Low dose formoterol administration improves muscle function in dystrophic mdx mice without increasing fatigue

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Abstract

The β2-adrenoceptor agonist (β2-agonist), formoterol, has been shown to cause muscle hypertrophy in rats even when administered at the micromolar dose of 25 μg/kg/day. We investigated whether a similar low dose of formoterol could improve muscle function in the dystrophic mdx mouse. Ten-week-old male mdx and wild-type (C57BL/10) mice were administered formoterol (25 μg/kg/day, i.p.) for 4 weeks. Formoterol treatment increased extensor digitorum longus (EDL) and soleus muscle mass, increased median muscle fibre size in diaphragm, EDL, and soleus muscles, and increased maximum force producing capacity in skeletal muscles of both wild-type and mdx mice. In contrast to other studies where β2-agonists have been administered to mice and rats, generally at higher doses, low dose formoterol treatment did not increase the fatiguability of EDL, soleus or diaphragm muscles. Although others have found formoterol can decrease ubiquitin mRNA and proteasome activity when administered to tumour bearing rats at high doses (2 mg/kg/day), in the present study low dose formoterol treatment did not alter ubiquitin or the E1 and E3 ubiquitin ligases in diaphragm muscles of wild-type or mdx mice, but it did reduce the level of ubiquitinated proteins in diaphragm of wild-type mice. The findings indicate that formoterol has considerably more powerful anabolic effects on skeletal muscle than older generation β2-agonists (like clenbuterol and albuterol), and has considerable therapeutic potential for muscular dystrophies and other neuromuscular disorders where muscle wasting is indicated.

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1. Introduction

Although a cure for the muscular dystrophies eventually looks likely to be derived from gene therapy, problems continue to plague this research, including: the limitation and longevity of expression from injection sites, the need for systemic delivery, vector design and carrying capacity, and difficulties with immunosuppression [1]. Although considerable progress has been achieved with gene therapies for neuromuscular disorders [2], there remains a profound need for alternative therapeutic strategies that can improve muscle mass and strength, ameliorate the dystrophic pathology, and enhance patient quality of life. A successful pharmacological approach may enable patients to survive and thus take advantage of gene therapies when they eventually become available [3–5].

Although traditionally used as bronchodilators for the treatment of asthma, β2-adrenoceptor agonists (β2-agonists) also have powerful anabolic effects on skeletal muscle [6]. Not surprisingly, there have been numerous studies that have focused on therapeutic applications of the anabolic properties of β2-agonists, for ameliorating muscle wasting and improving muscle function, in disorders such as muscular dystrophy [7]. Several clinical trials have investigated the potential of the β2-agonist, albuterol, to improve skeletal muscle
function in different neuromuscular disorders. Preliminary, trials using albuterol to treat young boys with facioscapulohumeral dystrophy, found that year-long administration at doses of 16 and 32 mg/day had only limited beneficial effects on strength, and was associated with some adverse cardiac related events [8]. Fowler and colleagues [9] administered albuterol at a lower dose of 8 mg/day for 28 weeks to boys with Duchenne or Becker muscular dystrophy, and found modest increases in strength with no side effects [9]. These results suggested that albuterol was well tolerated, but elicited only modest improvements in skeletal muscle mass and strength. Thus, a more efficacious β2-agonist may be required where there is severe wasting and weakness.

