Albuterol increases lean body mass in ambulatory boys with Duchenne or Becker muscular dystrophy

C.L. Skura, DPT
E.G. Fowler, PhD, PT
G.T. Wetzel, MD, PhD
M. Graves, MD
M.J. Spencer, PhD

ABSTRACT

Background: Albuterol is a beta-2 agonist that has been demonstrated to increase muscle strength in studies in animals and humans. Based on a pilot study of extended-release albuterol Repetabs in children with dystrophinopathies, the authors conducted a randomized, double-blind, placebo-controlled study with a crossover design.

Methods: Fourteen boys with Duchenne or Becker muscular dystrophy, 6 to 11 years old, completed two treatment periods (albuterol and placebo), 12 weeks each, separated by a 12-week washout period. As the albuterol Repetab formulation was no longer available, an alternate extended release albuterol was used (Volmax, 12 mg per day). Outcome measurements included 1) lean body mass, 2) fat mass, 3) isometric knee extensor and flexor moments, 4) manual muscle testing, and 5) timed functional tests.

Results: Lean body mass was significantly higher for subjects following albuterol treatment compared to placebo treatment, while fat mass was significantly lower. No differences were found in isometric knee moments or manual muscle tests. Time to run/walk 30 feet was improved following albuterol.

Conclusions: Short-term treatment with extended release albuterol may increase lean body mass, decrease fat mass, and improve functional measures in patients with dystrophinopathies. However, the significant change in strength of specific muscle groups found in the pilot study was not observed in the present study. These findings may be attributed to differences in the drug release and kinetics between Repetab and Volmax formulations as they affect the concentration of available beta-2 receptors on the muscle cell surface differently.

GLOSSARY

beta-2 agonists; BMD = Becker muscular dystrophy; DMD = Duchenne muscular dystrophy; LBM = lean body mass; MMT = manual muscle test; PODCI = Pediatric Outcomes Data Collection Instrument.

Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder caused by mutations in dystrophin, a protein that plays a role in the stability of the muscle membrane.1 Becker muscular dystrophy (BMD) is a less severe form of the disorder due to mutations in the dystrophin gene that produce either in-frame truncations or a reduced quantity of dystrophin compared to normative values. Both diseases are characterized by loss of skeletal muscle, progressive weakness, and cardiomyopathy, leading to a loss of ambulation for most children with DMD between 12 and 15 years. Children with DMD tend to succumb to respiratory or cardiac complications in their early to mid-20s, while children with BMD can survive into late adulthood. Treatments currently being studied for DMD include gene replacement or repair, stem cell transfer, and various pharmacologic regimens. Standard drug treatments include corticosteroids such as prednisone and deflazacort; however, these drugs tend to have side effects such as weight gain, growth inhibition, decreased bone density, hypertension, and behavioral changes.2-4
Beta-2 agonists (β2A) are a class of drugs that have been shown to improve the strength and function of healthy and diseased muscle.\(^5\)\(^-\)\(^8\) Numerous studies have shown that mdx mice (an animal model of DMD) treated with the β2A clenbuterol were stronger and exhibited greater muscle mass compared to appropriately matched control mice.\(^9\)\(^-\)\(^14\) One mechanism of action of β2As appears to be suppression of calpain-mediated proteolysis by upregulation of the specific calpain inhibitor calpastatin.\(^15\) The finding that calpastatin upregulation is beneficial for dystrophic pathogenesis was demonstrated following transgenic upregulation of this inhibitor in mdx muscles.\(^16\)

Patients with muscular dystrophies may also benefit from β2A treatment.\(^17\)\(^-\)\(^20\) In particular, a pilot study was conducted to evaluate the effectiveness of albuterol in ambulatory boys with dystrophinopathies,\(^20\) using the same form of the drug that was used successfully in a pilot study of adults with facioscapulohumeral muscular dystrophy\(^17\) (Proventil Repetabs). Albuterol is a β2A that has been used successfully to treat asthma in adults and children with few side effects, but had not been tested before in subjects with DMD. In this pilot study, nine subjects with DMD or BMD received albuterol (4 mg, twice a day) or placebo for 12 weeks in a randomized, double-blind, crossover investigation. Muscle strength was increased significantly in participants taking albuterol. The goal of the present research was to conduct a larger, double-blind, randomized study to confirm these results and to examine lean body mass and fat mass as additional outcome measures.

