The Effects of Voluntary Exercise on the Proximal Femur of Control and Mdx Mice

David J. Nye, Jeffrey M. Costas, Jessica B. Henley, Jin-Kwang Kim, Jeffrey H. Plochocki

1Arizona College of Osteopathic Medicine, Midwestern University, Glendale, AZ 85308, USA
2The Pennsylvania State University, University Park, PA 16802, USA

§Corresponding author

Email addresses:

DJN: david.nye@azwebmail.midwestern.edu
JMC: jeffrey.costas@azwebmail.midwestern.edu
JBH: jhenle@midwestern.edu
J-KK: jok5099@psu.edu
JHP: jploch@midwestern.edu

Submitted as an Original Article

Sources of Funding: Midwestern University, Glendale, AZ, USA.

Keywords: Mdx; Exercise; Femur; Dystrophin; Cartilage
Abstract

Background
Submaximal exercise is used in the management of muscular dystrophy. The effects of mechanical stimulation on skeletal development are well understood, although its effects on cartilage growth have yet to be investigated in the dystrophic condition. The objective of this study was to investigate the chondrogenic response to voluntary exercise in dystrophin-deficient mice, which exhibit weakened muscle contractile force.

Methods
Control and dystrophin-deficient (mdx) mice were divided into sedentary and exercise-treated groups and tested for chondral histomorphometric differences at the proximal femur.

Results
Exercised control mice exhibited significantly enlarged femur head diameter, articular cartilage thickness, articular cartilage tissue area, and area of calcified cartilage relative to sedentary controls and exercised mdx mice \( (P < 0.05) \). No differences were found between other treatment groups.

Conclusions
Mdx mice exhibit a reduced chondrogenic response to increased mechanical stimulation relative to controls. However, no significant reduction in articular size was found, indicating loss of chondral tissue may not be a clinical concern with dystrophinopathy.
Background

Genetic mutations affecting the expression of the dystrophin gene, as with Duchenne muscular dystrophy (DMD), impair cellular ability to resist muscle contractile forces and result in striated muscle cell death and fibrosis of the investing connective tissue [1,2]. Although there is no cure for muscular dystrophy, exercise has long been prescribed as a treatment modality [3,4]. Submaximal, low-intensity exercise has been shown to improve skeletal muscle performance [5], while rigorous exercise accelerates the dystrophic process [6-8]. Maintenance of muscle mass through exercise has also been shown to have musculoskeletal benefits related to gait, prolonged ambulation, and joint contracture [3,9,10]. While dystrophin deficiency does not directly affect bone and cartilage growth, the growth of these tissues is mechanically regulated and therefore indirectly affected by strains from muscle contraction [11]. Bone fractures, low bone mineral density, pelvic obliquity, and kyphoscoliosis have been attributed to the effects of muscular degeneration and joint contractures on bone growth and maintenance in dystrophin-deficient patients [12-16]. However, the precise effects of moderate exercise on articular cartilage growth with dystrophinopathy-related muscle degeneration have yet to be studied.

We examine the effects of voluntary exercise activity on the proximal femurs of juvenile dystrophin-deficient (mdx) mice. Mdx mice do not have DMD, but exhibit a similar X-linked myopathy caused by dystrophin deficiency. Mdx mice have significantly reduced skeletal myocyte diameter, numerous necrotic myocytes, and abundant fibrosis leading to significantly weaker muscle force generation relative to wild-type mice and thus serve as a useful model for testing the effects of dystrophin-related muscle weakness [17,18]. Voluntary, rather than forced, exercise is used in our study because it has been shown to help maintain muscle strength in mdx mice and is similar to the submaximal, low-intensity exercise prescribed by some physicians in the management of human dystrophinopathies [19,20].
Methods
Twenty mice of the control strain C57BL/10ScSn (000476; Jackson Laboratory, Bar Harbor, ME) and twenty mice of the dystrophin-deficient strain C57BL/10ScSn-Dmd\textsuperscript{mdx} (mdx mice; 001801; Jackson Laboratory, Bar Harbor, ME) were used in the experiment. The use of animals in this study was approved by the Institutional Animal Care and Use Committee at Midwestern University and follows NIH guidelines for animal research. All mice were 7-week-old virgin females that were housed individually and provided with food and water ad libitum. After a one-week acclimatization period, the mice were separated into four groups of equal size: sedentary control mice, exercise-treated control mice, sedentary mdx mice, and exercise-treated mdx mice. Exercise treatment consisted of voluntary access to a running wheel that lasted four weeks. Individual running distances were monitored using digital counters attached to each wheel. Following the four-week treatment period, the mice were sacrificed using compressed CO\textsubscript{2} at the age of 11 weeks.