Recently, the β2-agonist, formoterol, was shown to elicit significant skeletal muscle hypertrophy in rats when administered daily for four weeks even at very low (micromolar) doses [10]. Formoterol is described as a “new generation” β2-agonist and characterized by its relatively high lipophilicity, a property conferred by a long carbon side chain [11], its high affinity for the β2-adrenoceptor, and consequently its long duration of action [12]. The duration of formoterol’s action is determined principally by its physicochemical interactions with membrane lipid bilayers (the “plasmalemma diffusion microkinetic model”), rather than putative distinct exosite/excceptor binding sites in or near the β2-adrenoceptor [13]. Previous studies on β2-agonist administration in the mdx mouse, an animal model of Duchenne muscular dystrophy (DMD) have used high (millimolar) doses of older generation β2-agonists (e.g. clenbuterol and albuterol) and although they have produced significant muscle hypertrophy [14–16], they have been generally associated with a decrease in muscle fatigue resistance [17]. Since boys with DMD have poor muscle endurance and their respiratory muscles are susceptible to fatigue [18,19], an intervention that exacerbates this problem is not desirable [20]. For neuromuscular disorders, especially those associated with muscle wasting, anabolic agents such as formoterol may have therapeutic potential if low dose treatment can improve muscle function without affecting muscle fatigue resistance deleteriously.

The aims of this study were to determine whether a low, clinically relevant dose of formoterol could improve the functional and morphological properties of skeletal muscles from mdx dystrophic mice, and to determine whether treatment was associated with changes in muscle fatigability.

2. Methods

2.1. Animals

All procedures were approved by the Animal Experimentation Ethics Committee of The University of Melbourne and conformed to the guidelines for the care and use of experimental animals described by the National Health and Medical Research Council of Australia. Eight-week-old male C57BL/10 ScSn (C57BL/10, wild-type) and C57BL/10ScSn-mdx/J dystrophic (mdx) mice obtained from the Animal Resource Centre (Canning Vale, Western Australia) were randomly assigned to either saline control or formoterol treated groups (n = 9–10 mice/group). The mice were housed in the Biological Research Facility at The University of Melbourne and maintained on a 12 h-light/12 h-dark cycle, with standard mouse chow and water provided ad libitum. Treated C57BL/10 and mdx mice received daily i.p. injections of formoterol for 4 weeks (Astra-Zeneca, Molndal, Sweden; 25 µg/kg/day) and control mice received an equivalent volume of sterile saline. This dose of formoterol causes increases in muscle mass in rats, with minimal effects on cardiac mass [10].

2.2. Muscle function

At the completion of the 4-week treatment period, mice were anaesthetised deeply with 100 mg/kg Ketamine and 10 mg/kg Xylazine, with supplemental doses administered as necessary to prevent any response to tactile stimulation. Assessment of isometric contractile properties of EDL, soleus and diaphragm muscles was conducted in vitro as described in detail previously [21,22]. Maximum isometric tetanic force (P0) production was determined from the plateau of a full frequency-force relationship. Since the length and width (and consequently, mass) of individual diaphragm muscle strips can vary between preparations, comparisons of absolute values for peak twitch (Pt) and tetanic force (P0) are not valid, and all data must be normalized with respect to the muscle fibre cross-sectional area for appropriate comparisons of specific force (sP0).

Following determination of in vitro contractile parameters, muscles were subjected to a 4 min repeated stimulation protocol to induce muscle fatigue, as described in detail elsewhere [21,22]. EDL, soleus and diaphragm muscles were stimulated maximally (at the frequency which caused maximal P0) once every 4 s for 4 min, after which time stimulation was stopped and the preparations were rested. Following a rest interval of 5 and 10 min, muscles were again stimulated maximally in order to determine the recovery force after fatigue.

At the completion of functional testing, muscles were trimmed of tendon and any adhering non-muscle tissue, blotted once on filter paper, weighed on an analytical balance, snap frozen in thawing isopentane and stored at −80 °C. Transverse cryosections of EDL, soleus and diaphragm muscles were stored for histological, histochemical and immunohistochemical analyses.
2.3. Morphological properties

Muscle sections were stained with haematoxylin and eosin (H & E) for routine qualitative and quantitative examination of tissue morphology. Coloured digitized images of H & E sections were captured at 200× magnification with a digital camera (Spot, v2.2, Diagnostic Instruments, Sterling Heights, MI) mounted to an upright microscope (BX-51, Olympus, Tokyo, Japan). Median fibre cross-sectional area (CSA) was calculated by measuring the area of ~100 fibres per muscle section. A minimum of 100 muscle fibres from diaphragm muscle strips were analyzed for the proportion of centrally nucleated fibres (a marker of regeneration), and mean diaphragm thickness/width was determined from the centre of each strip.

