Again, this hypertrophy was not accompanied by an increase in Pdi in fresh diaphragm, but, after inspiratory resistive loading, tetanic Pdi was better preserved (10).

These morphological findings in diaphragm and skeletal muscles are in agreement with the results found in the present study. However, it is important to note that the aim of the present study was to measure functional effects of clenbuterol treatment on diaphragm morphology and not the direct inotropic effects of clenbuterol. The discrepancies between the clenbuterol effects on contractile properties and fatigue resistance described in these studies (10, 22, 25) and the results found in skeletal muscles (34) can very well be influenced by the actual presence of clenbuterol during the assessment of these contractile properties, because clenbuterol has a clear inotropic effect on diaphragm function (19).

Methodological Considerations

The effects of emphysema on diaphragm force generation are not exactly clear. Previous studies by Farkas and Roussos (7, 8), Kelsen et al. (11), and Supinski and Kelsen (28) did not show a reduction in specific force in emphysematous diaphragm. In contrast, Lewis et al. (12, 13) reported a reduction in specific force of ~25% in emphysematous hamsters, compared with control-treated animals. In these studies, emphysema was induced by instillation of elastase at 7–9 wk of age.
and function. Long-term clinical studies are needed to evaluate whether clenbuterol improves respiratory muscle function in patients with COPD.

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