Femurs were immediately excised and placed in decalcifier (Surgipath, USA) for 3 days. Once decalcification was complete, the femurs were frozen in liquid nitrogen and cryosectioned at a thickness of 12 \( \mu \text{m} \) in the coronal plane. Sections were stained with toluidine blue to distinguish cartilage, calcified cartilage, and bone. Measurements were taken on digital images captured using an Eclipse 55i microscope (Nikon Inc.). Measurements included medial-lateral femoral head diameter, cartilage thickness at midjoint, area of the calcified cartilage zone, and cartilage tissue area excluding the calcified cartilage zone. Because of differences in body mass, statistical treatment of the data consisted of a general linear model of covariance (ANCOVA) with body weight as the covariate. Statistical significance was set at \( P < 0.05 \).

Results and Discussion
Average daily running distance did not differ significantly between control and mdx mice (\( P > 0.05 \), Fig. 1). On average, control mice ran 1.01 km/day more than mdx mice. One mdx mouse did not run at all
and was excluded from the analysis. Mdx mice were significantly heavier than controls in both the sedentary and exercise groups (Fig. 2). However, body mass did not differ significantly between sedentary and exercised mice of the control strain or between sedentary and exercised mdx mice.

Statistical comparisons of histomorphometric parameters of the proximal femur corrected for body mass are displayed in Table 1 (see Fig. 3 for representative histological sections from each treatment group). Our data show that proximal femoral tissue of juvenile mdx mice is less responsive to mechanical stimulation in comparison with controls. No significant differences between sedentary and exercised mdx mice were found for any dependent variable included in the study ($P > 0.05$). However, femur head diameter, cartilage thickness, and cartilage tissue area are significantly larger in exercised controls relative to sedentary controls and exercised mdx mice ($P > 0.05$). There is an abundance of data demonstrating articular cartilage growth, bone growth, and the size of the calcification zone are sensitive to moderate exercise in healthy subjects [21-25]. In vitro studies confirm that mechanical loading of cartilage stimulates cell division and matrix synthesis [26,27], even in the moderate range of 10 MPa [28-29]. In vivo, these effects translate to elevated chondral tissue expansion under increased mechanical stimulation [23,30]. The lack of a chondrogenic response to voluntary exercise in the mdx mice in our study suggests the weakened muscle contractions of the mdx mice do not provide sufficient mechanical pressure to initiate significant chondral expansion. Skeletal muscle of mdx mice of similar age to the ones used in this study have been shown to contain degenerative and necrotic myocytes with smaller diameters, as well as extensive skeletal muscle fibrosis, leading to reduced force production and power output [17,31,32]. This reduction in force likely explains the lack of tissue enlargement at the proximal femurs in mdx mice treated with voluntary running activity.