2.4. Immunohistochemistry

Anti-myogenin antibody was used to quantify the number of myogenin positive nuclei in transverse sections of diaphragm muscles. Myogenin expression is associated with differentiation and was used to determine whether formoterol treatment was associated with altered muscle fibre regeneration [23]. Tissue preparation and immunohistochemical procedures were based upon a previously described protocol [24]. Briefly, sections were air-dried overnight and stored at −80 °C. Sections were fixed in ice-cold acetone for 15 min, washed in phosphate buffered saline (PBS) then placed in 1.5% H2O2 (diluted in PBS) to block endogenous peroxidase activity for 10 min. Sections were blocked with 50% normal sheep serum for 30 min to prevent non-specific binding. Muscle sections were reacted for 1 h with a polyclonal anti-myogenin antibody (#sc-576, Santa Cruz, Biotechnology Inc.). Sections were washed in PBS and then incubated for 30 min with a goat anti-rabbit antibody. Sections were developed using a 3-amino-9-ethyl carbazole (AEC, K3469; Dako, Sydney, Australia) for 2 min. Sections were counterstained with haematoxylin to emphasize muscle architecture. The number of myogenin positive nuclei in each section were counted and expressed as the number of positive nuclei per mm² of muscle.

2.5. Immunoblotting for ubiquitin and ubiquitinated proteins

Crude muscle homogenates were prepared by homogenizing diaphragm muscles 1:10 (wt:vol) in ice-cold buffer, as described previously [24]. Total muscle protein was determined by Bradford assay and an equal amount of protein for each condition was separated by sodium dodecyl sulfate–poly-acrylamide gel electrophoresis (SDS-PAGE) on 4–12% gradient gels. Proteins were transferred to polyvinylidene fluoride (PVDF) membranes and then blocked for 1 h with 5% non-fat milk and 2.5% bovine serum albumin (BSA). Immunoblotting was performed for 1 h at room temperature using a mouse monoclonal antibody (#sc s17, Santa Cruz Biotechnology, Santa Cruz, CA) which detects ubiquitin and ubiquitinated proteins. Samples were washed thoroughly in PBS plus 0.1% Tween and specific proteins were detected with a mouse horseradish peroxidase-conjugated secondary antibody and enhanced chemiluminescence as described previously [24]. Gels were digitized and relative protein levels were determined by scanning densitometry. Values for each condition (n = 4) were expressed as a percentage of the optical density for diaphragm muscles from control mice.

2.6. mRNA analysis of ubiquitin and related ligases

Total RNA from diaphragm muscles was isolated using a commercially available kit according to the manufacturer’s instructions (#74704; Qiagen, Valencia, CA). RNA concentration was determined by UV absorption at 260 nm and samples were stored at −80 °C. Analysis of mRNA levels for ubiquitin and related ligases was performed using gene specific primers. Ubiquitin (Ubd, Accession BC027627) used primers 5'-CCAATGGCG GTTAATGACCTT-3' and 5'-TTTCGATGGGGCTTGG AGGATT-3' resulting in a 141-bp product. Ubiquitin-activating enzyme E1 (Ubelx, Accession BC058630, E1 ligase) used primers 5'-GGGCTTGGGTGTAGAAA TTGC-3' and 5'-CTTCCCGAAGGTAAAACTGGG-3' resulting in a 228-bp product. Ubiquitin-protein ligase E3-alpha (UBR1, Accession AF061556) used primers 5'-CAGGTCTCCTCATTATGTGG-3' and 5'-CA CCTGGTTTCATTAATGTGGC-3'resulting in a 210-bp product. 18S was used as a loading control and the primers were 5'-AACGCCCATACACACTCCAA3' and 5'-CCCTCTAATCAGCCCTCA-3' resulting in a 479-bp product. Semi-quantitative reverse transcriptase (RT)-polymerase chain reaction (PCR) was performed on 100 ng total RNA using a commercially available kit according to manufacturer’s instructions (#74704, Qiagen). A standard RT-PCR protocol was used and consisted of 30 min at 50 °C, 15 min at 95 °C, and repeat cycles of denaturation (94 °C, 45 s), annealing (various temperatures, 45 s) and extension (72 °C, 45 s). The annealing temperatures were 50 °C for ubiquitin and 18S and 52 °C for E1 and E3 ligases. The RT-PCR products were electrophoresed on 2% agarose gels in Tris–acetate, ethylene-diaminetetra-acetic acid buffer and photographed under UV light after staining with ethidium bromide.