**METHODS** At the start of this clinical trial, the Schering Corporation no longer manufactured the Proventil Repetabs that were administered during the pilot study. A substitution was made with another form of albuterol extended release tablets, Volmax or VoSpire (Muro Pharmaceutical, Inc., Tewksbury, MA; Odyssey Pharmaceuticals, Inc., East Hanover, NJ). The dosage was 12 mg per day, two pills in the morning and one pill in the evening (each pill 4 mg).

Ambulatory boys with dystrophin mutations were recruited through Southern California Muscular Dystrophy Association sponsored clinics and via flyers and Web sites. The study was conducted within the UCLA Human Subject Protection Committee guidelines and all procedures performed on human subjects were approved by the Institutional Review Board. Parents or guardians gave informed consent and participants over 7 years old gave assent for their participation. Inclusion criteria were 1) male and between the ages of 6 and 11 years, 2) ability to walk independently, 3) minimum quadriceps manual muscle test (MMT) grade of 4 out of 5, and 4) diagnosis of DMD or BMD confirmed by muscle biopsy and immunohistochemistry for dystrophin, muscle biopsy and Western analysis of dystrophin, PCR or southern blot of blood DNA for dystrophin, or X-linked family history of muscular dystrophy. While some of these patients were clinically diagnosed with either DMD or BMD, placement of patients into these categories represents a continuum and was not strictly defined in this study. Exclusion criteria were 1) use of corticosteroids, or any other drug for the treatment of DMD or BMD within a 3-month period prior to study entry, 2) use of sympathomimetic agents or any beta antagonists, 3) cardiovascular disease or cardiovascular risk as determined by a pediatric cardiologist during the baseline examination, or 4) inability of the child to follow verbal instructions for testing.

General health was assessed by a neurologist during the initial baseline evaluation and at each treatment period evaluation. Assessments included height, weight, blood pressure, and heart rate. Parents and children were interviewed to determine the presence of adverse events and side effects. Compliance during each treatment period was determined by counting the number of remaining pills after the 12-week treatment period and a daily log, where parents or guardians recorded the number of pills given to their child. In addition, the child’s overall activity level during each day was recorded as low, moderate, or high based on parent perception. Respiratory testing was performed using a Renaissance II spirometer to measure forced expiratory volume in liters. Range of motion was assessed during each visit to monitor for contractures, including bilateral shoulder abduction, elbow extension, wrist extension, hip extension, hip adduction, knee extension, and ankle dorsiflexion.

Subjects completed two baseline evaluations and were then randomized by the pharmacist to one of two groups, albuterol first or placebo first. Examiners, patients, and their families were blinded to the randomization. Treatment periods were 12 weeks in length. Subjects were given either albuterol or placebo pills (contained within the same blue capsules) and instructed to swallow them whole, two pills in the morning and one pill in the evening. During the albuterol treatment period, each capsule contained 4 mg of extended release albuterol for a total of 12 mg per day. During the placebo period, the capsules contained candy. The two treatment periods were separated by a 12-week washout period.

Assessments of strength and function were carried out by the same physical therapist during each visit. Parents or guardians were instructed to minimize their child’s physical exertion the day before evaluations. A consistent testing sequence was used for each evaluation session. Small toys were given as rewards to provide positive reinforcement and to encourage the children to utilize maximum effort. Data were obtained at two time points during each of the collection periods (baseline, treatment period one, washout, treatment period two). Data from the two time points were averaged for analysis.

Strength was assessed using two methods: 1) MMT and 2) peak isometric knee joint moments (torque). MMT of the
subjects was based on the design of the Clinical Investigation of Duchenne Dystrophy Group,23 with minor modifications made based on our pilot study.21 Because distal muscle groups, such as wrist, finger, and ankle muscles, did not show a change in MMT value over a 12-week time period in the pilot study, they were removed from data collection in the current protocol. MMT was completed on 18 muscle groups (bilateral shoulder abductors, shoulder external rotators, elbow extensors, hip extensors, hip flexors, hip abductors, knee extensors, knee flexors, and ankle dorsiflexors), which were measured on a scale of 0 (no palpable contraction) to 5 (normal). MMT grades (0, 1, 2, 2+, 3–, 3+, 4–, 4+, 5–, 5) were translated into numerical format by converting a “+” to a +0.3 and “−” to a −0.3. A composite muscle strength score was obtained, with a maximum possible score of 90. Peak knee extensor and flexor moments were obtained from isometric contractions using a KIN-COM II dynamometer (Chattanooga Group Inc., 4747 Adams Rd., Hixson, TN 37343). Patients were instructed to perform five isometric contractions at 60 degrees of knee flexion using maximal effort. Verbal cueing by the therapist and feedback from the computer monitor were used to encourage maximal effort. Knee extension contractions were followed by knee flexion contractions for each limb, with a brief rest period in between. The peak moment obtained for the left and right limbs were averaged for data analyses.

