Multi-scale Investigation of Weight-bearing Exercise on Bone Biomechanical Integrity in the Osteogenesis Imperfecta Model (oim/oim) Mouse

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INTRODUCTION

Osteogenesis imperfecta (OI) is a heritable connective tissue disorder in which mutations in type I procollagen genes result in bone deformity and fragility. The oim/oim mouse, homozygous for a null mutation in the COL1A2 gene of type I collagen, has significantly reduced bone biomechanical integrity as well as altered bone mineral composition. Heterozygous mice (+/oim) have a phenotype intermediate to oim and wildtype (WT) mice.

Bone is inherently mechanosensitive, responding and adapting to its mechanical environment. Bone formation occurs in response to high mechanical loads, with bone strength directly proportional to muscle mass. In humans, children attain 28% of their peak bone mass during the normal 2 year period of pubertal/pubertal growth period. Children who are physically active accrue 10-40% more bone than inactive children. This suggests that sedentary lifestyle choices of children with OI are particularly detrimental to their bone health. We postulate that even though the OI bone material is biomechanically weaker, the OI bone will respond to exercise loading and/or gravitational ground force, especially during pubertal growth.

The potential benefits of therapeutic exercise to OI patients are significant, but the risks are real. It is critical that we first demonstrate the feasibility and potential efficacy of an exercise therapy in mouse models of OI for it to be considered a viable therapy for patients.

To address this need we combined the unique strengths of two University of Missouri Campuses to create a collaborative research team from the Departments of Biochemistry (UMC), Veterinary Pathology (UMC) and Oral Biology (UMKC) to determine if weight bearing exercise improves bone strength in a mouse model of osteogenesis imperfecta (oim) and to investigate the molecular, biochemical, physiochemical, structural and biomechanical impact of exercise on bone at the macro-, ultra- and nano-structural levels.

MATERIALS AND METHODS

Exercise Regimen

Female WT and +/oim mice were divided into control and exercise (treadmill) groups at 7 weeks of age. Control animals were allowed normal cage activity, treadmill animals walked for 30 min/day, 5 days/week (1st-8th) for 8 weeks.

Contractile Tension-Generating Capacity

At 4 months of age, control and treadmill WT and +/oim mice were deeply anesthetized and the distal tendons of the left plantar and gastrocnemius were exposed, tied off with 4-0 silk and attached to a Grass force transducer of the PowerLab system. The sciatic nerve was placed on a bipolar stimulation electrode and maximal contractile tension-generating capacity (peak tetanic tension: Tp) was elicited using ~7 volts, 150 Hz, 250 msec. 0.3 trains/sec. Pt values were normalized to cross sectional area to determine muscle quality.

Muscle Mass, Morphometry and Histology Analysis

After contractile studies, mice were sacrificed and muscles dissected, weighed and fixed in 4% paraformaldehyde for 24 hours, transversely sectioned at the middle of the muscle belly and stained with hematoxylin & eosin. Muscle sections were imaged at 10X and analyzed with Image J. An average of 300 sectioned muscle fiber areas was determined per muscle. Muscle fibers were also evaluated for pathology using Bone Biomechanical Testing (Torsional Loading to Failure).

Figure 1. Effect of Treadmill Exercise on Plantar and Gastrocnemius Muscles of Non-exercised Control and Treadmill Exercised WT (Wildtype) and Heterozygous (HET) Mice

Weight-bearing exercise significantly reduced specific gravity in +/oim mice without impacting the gastrocnemius. No changes were seen in either muscle of wildtype mice. Treatment groups are denoted with either +oim or WT. * P ≤ 0.05 versus control. / P ≤ 0.05 versus treadmill.

CONCLUSIONS

Neither +/oim nor oim/oim plants or gastrocnemius muscles demonstrated any signs of necrosis, degradation or repigmentation in control or exercise animals (data not shown).

+/oim mice were able to tolerate moderate treadmill exercise at 8 weeks of age, but oim mice were not (data not shown).

The contractile generating capacity of the plantaris was significantly reduced following treadmill exercise in +/oim mice, while the gastrocnemius appeared unaffected.

+/oim mice had 11-64% improvements in whole bone and material strength as well as energy to failure following treadmill exercise, though not significant.

+/oim mice also had significant reductions in whole bone and material stiffness following treadmill exercise.

+/oim mice appeared to benefit most from treadmill exercise when compared to WT mice, possible due to an increased mineral matrix ratio following treadmill following exercise.

+oim mice demonstrated tendon capsule thickness in comparison to +/oim mice.

FTIR analysis of WT and oim/oim femora as compared to WT.

Urine analysis of WT and oim/oim mice after 8 weeks of treadmill exercise.

Figure 2. Femoral Geometry and Torsional Loading to Failure.

A) Reconstruction of uC. Data to determine total femur length. Image movies were taken from the real-time uC (path C). B) The best-fit freeware fits the top of the femur holder is driven for any desired moment. C) Vertical cross section of a single femur with a high osteotomy fracture. D) Representative graph showing femur torque versus angular displacement for a WT femur.

Table 1. Femoral Geometry of Non-exercised Control and Treadmill Exercised WT and +/oim Mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Femur Length (mm)</th>
<th>Marrow Cavity Diameter (mm)</th>
<th>Marrow Cavity Width (mm)</th>
<th>Cortical Bone Area (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT Control</td>
<td>15.78 ± 0.11</td>
<td>0.75 ± 0.02</td>
<td>0.42 ± 0.01</td>
<td>19.1 ± 0.75</td>
</tr>
<tr>
<td>+/oim Control</td>
<td>15.59 ± 0.09</td>
<td>0.78 ± 0.06</td>
<td>0.47 ± 0.02</td>
<td>19.4 ± 0.56</td>
</tr>
<tr>
<td>+/oim Treadmill</td>
<td>16.70 ± 0.12</td>
<td>0.76 ± 0.02</td>
<td>0.44 ± 0.01</td>
<td>20.0 ± 0.56</td>
</tr>
</tbody>
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FUTURE STUDIES

- Continue characterizing the influence of weight bearing (treadmill) and non-weight bearing (swimming) exercise on the whole bone and material biomechanical properties of bone from male and female oim mice using multi-scale analyses.
- Use the G970C OI mouse, modeled after a human population with osteogenesis imperfecta, to determine if weight bearing (treadmill) and/or non-weight bearing (swimming) exercise may be a potential therapeutic target to improve bone strength in this population.

REFERENCES


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