2.7. Statistical analyses

All values in the text and tables are reported as means ± SEM unless indicated otherwise. Comparisons of formoterol treated and control C57BL/10 and mdx mice...
were made using a General Linear Model, two factor analysis of variance (ANOVA). Tukey’s posthoc multiple comparison procedure was used to identify differences between groups when significance was detected. Significance was set at \( P < 0.05 \). Data for muscle fibre CSA were first assessed for normality using a Kolmogorov–Smirnov test. Since the data for CSA was not normally distributed, they are presented as median CSA (95% confidence interval). Values were considered different if there was no overlap between the 95% confidence interval of the median.

3. Results

3.1. Morphological properties

Consistent with previous findings, EDL and soleus muscle mass was greater in mdx than C57BL/10 mice (18% and 22% respectively, \( P < 0.05 \), Table 1). Four weeks of formoterol treatment was associated with a 16% and 17% increase in the EDL muscle mass of C57BL/10 and mdx mice, respectively (\( P < 0.05 \)), and a 14% and 17% increase in the soleus muscle mass in C57BL/10 and mdx mice, respectively (\( P < 0.05 \)). Diaphragm thickness was not different between C57BL/10 and mdx mice and was not altered by formoterol treatment. Heart mass was not different after formoterol treatment in either C57BL/10 mice (untreated: 117 ± 2 mg vs treated: 122 ± 4 mg) or mdx mice (untreated: 114 ± 5 mg vs treated: 112 ± 4 mg).

EDL and soleus muscles from mdx mice exhibited a greater median fibre CSA than C57BL/10 mice (20% and 11%, respectively, Fig. 1). Median diaphragm muscle fibre CSA was smaller in mdx than C57BL/10 mice (40%, \( P < 0.05 \)). Formoterol treatment increased median fibre CSA 52% and 32% in EDL muscles of C57BL/10 and mdx mice, respectively, and by 34% and 16% in soleus muscles from C57BL/10 and mdx mice, respectively. In the diaphragm muscle, formoterol treatment increased median fibre CSA by 22% and 34% in C57BL/10 and mdx mice, respectively.

3.2. Muscle function

EDL muscles from C57BL/10 and mdx mice had a similar absolute force producing capacity (\( P_o \), Table 1), but when \( P_o \) was corrected for muscle CSA (\( sP_o \)), \( sP_o \) was 15% lower in mdx than C57BL/10 mice. For soleus muscles, \( P_o \) was 10% greater in mdx than C57BL/10 mice (\( P < 0.05 \)), but \( sP_o \) was 11% lower in mdx than C57BL/10 mice (\( P < 0.05 \)). For diaphragm muscle strips, \( sP_o \) was 40% lower (\( P < 0.05 \)) in mdx than C57BL/10 mice. Formoterol treatment increased \( P_o \) of the EDL muscle by 12% and 11% in C57BL/10 and mdx mice, respectively. For soleus muscles, treatment increased \( P_o \) by 20% and 22% in C57BL/10 and mdx mice, respectively. For the diaphragm muscles, \( sP_o \) was not different in either C57BL/10 or mdx mice after formoterol treatment. No differences were observed in time-to-peak twitch (TPT) tension, one-half relaxation time (1/2RT) or the peak rate of twitch force development (\( dP_o/dt \)) in EDL or soleus muscles. Diaphragm muscle strips from mdx mice exhibited a 21% prolongation of 1/2RT compared to C57BL/10 mice (\( P < 0.05 \)).