Timed functional tests included the time to walk or run 30 feet, the time to ascend four steps, and the time to rise from supine to a standing position. Each test was performed three times and the fastest time for each test was used for data analysis.

Body composition data were collected using DEXA at baseline and at the end of the three phases (treatment period one, washout, treatment period two). The DEXA model used was a Hologic 4500a fan-beam scan. Data collected from the whole body scan included lean body mass (LBM) and fat mass.

At the end of each treatment phase, parents completed the Pediatric Outcomes Data Collection Instrument (PODCI).22 This questionnaire contains scaled measures indexing the parents’ perception of their child’s function. There are eight scales: upper extremity and physical functioning, pain/comfort, treatment expectations, happiness, satisfaction with symptoms, and a global functioning scale (a combined measure calculated from the first four scales). All components are transformed to a 0 to 100 scale for analyses.

Statistical analysis was conducted by Jeffrey A. Gornbein, DrPH, Senior Statistician, SBCC. A repeated measures analysis of variance model for a crossover design was used to examine the change in outcome means at the end of each treatment period as compared to the preceding baseline or washout value, while adjusting for period and order effects. A p value of 0.05 was considered significant while p values between 0.05 and 0.10 were considered suggestive of a clinical effect. Data collected from the two time points in each treatment period were averaged.

Participants who completed the 36-week study were given the opportunity to continue taking albuterol during an open-label period for 6 months. They returned for follow-up appointments at 3 and 6 months in order to monitor for any change in function or adverse effects. Albuterol dosage during the follow-up period was the same as during the treatment phase.

**RESULTS** A total of 15 subjects were enrolled in the study. Fourteen boys with a mean age of 8.4 years at baseline (range 6 to 11 years; SD 1.46) completed the 36-week study. Seven were randomized to the albuterol-first treatment group, and seven to the placebo-first group (figure E-I on the Neurology Web site at www.neurology.org). Two of the subjects with milder symptoms had been given a clinical diagnosis of BMD (one was randomized to each treatment group), while the other 12 participants had a diagnosis of DMD. Baseline characteristics for the participants who completed the trial are described in table 1. In addition to all of the subjects meeting the exclusion criteria prohibiting the use of any drug for the treatment of DMD or BMD within the 3 months prior to the study, none had previously been treated with steroids at a therapeutic dose at any time before participating in the study. Compliance data were returned for 12 participants during the albuterol treatment phase and 13 participants during placebo. During both periods, the average medication compliance was 94% (albuterol range 82 to 100%, SD 6%; placebo range 80 to 101%, SD 7%).

Drug treatment (albuterol vs placebo) produced a significant improvement in body composition measurements for 12 out of the 14 subjects during the albuterol treatment phase. On average, participants taking albuterol demonstrated a significant increase in LBM (4% on albuterol compared to 1% on placebo) and decrease in fat mass (2% on albuterol compared to 6% increase on placebo) (table 2; figure E-2).

No significant difference was observed bet-

### Table 1  Baseline characteristics of subject population (n = 14)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>8.4</td>
<td>1.5</td>
</tr>
<tr>
<td>Height, cm</td>
<td>123.4</td>
<td>11.2</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>27.2</td>
<td>10.6</td>
</tr>
<tr>
<td>Composite MMT (90 total possible)</td>
<td>77.5</td>
<td>4.5</td>
</tr>
<tr>
<td>Composite PODCI (100 total possible)</td>
<td>75.8</td>
<td>9.9</td>
</tr>
<tr>
<td>Time to run 30 feet, sec</td>
<td>5.6</td>
<td>1.3</td>
</tr>
<tr>
<td>Time to climb four standard stairs, sec</td>
<td>8.7</td>
<td>6.0</td>
</tr>
<tr>
<td>Time to stand from supine, sec</td>
<td>4.9</td>
<td>2.8</td>
</tr>
<tr>
<td>Peak isometric knee extensor moment, Nm</td>
<td>23.0</td>
<td>22.7</td>
</tr>
<tr>
<td>Peak isometric knee flexor moment, Nm</td>
<td>12.5</td>
<td>9.3</td>
</tr>
<tr>
<td>Lean body mass, kg</td>
<td>19.0</td>
<td>4.8</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>7.6</td>
<td>5.7</td>
</tr>
</tbody>
</table>