Although not enlarged, the tissues of the mdx mice are also not significantly reduced in size relative to sedentary controls (Table 1). Reduced mechanical stimulation can retard articular tissue growth, leading to thinner articular cartilage and smaller joints [25,33,34]. Our data suggests that the mechanical
stimulation in both the sedentary and exercised mdx mice aged 11 weeks is still sufficient to maintain articular tissue size comparable to sedentary controls. These findings are of interest to clinicians because, 1) low intensity exercise is sometimes used in the management of muscular dystrophy, and 2) muscular dystrophy patients lead a more sedentary lifestyle. Although the mdx mouse model does not perfectly reproduce the progression and severity of the dystrophic process observed in humans, by 6 weeks of age mdx mice exhibit significant muscle weakness and thus serve as a useful model for studying dystrophin-related muscle-skeletal tissue interactions [11]. Our findings suggest that the addition of articular tissue during periods of growth is limited following the onset of significantly reduced muscle force. However, chondral tissue area was preserved in the sedentary groups, indicating loss of chondral tissue is not a major concern with dystrophin deficiency. This may explain the lack of articular cartilage involvement in dystrophinopathies.

Conclusions
The results of this study show mdx mice exhibit a reduced chondrogenic response to increased mechanical stimulation relative to controls. Voluntary running exercise does not significantly affect femur head diameter, cartilage thickness, cartilage tissue area, and calcified cartilage tissue area in mdx mice as it does in controls. However, articular size was not reduced in mdx mice in comparison with controls, suggesting loss of chondral tissue may not be a clinical concern with dystrophinopathy.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
DJN, MJC, JBH, and JHP contributed to the design of the experiments. JBH was primarily responsible for animal care and treatment. DJN was responsible for data collection. DJN and
JHP were equally responsible for data analysis and drafting the manuscript. J-KK aided in the revision of the manuscript and interpretation of the data. All authors have read and approved the final manuscript.

Acknowledgements
The authors are thankful to Midwestern University for supporting this research.

References


Figure Legends

Fig. 1. Mean daily running distance of exercised control and exercised mdx mice. No significant difference was found between mouse strains, although control mice ran an average of 1.01 km/day more than exercised mdx mice. Error bars depict mean ± standard deviation.

Fig. 2. Body mass in control and mdx by treatment groups at 11 weeks of age. Mdx mice have significantly greater body mass than treatment-matched controls. Error bars depict mean ± standard deviation.

Fig. 3. Representative photomicrographs of femoral head articular cartilage of A) sedentary control mice, B) exercised control mice, C) sedentary mdx mice, and D) exercised mdx mice. Exercised control mice had the largest femur head diameter, cartilage tissue area, and area of calcified cartilage in comparison to all other groups when corrected for differences in body mass. Toluidine blue, 40x.
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Toluidine blue, 40x.
Table 1. Comparison of histomorphometric parameters of the proximal femur between treatment groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Mdx</th>
<th>Sedentary control vs. sedentary mdx</th>
<th>Exercise control vs. exercised mdx</th>
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<tbody>
<tr>
<td></td>
<td>Sedentary</td>
<td>Exercised</td>
<td>P</td>
<td>Sedentary</td>
</tr>
<tr>
<td>Femur head diameter (mm)</td>
<td>1.363 ± 0.066</td>
<td>1.377 ± 0.042</td>
<td>0.03</td>
<td>1.273 ± 0.112</td>
</tr>
<tr>
<td>Cartilage thickness (µm)</td>
<td>6.081 ± 1.560</td>
<td>5.878 ± 0.651</td>
<td>0.01</td>
<td>5.250 ± 1.118</td>
</tr>
<tr>
<td>Calcified cartilage area (µm²)</td>
<td>0.143 ± 0.041</td>
<td>0.206 ± 0.022</td>
<td>0.01</td>
<td>0.172 ± 0.038</td>
</tr>
<tr>
<td>Cartilage Area (mm²)</td>
<td>0.909 ± 0.170</td>
<td>0.949 ± 0.082</td>
<td>0.01</td>
<td>0.811 ± 0.131</td>
</tr>
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Values are mean ± standard deviation; ANCOVA for dependent variables with body mass as a covariate.
Figure 1

Mean Running Distance (km)

Control

Mdx

n.s.
Figure 2: Comparison of body mass (g) between control and mdx groups in sedentary and exercised conditions. The bars represent the mean body mass with error bars indicating standard deviation. Statistical significance is indicated by $P < 0.05$.