**Table 1**

<table>
<thead>
<tr>
<th>C57BL/10</th>
<th>Formoterol (n = 10)</th>
<th>mdx</th>
<th>Formoterol (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 10)</td>
<td>10.1 ± 0.4</td>
<td>12.0 ± 0.4*</td>
<td>12.3 ± 0.4#</td>
</tr>
<tr>
<td>TPT (ms)</td>
<td>20.1 ± 0.5</td>
<td>18.4 ± 0.4</td>
<td>20.0 ± 0.5</td>
</tr>
<tr>
<td>( dP_o/dt ) (mN/ms)</td>
<td>20.7 ± 0.7</td>
<td>21.7 ± 1.0</td>
<td>21.8 ± 0.8</td>
</tr>
<tr>
<td>1/2 RT (ms)</td>
<td>24.7 ± 1.7</td>
<td>22.4 ± 0.5</td>
<td>25.3 ± 0.9</td>
</tr>
<tr>
<td>( P_o ) (mN)</td>
<td>445.0 ± 13.9</td>
<td>500.7 ± 12.1*</td>
<td>458.5 ± 14.7</td>
</tr>
<tr>
<td>( sP_o ) (kN/m²)</td>
<td>254 ± 6</td>
<td>239 ± 7</td>
<td>217 ± 5.2#</td>
</tr>
</tbody>
</table>

**Soleus**

<table>
<thead>
<tr>
<th>C57BL/10</th>
<th>Formoterol (n = 10)</th>
<th>mdx</th>
<th>Formoterol (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 9)</td>
<td>7.5 ± 0.3</td>
<td>8.7 ± 0.33</td>
<td>9.6 ± 0.4#</td>
</tr>
<tr>
<td>TPT (ms)</td>
<td>38.7 ± 1.3</td>
<td>35.0 ± 1.8</td>
<td>38.7 ± 1.8</td>
</tr>
<tr>
<td>( dP_o/dt ) (mN/ms)</td>
<td>10.2 ± 0.9</td>
<td>9.6 ± 0.8</td>
<td>9.5 ± 0.9</td>
</tr>
<tr>
<td>1/2 RT (ms)</td>
<td>51.7 ± 2.0</td>
<td>53.5 ± 2.0</td>
<td>56.0 ± 3.6</td>
</tr>
<tr>
<td>( P_o ) (mN)</td>
<td>205.0 ± 8.6</td>
<td>245.2 ± 9.8*</td>
<td>226.2 ± 9.1#</td>
</tr>
<tr>
<td>( sP_o ) (kN/m²)</td>
<td>231 ± 9</td>
<td>230 ± 9</td>
<td>205 ± 7#</td>
</tr>
</tbody>
</table>

**Diaphragm**

<table>
<thead>
<tr>
<th>C57BL/10</th>
<th>Formoterol (n = 10)</th>
<th>mdx</th>
<th>Formoterol (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 9)</td>
<td>360 ± 15</td>
<td>401 ± 25</td>
<td>448 ± 26</td>
</tr>
<tr>
<td>TPT (ms)</td>
<td>40.4 ± 1.8</td>
<td>38.6 ± 1.3</td>
<td>43.3 ± 0.8</td>
</tr>
<tr>
<td>( dP_o/dt ) (mN/ms)</td>
<td>10.0 ± 2.3</td>
<td>10.6 ± 1.2</td>
<td>7.8 ± 1.5</td>
</tr>
<tr>
<td>1/2 RT (ms)</td>
<td>57.4 ± 2.1</td>
<td>63.4 ± 2.8</td>
<td>68.6 ± 3.1#</td>
</tr>
<tr>
<td>( sP_o ) (kN/m²)</td>
<td>215 ± 5</td>
<td>222 ± 8.2</td>
<td>129 ± 7#</td>
</tr>
</tbody>
</table>

BM, body mass; TPT, time to peak twitch tension; \( dP_o/dt \), rate of force development during twitch contraction; 1/2RT, one-half twitch relaxation time; \( P_o \), maximum isometric force; \( sP_o \), specific maximum isometric tetanic force; width, overall diaphragm cross-sectional thickness; *\( P < 0.05 \), formoterol vs control; #\( P < 0.05 \), mdx vs C57BL/10.
and this effect was unchanged by formoterol treatment (Table 1).