MMT = manual muscle test; Nm = Newton meters; PODCI = Pediatric Outcomes Data Collection Instrument.
tween albuterol and placebo treatment arms for MMT or isometric knee extensor or flexor moments (table 2). A change in functional ability was observed while the subjects were taking drug, as seen by the timed tests. Subjects on placebo demonstrated an increase in the time required to perform functional tests over the course of the study as expected, due to the degenerative nature of the disease. However, the change in time to walk or run 30 feet was significantly lower following albuterol (table 2; see also online supplemental data). The average time to get up from the floor and to ascend four steps increased to a greater extent following placebo as compared to albuterol treatment periods, with a tendency toward significance (table 2). A difference in the PODCI parent questionnaire was not found between albuterol and placebo treatment periods.

Participants demonstrated very little change in range of motion throughout the course of the study. Although most subjects tended to lose a small amount of dorsiflexion range over time, the average loss was less than five degrees. Few minor side effects were reported for the participants who completed the study. Five adverse events were reported. Three participants exhibited an elevated heart rate compared to baseline measures while on albuterol. The cardiologist participating in the study concluded that their heart rates were within normal limits and they remained in the study. One participant had difficulty breathing and was evaluated by his local pediatrician. He was diagnosed with cold symptoms and treated with over-the-counter decongestants. A fifth participant developed a skin rash during the first treatment arm, shortly after starting placebo medication. The suspected cause of the rash was the blue dye contained in the capsule and this child withdrew from further participation in the study.

Seven of the 14 participants continued to take albuterol on an open-label basis after the trial and returned for follow-up assessment at 3 months. Four of these subjects also returned for evaluation at 6 months. For these subjects, MMT scores declined slightly during the follow-up period (1% at 3 months and 2% at 6 months), knee joint moments declined (7% at 3 months and 6% at 6 months), and the time to perform functional tests became slower (18% at 3 months and 23% at 6 months). Despite a decline in these clinical strength and functional measurements, parents reported an improvement in global function (3% at 3 months; 9% at 6 months) per the PODCI during the open-label trial. Information on body composition is not available for follow-up data, as DEXA scans were not completed during these visits.

**DISCUSSION** The results of this study suggest that 12 weeks of daily albuterol treatment may be beneficial for retaining muscle mass in patients with muscular dystrophinopathies. Participants in this study exhibited higher lean body mass and reduced fat mass while on albuterol compared to placebo. Since DMD is a progressive, degenerative disease, it is expected that the amount of time needed to perform functional activities would increase as the disease progressed. However, the timed test results changed very little over time while the subjects were taking albuterol compared to an increase in the time required to perform the activities when they were on placebo. This suggests that albuterol treatment slowed the normal decline in function associated with DMD. In contrast, an improvement in strength of spe-

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Mean (SD) and p values for changes from preceding baseline (albuterol first) or washout (albuterol second) in outcome measurements during treatment periods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo change</td>
</tr>
<tr>
<td>Lean body mass, kg</td>
<td>2.7 (6.7)</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>4.6 (6.4)</td>
</tr>
<tr>
<td>Composite manual muscle test (score)</td>
<td>−1.3 (2.2)</td>
</tr>
<tr>
<td>Peak isometric knee extensor moment, Nm</td>
<td>−0.5 (2.0)</td>
</tr>
<tr>
<td>Peak isometric knee flexor moment, Nm</td>
<td>−0.05 (1.8)</td>
</tr>
<tr>
<td>Timed run/walk, s</td>
<td>0.3 (0.5)</td>
</tr>
<tr>
<td>Timed stairs, s</td>
<td>0.8 (2.0)</td>
</tr>
<tr>
<td>Timed up from floor, s</td>
<td>1.2 (2.0)</td>
</tr>
<tr>
<td>Composite PODCI (score)</td>
<td>−3.3 (8.2)</td>
</tr>
</tbody>
</table>

*Significant (p < 0.05).
†Approaching significance (0.05 < p < 0.10).
PODCI = Pediatric Outcomes Data Collection Instrument.
Specific muscle groups was not measured using MMT or joint moment measurements following albuterol treatment. This apparent contradiction may be due to the inability of MMT or KINCOM assessments to detect small changes in strength in individual muscle groups. Measurements such as DEXA or functional testing may be more sensitive for detecting collective muscle mass and strength changes.

Possible explanations for the difference in findings between muscle strength and body composition tests include the reliability and sensitivity of the measures. Subject fatigue and variability in testing performance has been reported in the literature for children with DMD. Clinical assessment protocols such as MMT or joint moment measurements require that the subject produce voluntary, maximal effort, whereas for DEXA scanning, the only requirement is that the subject remain still. Although all of the participants in the current study met the inclusion criteria of being able to follow simple directions, some of the subjects had difficulty sustaining the level of exertion that was required to generate maximal effort with each test. Although interrater reliability can be a concern when using MMT as an outcome measure, good intrarater reliability for this test in children with DMD has been demonstrated. In the present study, interrater reliability was not a factor, as one physical therapist with considerable training and experience using a standardized protocol performed all tests.

In children with DMD, the percentage of body fat has been shown to increase with age, while the percentage of lean body mass and functional ability have been shown to decline. Previous studies have suggested that there is a significant positive correlation between the percentage of LBM and muscle function. Subjects in the current study exhibited reduced LBM at baseline compared to age-matched normative data, ranging from 11% to 18%, which is consistent with other studies of children with DMD. Although LBM increased during this 36-week study, due to both albuterol treatment and normal growth, it remained lower than published norms.

DMD and BMD are considered to represent a continuum of the same disease, and the distinction between the two is a clinical one. Because the pathogenic mechanisms involved in both diseases are based on a common molecular defect, therapeutic interventions are expected to be the same. In this study, 2 of the 14 children had a clinical diagnosis of BMD. With removal of these children from statistical analyses, the positive effect of albuterol was maintained for lean body mass (p = 0.045), fat mass (p = 0.100), and the timed walk/run test (p = 0.010). Although it is difficult to draw definitive conclusions based upon the small sample size in the current study, these data indicate that the response to albuterol treatment was similar for the children with DMD and BMD.

The mechanism of albuterol action is through binding to β2 receptors on the sarcolemmal membrane resulting in increased cytosolic cyclic AMP. These receptors are predominantly found in bronchial smooth muscle but they also appear on skeletal muscle. While the specific mechanism by which skeletal muscle mass increases are attained after treatment with this drug is unknown, there is evidence to suggest that it is through both increased muscle protein synthesis and decreased muscle protein degradation. Suppression of protein degradation is most likely accomplished through inhibition of calpains, which are calcium dependent proteases that have been demonstrated to play a role in the pathogenic process of mdx dystrophy. The suppression of calpain proteolysis by β2As occurs through upregulation of calpastatin, a calpain 1 and 2 inhibitor. Because calpains are ubiquitous proteins found in all tissues including platelets, the specific inhibition of muscle calpain is difficult to achieve without deleteriously altering other organ systems. Albuterol and other beta agonists are the only known class of drugs that can specifically up-regulate muscle calpastatin; therefore, this class of drug provides a promising treatment option for muscle-specific suppression of calpain activity. β2As may also affect other proteolytic systems of muscle (such as lysosomal proteases or the proteosome); however, the effect of these drugs on these other proteolytic systems has not been specifically tested.

Drug kinetics appear to be an important factor in the effectiveness of albuterol treatment. The results of the current study may have differed from the pilot study because either Volmax or VoSpire were used instead of albuterol Repetabs. These formulations have different mechanisms of drug release that can impact the drug concentration in the blood. Proventil Repetabs contain albuterol both in the tablet coating and in the core and have a repeat action release. The dose in the coating is immediately released and that in the core is released several hours later. In contrast, Volmax or VoSpire have an oral osmotic drug delivery system, releasing the albuterol at a constant rate through a laser-drilled hole. Repetabs cause more imme-
mediate elevations in blood plasma concentrations, while Volmax or VoSpire exhibit a more controlled release with less peaks and troughs. Since β2 receptors downregulate following ligand binding, a higher steady concentration of albuterol may result in fewer β2 receptors than would be available if the albuterol were administered in a “pulsed” manner. In addition, the albuterol dosage was increased in the present study compared to the pilot. These two changes in the protocol may have resulted in a higher level of albuterol in the blood with less fluctuation in concentration over time with potential adverse consequences for the concentration of cell surface β2 receptors.

β2As have promise as a therapeutic alternative to prednisone or perhaps as part of an armamentarium of drugs taken in conjunction with prednisone that could target different pathogenic pathways occurring in dystrophinopathies. Clinical studies involving albuterol treatment of patients with facioscapulohumeral muscular dystrophy, as well as the current study with DMD and BMD, have had positive outcomes, although further study is needed to determine the optimal dosing regimens for these patient populations. While pathogenic mechanisms in these diseases are different, they may involve a final common pathway that relates to increases in intracellular calcium and calpain activation.

Received February 11, 2007. Accepted in final form June 26, 2007.

REFERENCES