3.3. Muscle fatigue and recovery

There was no difference in fatigue resistance of the EDL and soleus muscles between C57BL/10 and mdx mice during the 4 min stimulation protocol, nor in the recovery forces after the five and ten minute rest periods (Fig. 2). Formoterol treatment had no effect on the susceptibility of EDL and soleus muscles to fatigue in either C57BL/10 or mdx mice (Fig. 2). Compared to wild-type control, diaphragm muscles from control mdx mice exhibited increased fatiguability after 1 min into the stimulation protocol, but no differences were observed at any other time (Fig. 2). Similarly, formoterol treatment did not alter the susceptibility to fatigue of isolated diaphragm muscle preparations from C57BL/10 or mdx mice.

Fig. 1. Box-and-whisker plots showing the median and interquartile range of the distribution of fibre CSA from (A) EDL, (B) soleus and (C) diaphragm muscles from control and formoterol treated C57BL/10 and mdx mice. Formoterol treatment was associated with a significant increase in the median fibre CSA in all three muscles examined for both C57BL/10 and mdx mice.

Fig. 2. Fatigue and recovery for (A) EDL, (B) soleus and (C) diaphragm muscles from control and formoterol treated C57BL/10 and mdx mice. Muscles were maximally stimulated once every 4 s for 4 min to elicit fatigue, and recovery (of force) was determined at 5 and 10 min after the completion of the fatigue protocol. No differences in fatigue or recovery were observed between muscles of control C57BL/10 and mdx after formoterol treatment.
3.4. Markers of regeneration in the diaphragm

Central nucleation is an indication of muscle fibre regeneration and so we examined the proportion of centrally nucleated muscle fibres in the diaphragm. There was a significantly greater proportion of centrally nucleated fibres in the diaphragm muscles of *mdx* than C57BL/10 mice (24.9 ± 2.1% vs 0.1% ± 0.1%, \(P < 0.05\), Fig. 3). Formoterol treatment did not alter the proportion of centrally located nuclei in either C57BL/10 or *mdx* mice.

No discernible levels of myogenin positive nuclei were observed in the diaphragm muscles from control and formoterol treated C57BL/10 mice (Fig. 4). Diaphragm muscles from both untreated *mdx* and formoterol treated *mdx* mice had similarly elevated levels of myogenin positive nuclei (22.9 ± 5.6/mm\(^2\) and 29.4 ± 12.5/mm\(^2\) respectively), compared to C57BL/10 mice (\(P < 0.05\)).

3.5. Ubiquitin, ubiquitin ligase mRNA and ubiquitinated proteins in the diaphragm

The relative amounts of ubiquitin and ubiquitin related ligase (E1 and E3) mRNA in the diaphragm were not different between C57BL/10 or *mdx* mice (Fig. 5), and were not altered with formoterol treatment.

The relative amount of ubiquitin protein in the diaphragm was not different between C57BL/10 and *mdx* mice, and this was not altered by formoterol treatment (Fig. 6). The level of ubiquitinated proteins was significantly greater in the diaphragm muscle of *mdx* compared to C57BL/10 mice (46%, \(P < 0.05\)). Formoterol treatment significantly reduced the level of ubiquitinated proteins in diaphragm muscles from C57BL/10 mice (27%, \(P < 0.05\)) but not in *mdx* mice.

4. Discussion

The most important finding of this study was that low dose formoterol administration improved the functional and morphological properties of EDL, soleus and diaphragm muscles of *mdx* dystrophic mice. Importantly, formoterol treatment did not affect fatigue resistance of any of the muscles examined, nor did it affect cardiac mass. The findings highlight therapeutic potential of formoterol for muscular dystrophy and other muscle wasting disorders, and provide important information for current and future clinical trials using \(\beta_2\)-agonists.

Low dose formoterol treatment increased the mass and force producing capacity of fast and slow muscles from wild-type and dystrophic mice, changes that were attributed to increases in fibre CSA in both muscles. These findings are in contrast to previous studies where administration with other \(\beta_2\)-agonists was reported to have no effect or minimal effects on the function of slow-twitch skeletal muscles [25–27]. The finding that predominantly slow-twitch muscles responded to formoterol in a similar way to fast-twitch muscles is important, since muscles of boys with DMD have fewer type II fibres and a higher proportion of type I fibres [28,29].

Of functional and clinical significance was the finding that muscles from treated C57BL/10 and *mdx* mice exhibited no change in muscle fatigue resistance nor in their ability to recover after fatigue compared with
control mice. This is important since β2-agonist treatment has been associated with slow-to-fast muscle fibre transitions which can increase susceptibility to muscle fatigue [30–32]. As muscles from boys with DMD are already highly susceptible to fatigue [18] any deleterious shift in muscle metabolism could increase fatigability and so reduce the clinical merit of the proposed intervention. The dose of formoterol employed in the present study (25 μg/kg/day) was sufficient to elicit muscle hypertrophy, but was not associated with changes in muscle fatigue resistance. Further research is required to elucidate the dose-dependent mechanisms responsible for skeletal muscle hypertrophy, fibre type, and metabolic alterations after formoterol administration.

Myogenin is upregulated during myoblast differentiation and early muscle regeneration, and is elevated significantly in muscles of mdx mice [23]. The effect of β2-agonist administration on myogenic regulatory factors in dystrophic skeletal muscle has not been well characterised. Myogenin levels decrease in slow-twitch muscle and increase in fast-twitch skeletal muscle after β2-agonist administration [33,34]. In the present study, the diaphragm muscles of mdx mice exhibited elevated myogenin levels, which were not altered by formoterol treatment in either control or dystrophic mice. Thus, the formoterol-induced hypertrophy of diaphragm muscle fibres was not likely to have resulted from increased muscle fibre regeneration.
Although one study has shown no alteration in gene expression of the ubiquitin-proteosome proteolytic pathway in limb muscles of mdx mice [35], we have shown that this pathway was altered in the diaphragm of mdx mice as evidenced from increased levels of ubiquitinated proteins [22]. Since formoterol has been found to decrease ubiquitin mRNA and proteasome activity when administered at a high dose (2 mg/kg/day) to tumour bearing rats [36], it was important to determine whether formoterol would also reduce ubiquitin mRNA and the level of ubiquitinated proteins in control and dystrophic mice. We found that low dose formoterol treatment did not alter ubiquitin or the E1 and E3 ubiquitin ligases in diaphragm muscles of control or mdx mice. Instead, formoterol is likely to stimulate an increase in protein synthesis. Interestingly, formoterol treatment did reduce the level of ubiquitinated proteins in the diaphragm muscle from control mice. Further studies are required to determine whether there are dose-related changes or muscle specific differences in the signalling pathways leading to hypertrophy after formoterol administration.

In conclusion, low dose formoterol treatment elicited hypertrophy of EDL, soleus and diaphragm muscle fibres without increasing the susceptibility of these muscles to fatigue. Furthermore, low dose formoterol treatment did not affect heart mass – an important consideration if these compounds are to have application for treating muscle wasting in humans [37] and further evidence that this β2-agonist has greater therapeutic potential than older generation β2-agonists such as albuterol or clenbuterol [10]. These results provide important information for current and future clinical trials of β2-agonists for the muscular dystrophies and other muscle wasting disorders since formoterol is already FDA approved and even micromolar doses can produce significant functional improvements in dystrophic skeletal muscles.

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References


